EUROMAR 2013

Magnetic Resonance Conference

and

Specialized Colloque AMPERE:
"Advances in Solid State Broadband Magnetic Resonance"

Satellite Meetings

cost Spin Hyperpolarisation in NMR and MRI
28th June – 30th June 2013

IDPbyNMR workshop
"Looking at intrinsically disordered proteins through NMR: challenges and perspectives"

EMFL European Magnetic Field Laboratory
Round Table on NMR and EPR in Ultra-High Magnetic Fields

NCSR
"Demokritos"

30th June - 5th July
Hersonissos, Crete, Greece

A European Magnetic Resonance Meeting EUROMAR

Sponsors

NCSR
"Demokritos"

A European Magnetic Resonance Meeting EUROMAR

NATIONAL BANK
OF GREECE

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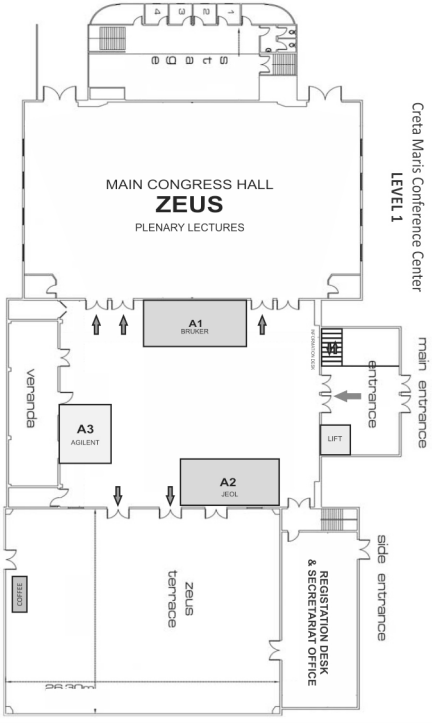
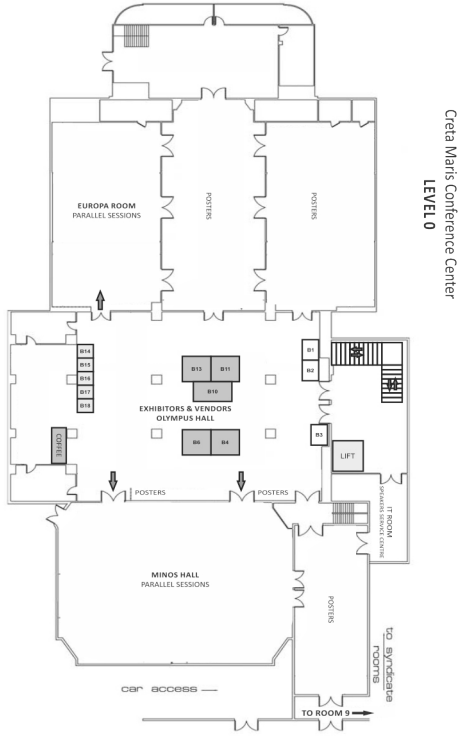
CRETA MARIS CONFERENCE CENTRE MAP

LEVEL 1



LEVEL 0



Registration	Level 1 - Zeus Hall (Secretariat)
Tutorial Lectures	Level 1 - Zeus Hall
Welcome Reception	Level 1 - Zeus Hall (Terrace)
Hospitality Suites	Level 1 - Zeus Hall (Terrace)
Plenary Lectures (PL)	Level 1 - Zeus Hall
Parallel Session Lectures (PS)	Level 0 - Minos Hall & Europa Room
Poster Presentations	Level 0
Exhibitors and Vendors	Level 1 – Zeus Hall & Level 0 – Olympus Hall

Committees

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Konstantina Yannakopoulou, NCSR Demokritos, Greece

WELCOME

ΚΑΛΩΣ ΗΛΘΑΤΕ ΣΤΟ ΣΥΝΕΔΡΙΟ EUROMAR 2013 ΣΤΗΝ ΚΡΗΤΗ

WELCOME TO EUROMAR 2013 in Crete, Greece

The National Center for Scientific Research "Demokritos", the oldest and largest research institution in Greece, is organizing the 9th European Magnetic Resonance Conference EUROMAR 2013 on the island of Crete, from June 30th- July 5th, 2013.

EUROMAR was established in 2004 through the merger of three major NMR conferences; the European Experimental NMR Conference, the AMPERE Congress and the United Kingdom RSC NMR Discussion Group. It is the preeminent European conference in the field of Magnetic Resonance with applications ranging from life sciences and clinical magnetic resonance imaging, to condensed matter physics, chemistry and materials science, as well as industry. The impact of this field on modern science and life is revealed by the fact that in the last 20 years 3 scientists have been awarded the Nobel Prize for achievements in Magnetic Resonance.

EUROMAR 2013 gathers leading scientists from around the world, who will present the latest developments in the field of Magnetic Resonance, a large number of participants and exhibitors, as well as representatives from the commercial and business world related to the field of Magnetic Resonance.

The EUROMAR 2013 Program Committee - whose contribution in organizing a stimulating conference is gratefully acknowledged - with the support of the local organizing committee has put together a robust scientific program. In addition to the main program, four satellite symposia on special topics of Magnetic Resonance will be running in parallel:

1) A Specialized Colloque Ampere entitled "Advances in Solid State Broadband Magnetic Resonance". The event will run jointly with "EUROMAR 2013" during the period of Sunday, June 30th until Thursday, July 4th, 2013.
2) A satellite COST-European Network Meeting on “Spin Hyperpolarization Physics and Methodology in NMR and MRI”, on June 28th and 29th, 2013.
3) The IDP (“Intrinsically Disordered Proteins”) by NMR workshop, organized in the frame of the IDPbyNMR Marie Curie ITN, on June 30th, 2013.
4) A Round Table on “NMR and EPR in Ultra-High Magnetic Fields” of the European Magnetic Field Laboratory, on July 2nd, 2013 (EMFL is part of the European Strategy Forum on Research Infrastructures, ESFRI).

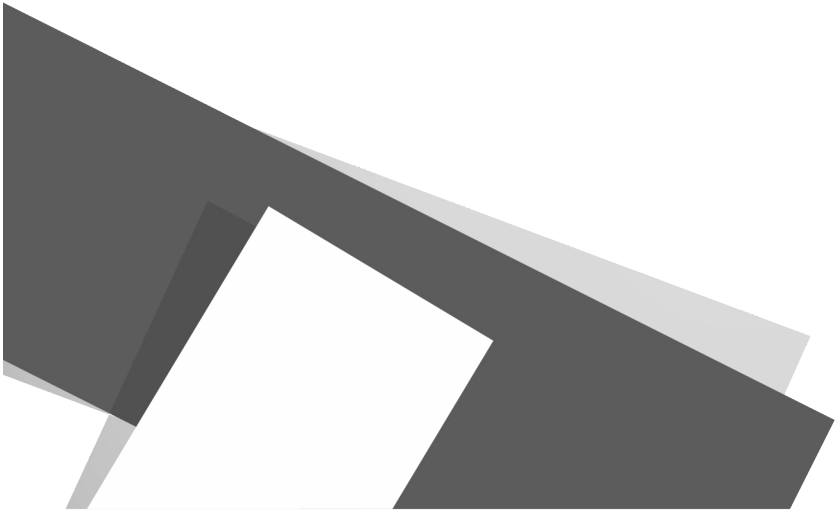
Welcome

Finally, in the framework of EUROMAR 2013, the Annual General Meeting of the International EPR/ESR Society will take place on July 4th, 2013.

Beyond the scientific dimension, EUROMAR 2013 represents a great opportunity for all participants to experience the natural beauty, culture and traditions of one of the most interesting places of Greece: Crete. Crete is the birthplace of the most important Bronze Age civilization in the Mediterranean Sea, the Minoan civilization (circa 2700–1420 BC) which was the first civilization in Europe. The city of Knossos (that will be visited on Wednesday, July 3rd, during the afternoon excursion) was one of the main cities of the Minoan era and the first European city with a palace. The palace is connected with thrilling legends, such as the Labyrinth, the Minotaur, and the story of Daidalos and Ikaros. In the centuries that followed (Classical, Hellenistic, Roman, Byzantine and Modern times) Crete experienced a stormy history, contributing a unique chapter in the history and heritage of Greece while retaining its own local cultural traits and traditions, still present in today's life.

With all the above in mind, the scientific program committee, the local organizing committee, and personally myself, welcome you to EUROMAR 2013 and wish you to enjoy a unique and memorable combination of science, culture, and fun on the beautiful island of Crete.

Georgios Papavassiliou,
Chairman EUROMAR 2013



General Information

REGISTRATION DESK AND SECRETARIAT OFFICE

The registration desk and secretariat office are located on Level 1 in the Zeus Hall (Secretariat area) and will be open on 30th June at 10:00 and will close at the end of the conference.

LOCATION OF LECTURES AND SESSIONS

All sessions will take place at the Creta Maris Conference Centre.
Tutorial sessions will take place in the Zeus Hall (Level 1).
The plenary lectures will be held in the Zeus Hall (Level 1).
The parallel session lectures will be held in the Zeus Hall (Level 1), Minos Hall (Level 0) and Europa Room (Level 0).
Notifications will be present outside the lecture halls for identifying the sessions.
The poster presentations will be held on Level 0.

NAME BADGES

All delegates are kindly requested to wear the provided badge during the conference.

SPEAKERS

Stewards will be available to assist speakers in transferring their presentations to provided computers or setting up personal computers. Speakers are kindly requested to be present in the lecture hall 20 minutes before the session starts.

IT ROOM & SPEAKERS' SERVICE CENTRE

The IT room and speakers' service centre will be situated on Level 0 adjacent to the entrance and computers will be available for those without a laptop. Stewards will be on duty to provide assistance if required. The opening times will be the same as the secretariat office.

INTERNET

Wireless internet connection will be available in all areas of the convention centre. Usernames and passwords for connection to the network will be provided in the IT Room.

General Information

LUNCHES & COFFEE BREAKS

Lunch boxes will be provided on Monday 1st, Tuesday 2nd, Wednesday 3rd & Thursday 4th between 12:45 and 13:30 in the Zeus Hall (Level 1) & the Olympus Hall (Level 0).
Coffee during breaks will be served in the Zeus Hall (Level 1)
& the Olympus Hall (Level 0).

WELCOME PARTY & CONFERENCE DINNER

The Welcome Party will take place on the Terrace in the Zeus Hall on Sunday 30th June at 19:00 – 21:30
The conference dinner will be held in the Cochlias Restaurant (Creta Maris Hotel) on Thursday 4th July at 20:30.
Tickets will be required for admission to the conference dinner.

TOURS

All tours will depart from and return to the Creta Maris Conference Centre. Please note the departure time of the tours below. Tours will depart on time and no refunds will be offered to late-comers.
We request that attendees are in the entrance of the Conference Centre 20 minutes before the departure time.

Tuesday 2nd July	09:00 – 19:30	Rethymnon, Arkadi & Chania
Wednesday 3rd July	14:00 – 19:00	Knossos Palace
"	14:00 – 19:00	Elounda & Spinalonga Island
"	14:00 – 19:00	Lassithi Plateau
Thursday 4th July	09:00 – 19:30	(South Crete) Phaestos, Gortys & Matala
Friday 5th July	09:30 – 17:00	Heraklion City Tour & Museum

VENDOR USER MEETING & ACTIVITIES

See Exhibitors Events Program on page 15

SPECIAL MEETINGS (INVITED ONLY)

The following meetings will be held each day from Monday 1st July to Thursday 4th July in the syndicate room 9 (ENIA) on Level 0.

Monday: 12:50	EUROMAR Board of Trustees Meeting
Tuesday: 12:50	AMPERE Bureau Meeting
Wednesday: 12:50	EUROMAR 2014 Program Committee Meeting
Thursday: 13:50	EUROMAR 2013 Local Committee Meeting

General Information

OTHER MEETINGS (OPEN TO ALL)

IDP by NMR Workshop

Sunday at 10:00 – 14:00 in the Minos Hall (Level 0)

10.00-10.10	Intro
10.10-11.30	Talks
11.30-11.50	Quick Coffee
11.50-12.50	Talks
12.50-13.20	Round Table and Brainstorming
13.20-13.30	Closing
13.30-14.00	Lunch Snacks

Round Table on NMR and EPR in Ultra-High Magnetic Fields

Tuesday at 14:30 – 16:00 in the Zeus Hall (Level 1)

14:30 - 14:40	Presentation of the EMFL (S. Krämer, Grenoble)
14:40 - 14:55	Ultra-high field NMR for Solid State Physics (M. Horvatić, Grenoble)
14:55 - 15:10	Ultra-high field NMR for Solid State Chemistry (A. Kentgens, Nijmegen)
15:10 - 15:25	Ultra-high field ESR (S. Zvyagin, Dresden)
15:25 - 16:00	Discussion

Annual General Meeting of the International EPR/ESR Society 2013

Thursday at 12:40 – 16:00 in the Zeus Hall (Level 1)

FUTURE EUROMAR CONFERENCES

The 10th EUROMAR conference will be held in Zurich, Switzerland,
29th June – 3rd July, 2014

General Information

CATEGORIES	POSTER NUMBERS
Biosolids	300 - 310
Computation and Theory	311 - 320
EPR Methods and Applications	321 - 343
Imaging	344 - 348
In Cell and In Vivo Studies	349 - 351
Industrial and Cultural Applications	352 - 357
Intermetallic and Composite Materials	358 - 360
Liquid State NMR methods	361 - 397
Magnetism and Superconductivity	398 - 407
Materials and Methods in the Nanoscale	408 - 414
Materials and Processes	415 - 423
Metabolomics	424 -440
New Methodologies and Instrumentation Advances	441 - 469
Paramagnetic Systems	470 - 478
Proteins and Nucleic Acids	479 - 537
Relaxation and Dynamics	538 - 569
Sensitivity Enhancement	570 - 583
Small Molecules and Pharmaceuticals	584 - 625
Solid State NMR Methods	626 - 643
Solid State Physics (other)	644
Spatially Resolved NMR and EPR of Solids	645
Transport and Diffusion	646 - 654

General Information

POSTER SESSIONS

There are three poster sessions, on Monday 1st, Tuesday 2nd and Thursday 4th July between 14:30 and 16:00 each day. Authors are asked to be present at their posters on the day they have been assigned. Poster session allocations are as follows:
Each abstract is assigned a poster number which indicates its category (see previous page) and also has a two letter code to denote the day of presentation by the author. MO-Monday, TU-Tuesday and TH-Thursday.
Examples:
344TH, would be a poster in the Imaging category and be presented on Thursday, 4th July.
640TU, is in the Solid State NMR Methods category and would be presented on Tuesday 2nd July.

SET-UP AND REMOVAL

Authors are kindly asked to have their posters mounted on the boards before 12:45 on Monday, 1st July and leave them on display for the duration of the conference. Poster areas are separated according to category and all boards will be labeled with the individual abstract code which can be found in this book in the list of abstracts. Posters should be removed before 17:00 on Thursday 4th July; any unclaimed posters will be discarded.

Remembrance

Ivano Bertini †

passed away untimely on July 7, 2012, at the age of 71, while still fully scientifically active. He was at the crossroad of many scientific communities, encompassing chemistry, biology, biophysics, spectroscopy and biomedicine. The academia and the entire society have lost a unique personality, one that will not be easily forgotten.

Starting from his training in chemistry, Ivano combined the characterization of biomolecules, and particularly their interaction with metal ions, with magnetic resonance techniques, and mostly NMR, for the description and understanding of their structure-function relationship and of the functional pathways in which they are involved. Ivano left his traces all the way from Magnetic Resonance through Inorganic Chemistry and Biochemistry to the Medical Sciences. His innovative approach to paramagnetic NMR allowed him and his group to solve the first solution structure of a paramagnetic protein and to develop approaches and programs which are now routinely used in NMR. He has continuously contributed methodological NMR advancements to study e.g. intrinsically disordered, fibrillar, or sedimented proteins.

His innovative mind and his vision lead him to exploit new approaches and applications of NMR. Through his forward thinking and his ability to coordinate different and diverse approaches, he contributed to the integration of NMR with other techniques to have a more comprehensive picture of biological systems.

Ivano always promoted coordination, interactions and exchanges among scientists, and he has been a tireless key player in the international scientific scene. His vision and determination lead him to initiate many initiatives, from the worldwide recognized NMR Center of the University of Florence CERM and the InterUniversity Consortium CIRMMP, to two biotech spin off companies, to Conference series, Scientific societies… a long list of actions which have been and are still impacting the international scientific community. Magnetic resonance community benefited enormosuly from his activity, among whom the impressive number of Europeans (and non Europeans too!) who had and are having access to the CERM/CIRMMP NMR facility and to its expertise. Thanks to his enthusiasm, driving personality, and the way he viewed international relationships, he was initiator and cofounder of several very successful Conference series, among which there are the *International Conferences on Bioinorganic Chemistry* (ICBICs) and the *Chianti Workshops*.

He was a true protagonist of new ideas: with him the Magnetic Resonance Community lost one of its giants!

There is no way to list here all the achievements of Ivano Bertini but I would like to conclude by saying: THANK YOU, Ivano, for what you did for magnetic resonances, the chemical sciences and the scientific community in general -- you will never be forgotten!

Lucia Banci

Awards and Prizes

THE RUSSELL VARIAN LECTURE AND PRIZE

Lucio Frydman, Weizmann Institute of Science, Israel
"Ultrafast Multidimensional NMR and MRI: Principles and Applications"

THE RAYMOND ANDREW PRIZE OF THE AMPERE GROUP

Michael Tayler, Southampton University, UK

Present address: Radboud University, Netherlands
"Singlet NMR highlights"

MRC AWARD FOR YOUNG SCIENTISTS BY JOHN WILEY & SONS

Francois-Xavier Theillet, Leibniz Institute of Molecular Pharmacology (FMP Berlin)
"In-Cell Dynamics, Interactions and Structural Features of the Human Amyloidogenic Protein Alpha-Synudein"

John Blanchard, Lawrence Berkeley National Laboratory & University of California, Berkeley, CA
"Developments in Zero-Field Nmr for Chemical Analysis"

Olivier Lafon, Univ. Lille 1, CNRS UCCS/LASIR, France
"Nanostructured Materials: An Ariadne's Thread to Understand Dynamic Nuclear Polarization"

Exhibitor Events

Sunday 30	Time	Monday 1	Tuesday 2	Wednesday 3	Thursday 4	Friday 5
	8:30-9:15	Plenary Lectures	Plenary Lectures	Plenary Lectures	Plenary Lectures	
	9:15-10:00					Plenary Lecture
	10:00-10:40	Coffee	Coffee	Coffee	Coffee	Coffee
	10:40-11:10	Parallel Sessions	Parallel Sessions	Parallel Sessions	Parallel Sessions	Parallel Sessions
	11:10-11:30					
	11:30-11:50					
	11:50-12:10					
	12:10-12:40					
10:00-14:00 Registration; IDPbyNMR Workshop	12:40-13:30	13:00-13:15 JEOL Resonance SSNMR in Academic & Industrial Researches & 13:15-13:30 Zero-Boil-Off NMR System - Solution to Helium Crisis (APOLLO WEST, main building of Creta Maris Hotel)	13:00-13:15 JEOL Resonance SSNMR in Academic & Industrial Researches & 13:15-13:30 Zero-Boil-Off NMR System - Solution to Helium Crisis (APOLLO WEST, main building of Creta Maris Hotel)	12:50 - 15:00 Agilent USER meeting (Europa meeting room)	13:00-13:15 JEOL Resonance SSNMR in Academic & Industrial Researches & 13:15-13:30 Zero-Boil-Off NMR System - Solution to Helium Crisis (APOLLO WEST, main building of Creta Maris Hotel)	Plenary Lecture; CLOSING
	13:30-14:30		13:30 - 16:00 THERMO SCIENTIFIC Vendor Workshop (Europa meeting room)			
14:00-16:15 Tutorial Lectures; 13:30-15:15 Bruker News Xpress (Europa meeting room); 14:00 - 18:00 Agilent EMEA NMR Team meeting (APOLLO WEST, main building of Creta Maris Hotel)	14:30-16:00	POSTER SESSION ONE	POSTER SESSION TWO		POSTER SESSION THREE	
	16:00-16:30	Parallel Sessions	Parallel Sessions	Excursion	Parallel Sessions	
16:30-18:45 Opening and Prize Session	16:30-16:50					
	16:50-17:10					
	17:10-17:30					
	17:30-18:00					
	18:15-19:00	Plenary Lecture	Plenary Lecture		Plenary Lecture	
19:00 Welcome party	19:00-22:00	Bruker Hospitality Suite			20:30 Dinner	

Program

Sunday 30th June	
10:00-16:30	Registration
	Zeus Hall
Tutorial Lectures	
14:00-14:45	**Geoffrey Bodenhausen** Principles and practice of Dynamic Nuclear Polarization in static and spinning solids
14:45-15:30	**Beat Meier** NMR structure determination for amyloid fibrils
15:30-16:15	**Gunnar Jeschke** DEER distance measurements and data analysis
Opening & Prize Session	
16:30-16:45	Welcome Remarks **Georgios Papavassiliou** **Lucio Frydman** **Bernhard Blümich**
16:45-17:15	Remembrance Ivano Bertini by **Lucia Banci**
17:15-18:10	The Russell Varian Prize Laudatio by **Krish Krishnamurthy**
	PL 1 - Lucio Frydman Ultrafast multidimensional NMR and MRI: principles and applications
18:10-18:45	The Raymond Andrew Prize Laudatio by **Beat Meier**
	PL 2 - Michael Tayler Singlet NMR highlights
19:00-21:30	Welcome Mixer

Program

Monday 1st July			
08:30-19:00	Registration		
	Zeus Hall		
Chair:	**Malcolm Levitt**		
08:30-9:15	**PL 3 - Daniel B. Vigneron** Clinical translation of hyperpolarized carbon-13 MRI for human research studies: challenges, successes, & future directions		
09:15-10:00	**PL 4 - Arno Kentgens** Nuclear magnetic resonance on the strip		
10:00-10:40	Coffee		
	Zeus Hall	**Minos Hall**	**Europa Room**
Chair: Session:	**C. Hewage** **Bioliquids I**	**A. Jerschow** **MRI / In Vivo**	**M. Bennati** **EPR Methods I**
10:40-11:10	**PS 101 - Angela Gronenborn** Synergy between NMR, cryo-EM and large-scale MD simulations - novel findings for HIV Capsid Function	**PS 106 - Hadassa Degani** Tracking tissue microstructure with diffusion tensor imaging : clinical application in breast cancer diagnosis	**PS 111 - John Morton** Donors in silicon: ESR clock transitions and hyperpolarisation
11:10-11:30	**PS 102 - Helge Meyer** A flexible partnership with amylogenic proteins	**PS 107 - Patrick Berthault** Hyperpolarized ^{129}Xe NMR-based sensors of biological events	**PS 112 - Pavel Baranov** Point defects in SIC as a promising basis for single-defect resonance spectroscopy with room temperature controllable spin quantum states
11:30-11:50	**PS 103 - Guillaume Mas** Specific isotopic labelling of methyl group: a tool box for the dynamics and structural studies of large proteins and complexes	**PS 108 - Eva Serrao** Non invasive detection of early precursor lesions of pancreatic cancer using hyperpolarized [1-^{13}C] pyruvate	**PS 113 -Frederic Mentink-Vigier** Increasing sensitivity of echo detected EPR experiments using CPMG
11:50-12:10	**PS 104 - David Neuhaus** Solution structure of Bud31, a yeast protein containing a highly unusual metal cluster	**PS 109 - Daniel Fiat** ^{17}O Methods for determination of regional cerebral metabolic rate of oxygen (rCMR(O_2)) in the human	**PS 114 - Gavin Morley** Quantum control of hybrid nuclear-electronic qubits
12:10-12:40	**PS 105 - Nicolas Wolff** Perturbation of neuronal signaling pathways by the rabies virus : Role of the PDZ domains	**PS 110 - Igor Koptyug** MMR imaging and spectroscopy in rodents: from transport and metabolism to cancer treatment and NMR signal enhancement	**PS 115 - Matvey Fedin** EPR of "breathing" materials with nitroxides
12:40-14:30	Lunch		

Program

14:30-16:00	POSTER SESSION ONE AND COFFEE		
	Zeus Hall	**Minos Hall**	**Europa Room**
Chair: Session:	**C. Kalodimos** Relaxation and Dynamics	**L. Frydman** Sensitivity Enchancement	**H. Ohta** Materials and Methods in the Nanoscale
16:00-16:30	**PS 116 - Eva Meirovitch** Internal mobility in proteins from the established perspective of restricted motions	**PS 121 - Christian Hilty** Dissolution DNP methods for chemistry	**PS 126 - Alexander Tartakovskii** Nuclear magnetic resonance in semiconductor quantum dots
16:30-16:50	**PS 117 - Donghan Lee** Molecular recognition through concerted backbone and side chain motion	**PS 122 - Hiroki Takahashi** Matrix-free dynamic nuclear polarization enables supramolecular structural studies on biosolids at natural ^{13}C abundance	**PS 127 - Olivier Lafon** Nanostructured materials: an ariadne's thread to understand dynamic nuclear polarization
16:50-17:10	**PS 118 - Jadwiga Tritt-Goc** Interesting features of solvents in the sugar-based organogels revealed by NMR: diffusive diffraction phenomenon and dispersion of the spin-lattice relaxation time	**PS 123 - Anne Lesage** Dynamic nuclear polarization surface enhanced NMR spectroscopy: the design of new biradicals enables application to challenging materials	**PS 128 - Salvatore Mamone** Investigation of the quantum rotation of a water molecule encapsulated into a fullerene cage by solid state NMR
17:10-17:30	**PS 119 - David Peat** High polarization of nuclear spins mediated by nanoparticles at millikelvin temperatures	**PS 124 - Vladimir Zhivonitko** New ways for revealing unobservable nuclear spin order and hyperpolarization	**PS 129 - Claudia Avalos** Towards room temperature DNP and low field NMR using nitrogen- vacancy centres in diamond
17:30-18:00	**PS 120 - Nikolai Skrynnikov** Ensemble-restrained MD simulations: accurate structure leads to accurate dynamics	**PS 125 - Sami Jannin** Some more or less successful attempts at improving dissolution DNP	**PS 130 -Fazhan Shi** Room temperature nanoscale magnetic sensing based on single elecltron spin in diamond
Chair:	**Jürgen Haase**		
18:15-19:00	**PL 5 - Dominique Massiot** Topologic, geometric, and chemical order in materials : insights from solid-state NMR		
19:00-22:00	Bruker Hospitality Suite		

Program

Tuesday 2nd July			
08:30 - 19:00	Registration		
	Zeus Hall		
Chair:	**Claude Berthier**		
08:30-9:15	**PL 6 - Anja Böckmann** Protein solid-state NMR: fibrils, sediments and membrane proteins		
09:15-10:00	**PL 7 - Stephen Hill** Controlled under pressure: high-field EPR studies of magnetostructural correlations in molecule-based magnetic materials		
10:00-10:40	Coffee		
	Zeus Hall	**Minos Hall**	**Europa Room**
Chair: Session:	**T. Meersmann** NMR Methods I	**I. Gerothanassis** Proteins & Nucleic Acids	**M. Hagiwara** Magnetism and Superconductivity
10:40-11:10	**PS 131 - Perunthiruthy Madhu** Heteronuclear spin decoupling in solid-state NMR	**PS 136 -Leo Spyracopoulos** Gate dynamics regulate the catalytic activity of ubiquitination enzymes	**PS 141 - Marc-Henri Julien** NMR in high magnetic fields and the puzzle of high temperature superconductivity
11:10-11:30	**PS 132 - Nicolas Giraud** Combination of J-edited and correlation spectroscopies within a multi-dimensional spatial frequency encoding	**PS 137 - Konstantin Pervushin** Structural and dynamic insights into substrate binding and catalysis in human lipocalin prostaglandin D synthase	**PS 142 - Anton Potočnik** Unconventional superconductivity of alkali-doped fullerenes
11:30-11:50	**PS 133 - Diego Carnevale** Solid-state proton NMR of paramagnetic metal complexes: DANTE spin echoes for selective excitation in inhomogeneously-broadened lines	**PS 138 - Harald Schwalbe** Time-resolved NMR studies of the light-activated states of rhodopsins	**PS 143 - Pietro Carretta** Anomalous behaviour of $1/T_2$ in the iron-based superconductors
11:50-12:10	**PS 134 - Maïwenn Beaugrand** NMR study of the effect of dilution on isotropic bicelles	**PS 139 - Eric Guittet** Integrated structural biology shows how scarce sequence elements control the function of single ß-thymosin/WH2 domains in actin assembly	**PS 144 - Vladislav Kataev** High-field ESR and NMR spectroscopy of the novel low- dimensional quantum magnet $BaAg_2Cu[VO_4]_2$
12:10-12:40	**PS 135 - Kiyonori Takegoshi** How to squeeze more signal intensities out of nuclear spins in solids ... Mehr Licht!	**PS 140 - Vladimír Sklenář** Order in disorder – atomic resolution studies of unstructured proteins	**PS 145 - Sergei Zvyagin** Spin dynamics in the spin-1/2 triangular-lattice antiferromagnet Cs_2CuBr_4
12:40-14:30	Lunch		

Program

14:30-16:00	POSTER SESSION TWO AND COFFEE		
	Zeus Hall	**Minos Hall**	**Europa Room**
Chair: Session:	**E. Mikros** Small Molecules & Metabolomics	**A. Bagno** Theory and Computation	**D. Fiat** Paramagnetic Systems
16:00-16:30	**PS 146 - James Prestegard** Monitoring enzymatic conversion of DNP-enhanced substrates by Indirect detection	**PS 151 - Alexandre Bonvin** A unified conformational selection and induced fit approach to the modelling of protein-peptide interactions	**PS 156 - Lucia Banci** NMR in molecular systems biology: from structures to function
16:30-16:50	**PS 147 - Alan Wong** Nanoliter HR-MACS NMR metabolic profiling of biopsy and microorganism	**PS 152 - Perttu Lantto** First-principles analysis of NMR isotope shifts in heavy-element systems	**PS 157 - Jozef Kowalewski** Paramagnetic NMR (pNMR): recent developments along the borderline of theory and experiments
16:50-17:10	**PS 148 - Elena Matei** Fluorinated carbohydrates as lectin ligands: dissecting glycan-cyanovirin interactions by ^{19}F-NMR	**PS 153 - Tobias Madl** De novo structure prediction of proteins using solvent PREs	**PS 158 - Miwa Murakami** Application of rotation-synchronized DANTE to paramagnetic solids under MAS
17:10-17:30	**PS 149 - Ulrich Guenther** Deciphering cancer metabolism for drug discovery using NMR	**PS 154 - Nikolaos Sgourakis** Advances in computational modelling of macromolecular assemblies using limited NMR data	**PS 159 - Frans Mulder** An optimal paramagnetic relaxation agent for NMR spectroscopy of intrinsically disordered proteins
17:30-18:00	**PS 150 - Cynthia Larive** Probing heparin structure through NMR measurements of exchangeable protons	**PS 155 - Frank Neese** Theoretical EPR spectroscopy of open-shell transition metal complexes with strong spin orbit coupling	**PS 160 - Joris van Slageren** Unconventional EPR investigations of molecular nanomagnets
Chair:	**Beat Meier**		
18:15-19:00	**PL 8 - Shimon Vega** What do we think we know about the DNP mechanism in solids		

Program

Wednesday 3rd July			
	Zeus Hall		
Chair:	**Geoffrey Bodenhausen**		
08:30-9:15	**PL 9 - Isabella Felli** New methods to study intrinsically disordered proteins based on 13C direct detection		
09:15-10:00	**PL 10 - Graham Smith** Very high sensitivity orientation-PELDOR		
10:00-10:40	Coffee		
	Zeus Hall	**Minos Hall**	**Europa Room**
Chair: Session:	**G. Boutis** Biosolids I	**S. Caldarelli** New Methodologies and Instrumentation Advances	**J. Dolinšek** Intermetallic and Composite Materials
10:40-11:10	**PS 161 - Bernd Reif** Amyloid aggregates and large soluble protein complexes	**PS 166- Ago Samoson** Spinning beyond 250 kHz	**PS 171 - Hae Jin Kim** Hydrogen dynamics in Zr- and Hf-based alloys studied by ^{2}D NMR
11:10-11:30	**PS 162 - Jean Paul Amoureux** Structure and dynamics of solid- state biomolecules: novel methods to probe short and long range ^{13}C-^{13}C proximities (up to 100 pm) and to measure ^{1}H-^{15}N and ^{1}H-^{13}C dipolar couplings	**PS 167 - Hitoshi Ohta** Developments of multi extreme high field ESR system and its application to honeycomb lattice antiferromagnet	**PS 172 - Ulrich Scheler** Polymer composite materials - structure, dynamics and interfaces
11:30-11:50	**PS 163 - Flemming Larsen** Multiphase NMR - a valuable tool for structural analysis of polysaccharides	**PS 168 - John Blanchard** Developments in zero-field NMR for chemical analysis	**PS 173- Magdalena Wencka** Complex magnetism of $MnCO_3$ small hollow nanospheres: from AFM/FM to spin glass
11:50-12:10	**PS 164 - Józef Lewandowski** Site-specific protein dynamics in the solid state probed by ^{13}C and ^{15}N relaxation measurements employing magic angle spinning frequencies up to 100 kHz	**PS 169 - Nicola Salvi** Towards para-water in bulk	**PS 174 - Miroslav Požek** Dynamic correlations in ionic conductor Cu_2HgI_4 probed by NMR
12:10-12:40	**PS 165 - Tatyana Polenova** Structure and dynamics of microtubule-associated and HIV-1 protein assemblies: methods and applications	**PS 170 - Dimitrios Sakellariou** A portable permanent-magnet analyzer for high-resolution ^{1}H MAS NMR spectroscopy	**PS 175 - Martin Klanjšek** NMR as a tool for checking the structural models of complex metallic alloys and quasicrystals
12:40-14:30	Lunch		

Program

Thursday 4th July			
	Zeus Hall		
Chair:	**Sabine van Doorslaer**		
08:30-9:15	**PL 11 - Daniella Goldfarb** High field and high spin - a different approach for long range distance measurements in biomolecules		
09:15-10:00	**PL 12 - Peter van Zijl** Chemical exchange saturation transfer (CEST) NMR and MRI		
10:00-10:40	Coffee		
	Zeus Hall	**Minos Hall**	**Europa Room**
Chair: Session:	**J. P. Amoureux** NMR Methods II	**I. Felli** In Cell and In Vivo Studies	**S. Misra** EPR Methods II
10:40-11:10	**PS 176 - Jeffrey Reimer** NMR distance measurements, along with molecular simulations, act as a blunt instrument for ascertaining linker distributions in metal-organic frameworks	**PS 181 - Volker Dötsch** In-cell NMR spectroscopy of larger proteins and G-quadruplexes	**PS 186 - Vasili Petrouleas** Water, light, and ... EPR spectroscopy
11:10-11:30	**PS 177 - Veniamin Shevelkov** Efficient CO-CA transfer in deuterated and protonated proteins by band-selective homonuclear cross-polarization	**PS 182 - Miquel Pons** Real time monitoring of in vivo multi-phosphorylation events in an intrinsically disordered protein	**PS 187 - Jack Freed** New developments in high sensitivity pulse dipolar ESR and protein structure determination
11:30-11:50	**PS 178- Yusuke Nishiyama** Sensitivity enhancement in solid- state NMR at very fast magic- angle sample spinning by RFDR mixing	**PS 183- Francois Xavier Theillet** In-cell dynamics, interactions and structural features of the human amyloidogenic protein alpha-synuclein	**PS 188 - Andriy Marko** Extraction of geometrical parameters from EPR/PELDOR data and their use for the verification of molecular structure obtained by NMR
11:50-12:10	**PS 179- Gang Zheng** Water control: the next generation solvent signal suppression in NMR	**PS 184 - Stefan Klippel** Xenon based hyper-CEST-MRI of cryptophane labeled cells	**PS 189 - Ryszard Narkowicz** Cryogenic receiver for low temperature ESR measurements
12:10-12:40	**PS 180 - Gianluigi Veglia** Allosteric regulation of the sarcoplasmic reticulum Ca^{2+}-atpase by phospholamban and sarcolipin using solid-state NMR spectroscopy	**PS 185 - Mathilde Lerche** Non-invasive in cell determination of NAD+/NADH ratios using hyperpolarized glucose show large variations in metabolic phenotypes	**PS 190 - Bela Bode** Accurate extraction of multiple pulse EPR distances in homo- oligomeric systems
12:40-14:30	Lunch		

Program

14:30-16:00	POSTER SESSION THREE AND COFFEE		
	Zeus Hall	**Minos Hall**	**Europa Room**
Chair: Session:	**C. Geraldes** Bioliquids II	**F. Milia** Industrial and Cultural Applications	**J. Tritt-Goc** Spatially Resolved NMR and EPR of solids
16:00-16:30	**PS 191 - John Christodoulou** Co-translational protein folding on the ribosome: using NMR spectroscopy to provide structure and dynamics of ribosome-nascent chains	**PS 196 - Donatella Capitani** NMR methodologies in cultural heritage	**PS 201 - Jerome Ackerman** Solid state MR spectroscopy and imaging of bone mineral and matrix
16:30-16:50	**PS 192 - Roberta Pierattelli** The heterogeneous structure of E7 from HPV16 revealed by NMR spectroscopy	**PS 197 - Andrey Andreev** Internal field ^{59}Co NMR for Fischer-Tropsch heterogeneous catalysts characterization	**PS 202 - Alexej Jerschow** In situ MRI of batteries and supercapacitors
16:50-17:10	**PS 193 - Ewen Lescop** Fast conformational exchange governs electron flux efficiency through a multidomain diflavin reductase	**PS 198 - Franz Dalitz** On-line process and reaction monitoring by low-field NMR spectroscopy	**PS 203 - Thomas Meersmann** In situ hp ^{129}Xe MRI of combustion
17:10-17:30	**PS 194 - Sonja Dames** Detailed characterization of the membrane-interactions of the TOR FATC domain by NMR, oriented CD spectroscopy and MD simulations	**PS 199 - Wasif Zia** Compact stray-field NMR of moisture: frescoes and polyethylene	**PS 204 - Marios Katsiotis** NMR and MRI analysis of rock core samples from oil wells
17:30-18:00	**PS 195 - Mikael Akke** ^{13}C relaxation dispersion experiments for aromatic side chains in proteins	**PS 200 - Lynn Gladden** Applications of MRI in imaging fluid flows and reaction engineering	**PS 205 - Aharon Blank** High sensitivity high resolution pulsed electron spin resonance in solids - technique and applications
Chair:	**Gil Navon**		
18:15-19:00	**PL 13 - Yi-Qiao Song** Magnetic resonance of porous media		
20:30	Conference Dinner		

Program

Friday 5th July			
	Zeus Hall		
Chair:	**Bernhard Blümich**		
09:15-10:00	**PL 14 - Dmitri Budker** Zero- and low-field NMR spectroscopy		
10:00-10:40	Coffee		
	Zeus Hall	**Minos Hall**	**Europa Room**
Chair: Session	**M. Pons** Biosolids II	**L. Gladden** Transport and Diffusion	**S. Stapf** Materials and Processes
10:40-11:10	**PS 206 - Marc Baldus** Ultra-high field DNP-ssNMR applied to cellular structural biology	**PS 211 - Daniel Topgaard** New diffusion MRI methods for characterization of microheterogeneous materials	**PS 216 - Robert Schurko** BRAIN-CP: broadband adiabatic inversion-cross polarization – applications to Solid-State NMR of Spin-1/2 and quadrupolar nuclei
11:10-11:30	**PS 207 - Guido Pintacuda** Resonance assignment and structure investigation by high resolution ^{1}H-detected solid-state NMR under ultra-fast MAS: from microcrystalline proteins to large protein assemblies	**PS 212 - Gisela Guthausen** Characterization of (double-)emulsions by NMR: diffusometry and relaxometry	**PS 217 - Oleg Poluektov** Photoinduced charge separation processes: from natural photosynthesis to organic photovoltaic cells
11:30-11:50	**PS 208 - Anne Jantschke** Dynamic nuclear polarization for understanding biomineralisation in diatoms	**PS 213 - Diana Bernin** ^{1}H and ^{19}F diffusion and relaxation imaging to monitor detailed drug release profiles through extended release films	**PS 218 - Eike Brunner** Chiral recognition in metal- organic frameworks studied by solid-state NMR spectroscopy using chiral solvating agents
11:50-12:10	**PS 209 - Amir Goldbourt** Magic angle spinning NMR studies of intact bacteriophage viruses	**PS 214 - Catherine Bessada** Towards a better undestanding of the local structure in molten salts : coupling NMR chemical shifts, self diffusion coefficients measurements at high temperature and molecular dynamics simulations	**PS 219 - Yan-Yan Hu** Solid state NMR studies of rechargeable battery materials
12:10-12:40	**PS 210 - Ann McDermott** Conformational exchange and ion binding in the ion channel KcsA	**PS 215 - Janez Stepišnik** Dynamics of polymers and liquids studied by the modulated gradient spin echo method	**PS 220 - Xianyu Xue** NMR crystallography of high-pressure silicate minerals and related inorganic materials
Chair:	**Gunnar Jeschke**		
13:00-13:45	**PL 15 - Charalampos Kalodimos** NMR of large proteins: molecular chaperones		
13:45-14:15	**CLOSING**		

Plenary Lectures

Plenary Lectures

PL 01

Ultrafast Multidimensional NMR and MRI: Principles and Applications

Lucio Frydman

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Over a decade ago we proposed and demonstrated a scheme enabling the acquisition of arbitrary multidimensional NMR spectra and/or images (MRI), within a single scan. This is by contrast to the hundreds or thousands of scans that are usually needed to collect this kind of data. Provided that the target molecule's signal is sufficiently strong, the acquisition time of NMR/MRI scans can thus be shortened by several orders of magnitude. This new "ultrafast" methodology is compatible with existing multidimensional pulse sequences and can be implemented using conventional hardware. The manner by which the spatiotemporal encoding of the NMR interactions proceeds will be summarized. The new horizons that are opened by these protocols will also be exemplified with a variety of NMR and MRI projects we are currently involved with in the fields of chemistry, biophysics, biology and medicine. The incorporation into these experiments of nuclear hyperpolarization procedures capable of increasing the sensitivity of these single-scan nD solution NMR and MRI acquisitions by factors ranging from 10^3-10^6, will also be assessed.

PL 02

Singlet NMR Highlights

Michael Tayler

School of Chemistry, Southampton University, Southampton, UK.
Present address: Institute for Molecules and Materials, Radboud University, Nijmegen, Netherlands

A singlet state is the unique exchange-antisymmetric quantum state formed between two spins-½. This system can be rather useful to an NMR spectroscopist: in many molecules, a singlet state involving two spin-1/2 nuclei relaxes more slowly than the magnetisation of each nucleus in isolation. Singlet states decay via mechanisms that are antisymmetric with respect to nuclear permutation. These are in most cases much weaker than those permutation-symmetric. A paradigm of this phenomenon is the slow (often days-long) ortho-para nuclear spin conversion in dihydrogen, H2. Nuclear singlet states offer to minimise decay of nonequilibrium spin order during long waiting periods in an NMR experiment, such as those required for diffusion, a chemical reaction or bulk sample transport.In this talk I will summarise two topics of current interest for polarisation storage using singlet states. I will overview some experimental measurements of singlet relaxation where additional spins (nuclei or electrons) are either added to or excluded from a spin-1/2 pair system, for instance by isotopic substitution or paramagnetic doping, and the rate contribution to the singlet lifetime determined by difference. I then discuss singlet state manipulations in regimes where the participating spin-1/2 pair nuclei are nearly equivalent, where the singlet is a near eigenstate of the system. Development in this area has numerous targets: high-symmetry molecules designed purposely for very long singlet lifetimes, polarisation storage in vivo and singlet NMR at low magnetic fields.

References:
1.M.C.D. Tayler, "Theory and practice of singlet nuclear magnetic resonance", PhD thesis, Southampton University (2012)
2.M.H. Levitt, "Singlet nuclear magnetic resonance", Annu. Rev. Phys. Chem. 63, 89-105 (2012)
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4.G. Pileio, "Relaxation theory of singlet states in two spin-1/2 systems", Progr. NMR Spectrosc. 56, 217-231(2010)

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Plenary Lectures

PL 03

Clinical Translation of Hyperpolarized Carbon-13 MRI for Human Research Studies: Challenges, Successes, & Future directions

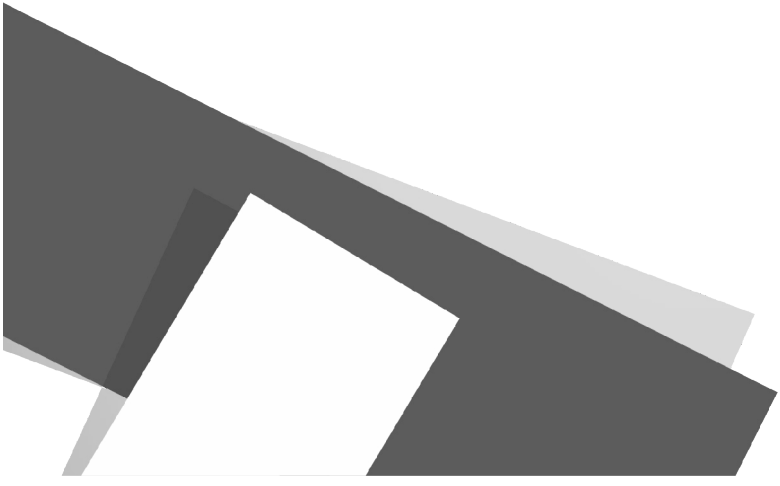
Daniel B. Vigneron

University of California San Francisco

Hyperpolarized carbon-13 MRI using the dissolution dynamic nuclear polarization (DNP) method can provide a >10,000 fold signal enhancement for detecting ^{13}C probes of endogenous, nontoxic, nonradioactive substances such as pyruvate to monitor metabolic fluxes through multiple key biochemical pathways (Ardenkjaer-Larsen et al PNAS 2003; 100:10158; Golman et al. Cancer Res. 2006; 66:10855).

The hyperpolarization of [1-^{13}C]pyruvate has demonstrated the ability to not only detect pyruvate uptake but also the *in vivo* enzymatic conversion to ^{13}C-lactate through the enzyme lactate dehydrogenase (LDH), ^{13}C-alanine through the alanine transaminase (ALT) pathway; and $^{13}CO_2$ & ^{13}C-bicarbonate through the pyruvate dehydrogenase (PDH) catalyzed metabolic pathway. In addition to basic science and animal studies utilizing HP ^{13}C MRI, we developed new techniques and coils for the first human studies in prostate cancer patients and tested them in preclinical murine prostate cancer and canine models with injections of HP ^{13}C-pyruvate. A proof-of-concept (POC) DNP instrument was constructed through an academic-industry collaboration with GE to operate in a sterile clean room next to a 3T MR scanner under the direction of UCSF Clinical Pharmacy investigators. Specialized C-13 MRI techniques were developed to provide extremely rapid volumetric imaging and serial dynamic acquisitions to monitor temporal metabolic changes in cancer patients following the injection of HP ^{13}C-pyruvate. Led by UCSF Cancer Center investigators, our multidisciplinary research group designed and conducted the world's first clinical trial of hyperpolarized carbon-13 MRI titled, “A Phase 1 ascending-dose study to assess the safety and tolerability and imaging potential of hyperpolarized Pyruvate (^{13}C) Injection in subjects with prostate cancer”. This study received FDA-IND approval and 31 patients were studied demonstrating feasibility and safety with no dose-limiting toxicity up to 0.42ml/kg of 250mM HP pyruvate.

Increased lactate conversion was observed in prostate cancers up to 70s following injection. Future plans include 3 new clinical research studies in prostate cancer and brain tumor patients, validation of the new GE commercial dissolution DNP instrument recently purchased through an NIH high-end instrumentation grant, revised IND applications, new acquisition/analysis developments, and future kidney and liver studies.



Plenary Lectures

PL 04

Nuclear Magnetic Resonance On the Strip

Jacob Bart[1,2], Anna-Jo Oosthoek – de Vries[1], Koen Tijssen[1], Gijs van der Heijden[1], Pjotr Kurek[1,2], Mithun Goswami[1], Vinod Nair[1], Vipin Agarwal[1], Lavinia Utiu[1], Han Gardeniers[2], Jan van Bentum[1] and Arno Kentgens[1]

[1]*Institute for Molecules and Materials, Radboud University, Nijmegen*
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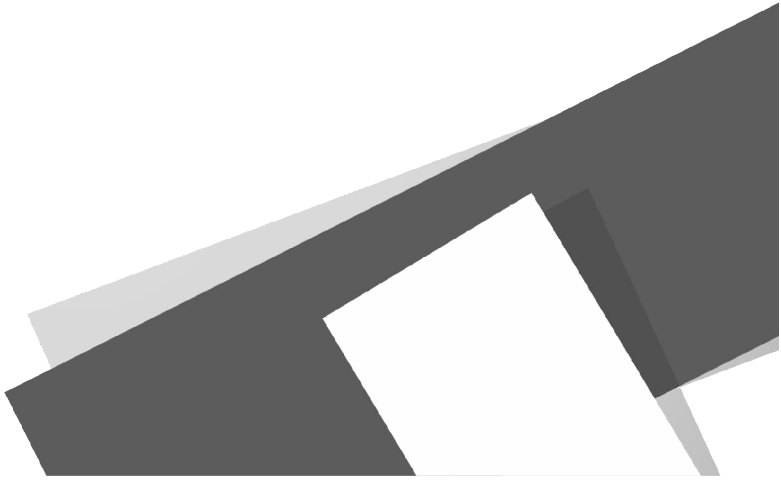
A route to enhance NMR sensitivity for mass limited samples, is the miniaturization of the detection coil. The state of the art of the newly developed stripline technology and its applications will be reviewed. The details behind this geometry will be described demonstrating the possibility to obtain high-sensitivity one and two-dimensional spectra in-flow. This is used in a variety of relevant applications in a microfluidic context such as monitoring of fast chemical reactions in-situ. Using a tapered stripline structure it proves to be possible to combine MRI (imaging) with MRS (spectroscopy) to perform localized spectroscopy or even faster kinetic experiments.
Dynamic Nuclear Polarization (DNP) may help to overcome sensitivity problems in NMR measurements of mass-limited samples. The development of on-chip hyper-polarization is pursued, integrating a newly developed microwave resonator for saturating the electron spin system of dissolved radicals such as TEMPOL with microcoil NMR detection. This allows efficient Overhauser DNP of water or ethanol in solution. The proton enhancement in water is well understood and consistent with independent diffusion and relaxation measurements. In more complex molecules such as ethanol we find similar enhancement levels for all functional groups (OH, CH_2 and CH_3). The CH_2 and CH_3 proton enhancement can be well understood in the same framework as proposed for the water-TEMPOL system. The situation for the OH proton is more complicated due to the fast dynamics of hydrogen bonding.
The stripline approach has also proven to be a fertile platform for more widespread applications in materials science studies. In the field of polymer research, the stripline technology is presently used to study phase composition of polymer materials as a function of strain applied to the polymer films. In the study of thin film materials for hydrogen storage and photochromic materials, the stripline technology provides a unique way to study local crystal structure and hydrogen dynamics in the amorphous state where X-ray and other techniques fail. Furthermore we studied thin-film semi-conductors providing insight in the local order and disorder in this type of technologically relevant materials. Here the aim is to link growth parameters to structural effects which in turn influence the performance of the materials.



Plenary Lectures

PL 05

Topologic, Geometric, and Chemical Order in Materials : Insights from Solid-State NMR

Dominique Massiot*, Sylvian Cadars, Michael Deschamps, Emmanuel Véron, Mounesha N.Garaga, Robert J. Messinger, Mathieu Allix, Pierre Florian and Franck Fayon

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Local order, as opposed to the long-range order of the ideal crystalline structures, can be considered as an intrinsic characteristic of real materials, being often the clue to the tuning of their properties and their final applications. While ordering can be easily assessed in two-dimensional imaging techniques with resolution approaching the atomic level, the diagnostic, description, and qualification of local order in three dimensional systems is much more challenging.
Solid-state nuclear magnetic resonance [NMR] and its panel of constantly developed new instruments and methods enable local, atom selective characterization of structures and assemblies ranging from atomic to nanometer length scales. This opens unique opportunities for characterizing a variety of materials, ranging from crystalline compounds to amorphous or glassy materials, for which we show that it becomes possible to separate topologic, geometric and chemical contributions to the order or disorder, in cooperation with other experimental techniques and in-silico approaches.
As identified by solid state NMR, the local structure of amorphous materials or glasses consists of well-identified structural entities up to at least the nanometer scale. Instead of speaking of disorder, we propose a new paradigm to describe their structures in terms of a continuous assembly of locally defined structures, reminiscent of locally favored structures (LFS). This draws a comprehensive picture of amorphous structures based on fluctuations of chemical composition and structure over different length scales. We hope that these new local or molecular insights could open new possibilities for considering key questions related to nucleation and crystallization, as well as chemically (spinodal decomposition) or density driven (polyamorphism) phase separation, which could enable future applications.

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4. P. Florian, E.Véron, T.Green, J.R.Yates, D.Massiot, Elucidation of the Al/Si ordering in Gehlenite Ca2Al2SiO7 by combined 29Si and 27Al NMR spectroscopy / quantum chemical calculations, Chem. Mater. 24 4068–4079 (2012)
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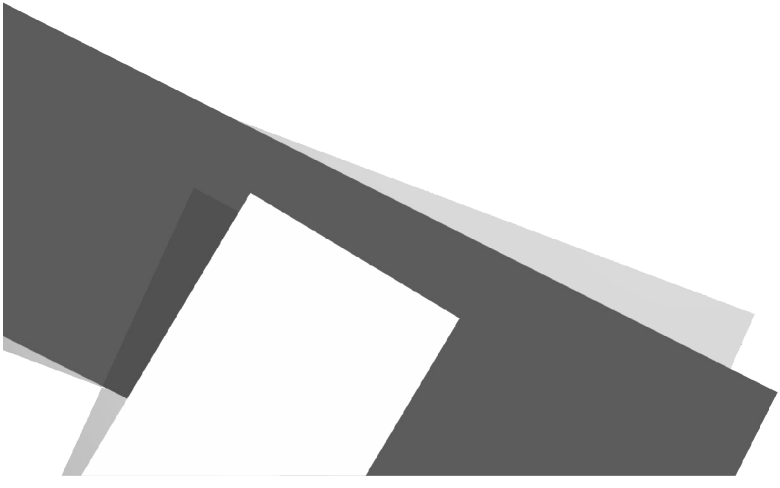
PL 06

PROTEIN SOLID - STATE NMR: FIBRILS, SEDIMENTS AND MEMBRANE PROTEINS

A. Böckmann, B. H. Meier, N. Luckgei, A. Schütz, B. Kunert, C. Gardiennet, B. Habenstein, P. Falson, J.-M. Jault, L. Terradot, L. Bousset, R. Melki

Institut de Biologie et Chimie des Protéines UMR 5086 CNRS/Université de Lyon

Solid-state NMR is advancing to become an important tool in biophysical studies. Proteins targeted by this methods include fibrillar proteins, membrane proteins, but also, as recently shown, simple sediments of large proteins or their complexes. I will present recent work and progress on the different types of protein preparations.
Sup35, a 685 amino-acid protein of *Saccharomyces cerevisiae*, is a yeast prion protein, which is at the origin of the [*PSI*$^{+}$] phenotype. It contains 685 amino acids and consists of three domains.
The fragment Sup35NM is often used as a convenient model to document the assembly and infectious properties of the full-length prion.
We investigated Sup35 and Sup35NM prion fibrils by two- and three- dimensional solid-state NMR spectroscopy, which allowed us to assign the rigid and well-ordered fibrillar core, and also to reveal static and dynamic disorder in the remaining protein sequence.
DnaB is a helicase from *Helicobacter pilori*. It is believed to function as a dodecamer, each subunit having a molecular mass of 55.7 kDa (488 residues).
We compared the fingerprints from the isolated domains to the functional full-length protein. Also, DnaB has different interaction partners, which however do not co-crystallize. We recently showed that sedimentation can be used as sample preparation, and illustrate how this approach can be a promising possibility to study such proteins and their partners.
BmrA is a membrane protein of the class of ABC transporters. It functions as a dimer of 2x 64.5 kDa (589 residues). It is a close bacterial homologue of the P-glycoprotein, which is involved in multidrug resistance in humans. In order to obtain stable preparations showing a high protein/lipid ratio of BmrA for solid-state NMR studies, we reconstituted it in its native lipids extracted from *B. subtilis*.
The obtained samples yield very good signal/noise ratio and linewidth.
The possibility to prepare the protein under different conditions in a lipid environment should allow to gain insight in the conformational changes between the open, closed, and drug-bound states.



Plenary Lectures

PL 07

Controlled Under Pressure: High-Field EPR Studies of Magnetostructural Correlations in Molecule-based Magnetic Materials

Stephen Hill,[1] C. C. Beedle,[1] K. Thirunavukkuarasu,[1] S. M. Winter,[2] J. A. Schlueter,[3] S. W. Tozer,[1] J. L. Manson,[4] R. T. Oakley,[2]
A. Prescimone,[5] S. Parsons,[5] E. K. Brechin[5]

[1]Florida State University and National High Magnetic Field Laboratory; [2]University of Waterloo; [3]Argonne National Laboratory; [4]Eastern Washington University; [5]University of Edinburgh

The application of high pressures in the study of molecule-based materials has gained considerable recent interest, in part due to their high compressibilities, but also because the relevant electronic/magnetic degrees of freedom are often very sensitive to pressure. For example, small changes in the coordination environment around a magnetic transition metal ion can produce quite dramatic variations in both the on-site spin-orbit anisotropy as well as the exchange interactions between such ions when assembled into clusters or 3D networks.
This has spurred the development of sophisticated spectroscopic tools that can be integrated with high-pressure instrumentation.
The study of magnetic structure/property relations, or magnetostructural correlations, requires not only precise crystallographic data, but also detailed spectroscopic information concerning the unpaired electrons that give rise to the magnetic properties. After a brief introduction to the high-field EPR facilities available to external users of the NHMFL, this lecture will describe the development and application of methods enabling EPR studies of oriented single-crystal samples subjected to hydrostatic pressures of up to 3.5 GPa. Two example applications will be discussed.
The first involves the magnetic coordination polymer $[CuF_2(H_2O)_2(pyz)]$ (pyz = pyrazine), which undergoes a pressure-induced structural transitions, involving a ~90° reorientation of the Jahn-Teller distortion associated with the Cu^{II} ions [1,2]. EPR studies provide crucial insights into the disposition of the magnetic d_{x2-y2} orbital and consequent changes in the effective dimensionality of the extended Cu···Cu exchange interactions (from 2D to 1D [3]) that occur at this transition.
A second example involves an organic radical ferromagnet that holds records for both the highest transition temperature and coercivity. The latter property is the result of an unexpectedly high magnetic anisotropy, attributable to anisotropic spin-orbit-mediated exchange (hopping) processes [4,5]. Ferromagnetic resonance measurements reveal an enhancement of the anisotropy field under applied pressures, which is attributed to increased π-orbital overlap that, in turn, increases the strength of the exchange anisotropy.

[1] Angew. Chem. Int. Ed. **49**, 419 (2010); [2] Angew. Chem. Int. Ed. **49**, 419 (2010); [3] Chem. Mater. **20**, 7408 (2008); [4] J. Am. Chem. Soc. **133**, 8126 (2011).

PL 08

WHAT DO WE THINK WE KNOW ABOUT THE DNP MECHANISM IN SOLIDS

Shimon Vega

Chemical Physics Department,Weizmann Institute of Science, Rehovot, Israel

In this presentation I will discuss our understanding of the Dynamic Nuclear Polarization mechanism responsible for the sensitivity enhancement of NMR signals of frozen solutions of molecules dissolved in organic solutions containing free radicals.
Since its introduction about sixty years ago the basic solid-DNP experiment has not changed and the cw MW irradiation remains the energy source driving the electron-nuclear polarization transfer process.
In recent years DNP went through a renaissance, mainly because of the important high field MAS-DNP development and the introduction of dissolution-DNP. However, the original three solid-DNP enhancement mechanisms, the solid effect1 (SE), the cross effect (CE) and thermal mixing (TM), remain responsible for the signal enhancements. Despite these facts we are still sometimes puzzled about how DNP works in our samples.
Thus, I will try to approach some of these questions by presenting our recent understanding of solid state DNP, based on experimental observations and numerical simulations, in terms of the competition between the SE-DNP and the CE-DNP process, the necessity of the TM formulation and the task of the relaxation assisted spin diffusion process in static and rotating samples.

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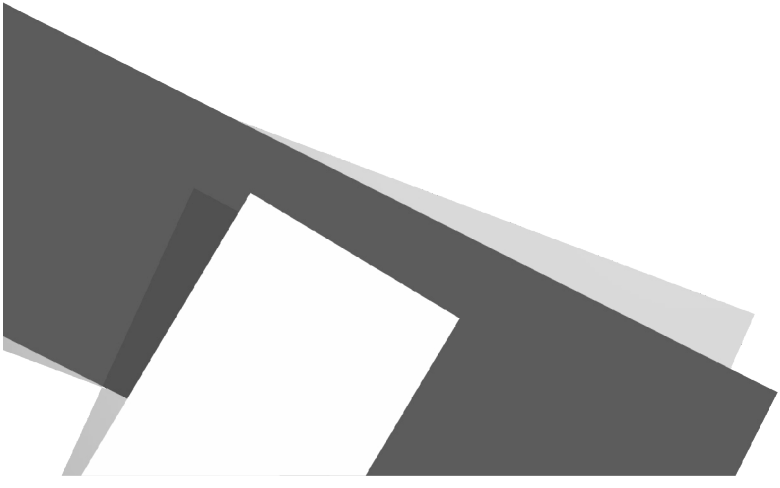

Plenary Lectures

PL 09

New Methods to Study intrinsically Disordered Proteins Based on ^{13}C Direct Detection

Isabella C. Felli

CERM and Department of Chemistry "Ugo Schiff", University of Florence, Sesto Fiorentino, Italy

Recent progress in NMR instrumentation, in parallel to the growing interest in understanding the functional role of protein intrinsic disorder and flexibility, have stimulated the development of a variety of new NMR methods to study intrinsically disordered proteins (IDPs)[1].
The high flexibility and largely solvent exposed backbone typical of IDPs influence NMR parameters causing reduced chemical shift dispersion and extensive broadening of amide proton resonances, in particular approaching physiological conditions.
These constitute general features of IDPs that need to be taken into account in the design of NMR experimental methods. ^{13}C detected NMR experiments now offer a valuable tool to address these peculiar features of IDPs. The experimental variants to improve the performance of ^{13}C detected NMR experiments to study IDPs include the design of multidimensional experiments[2], the exploitation of longitudinal relaxation enhancement[3], the design of experiments to alleviate the problem of extensive cross peaks overlap[4].
The new experiments are demonstrated on a paradigmatic IDP, α-synuclein.

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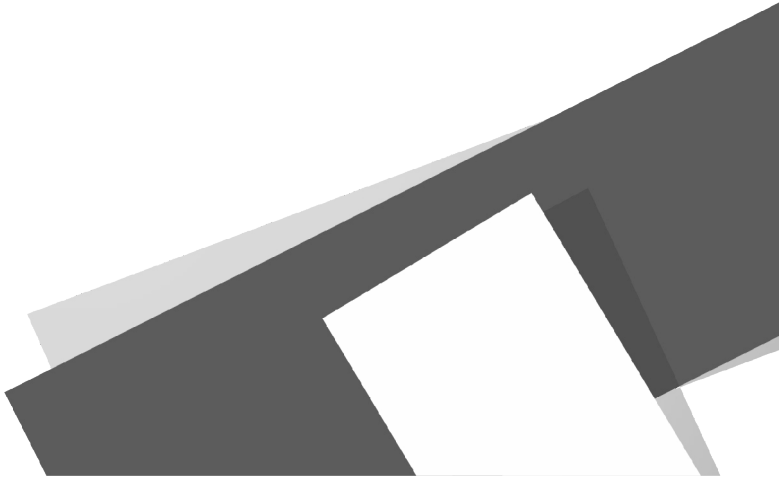


PL 10

VERY HIGH SENSITIVITY, ORIENTATION-PELDOR

Graham Smith[1], Robert Hunter[1], David Bolton[1], Paul Cruickshank[1], David Norman[2], Bela Bode[1], Olav Schiemann[3], Johannes McKay[1], Hassane El Mkami[1]

University of St Andrews[1], University of Dundee[2], University of Bonn[3]

Today PELDOR (Pulsed Electron Double Resonance) in conjunction with Site-Directed Spin Labelling is almost a standard characterisation technique in structural biology. PELDOR can be used to measure distance and distance distributions in biological structures beyond 10 nm. It is often used to understand conformational changes, protein-protein interactions and to provide additional long-range distance constraints that can remove ambiguities in structural models derived from NMR or X-ray crystallography data.

PELDOR measurements are usually ran at 50K at X-band (10GHz) at sample concentrations of 100uM and typically take 12-24 hours of averaging. In the talk we experimentally demonstrate:

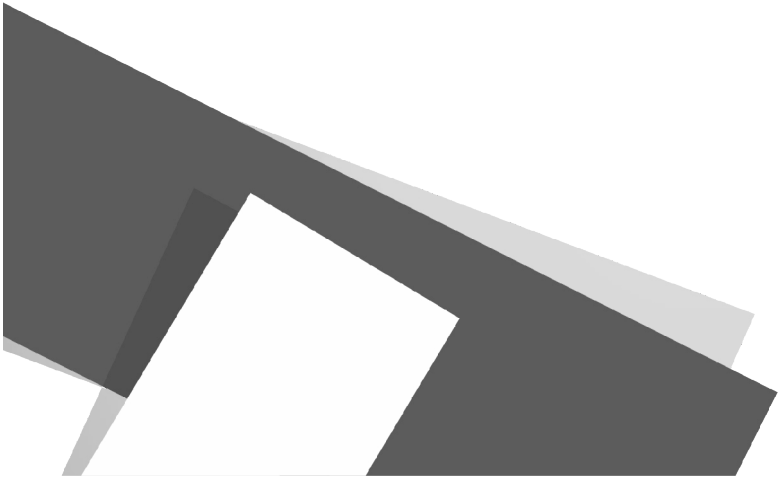
1) orders of magnitude improvement in concentration sensitivity, relative to X-band PELDOR, using a new type of spectrometer operating at high frequencies (W-band - 94 GHz), high powers (1kW) and using relatively high sample volumes (100uL+).

2) the importance of sample annealing to improve sensitivity (in this system), by reducing sample cracking induced by thermal stresses - with relevance for solid-state DNP

3) broadband operation with 1GHz instantaneous bandwidth at W-band

4) orientational-PELDOR where we show that in many cases the relative orientation and orientational distribution of spin labels can be quantitatively measured and correlated with distances and distance distributions using new spin labels that are rigidly attached to the protein backbone.

5) the application of orientation-PELDOR to a variety of biological systems showing its usefulness in studying biological structure, function and dynamics.



Plenary Lectures

PL 11

High Field and High Spin - a Different Approach for Long Range Distance Measurements in Biomolecules

Daniella Goldfarb

Weizmann Institute of Science

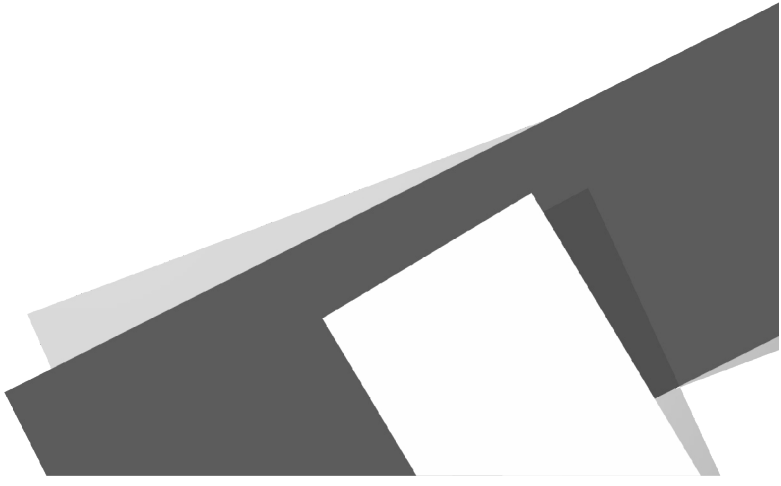
Methods for measuring nanometer scale distances between specific sites in biomolecules (proteins and nucleic acids) and their complexes are essential for analysis of their structure and function. In the last decade pulse EPR techniques, mainly pulse double-electron-electron resonance (DEER), have been shown to be a very effective for measuring distances between two spin labels attached to a biomolecule.

DEER is routine for distances up to 5 nm and with some extra effort and favorable conditions distances as high as 8 nm can be measured. So far, such measurements have been applied mostly to biomolecules labeled with nitroxide stable radicals and the measurements are usually carried out at standard X-band frequencies (~9.5 GHz, 0.35 mT).

Here we introduce a new family of spin labels that are based on Gd^{3+} chelates for DEER measurements at high frequencies, particularly W-band (95 GHz, ~3.5 T). Gd^{3+} has a spin of 7/2 and its unique EPR spectral properties turn it into an excellent spin label for distance measurements at high fields.

This will be discussed and examples on rigid models, proteins, DNA and trans-membrane peptides in model membranes will be presented. We also show that distance measured between a Gd^{3+} tag and a nitroxide spin label may be attractive for some application and present a spectroscopic approach to select distance distributions from a mixture consisting Gd^{3+}-Gd^{3+}, Gd^{3+}-nitroxide and nitroxide-nitroxide labeled proteins.

Finally, we will discuss the optimal experimental conditions and tag properties for achieving the best sensitivity.



Plenary Lectures

PL 12

Chemical Exchange Saturation Transfer (CEST) NMR and MRI

Peter C.M. van Zijl

Johns Hopkins University School of Medicine and Kennedy Krieger Research Institute

CEST agents exploit exchangeable protons to achieve MRI contrast. This is accomplished by using radiofrequency saturation at the resonance frequency of these protons and monitoring of the transfer of this saturation to the water protons imaged in MRI. When continuous saturation is applied, strong sensitivity enhancements (factors of hundred to hundreds of thousands) can be attained to image micromolar compounds.

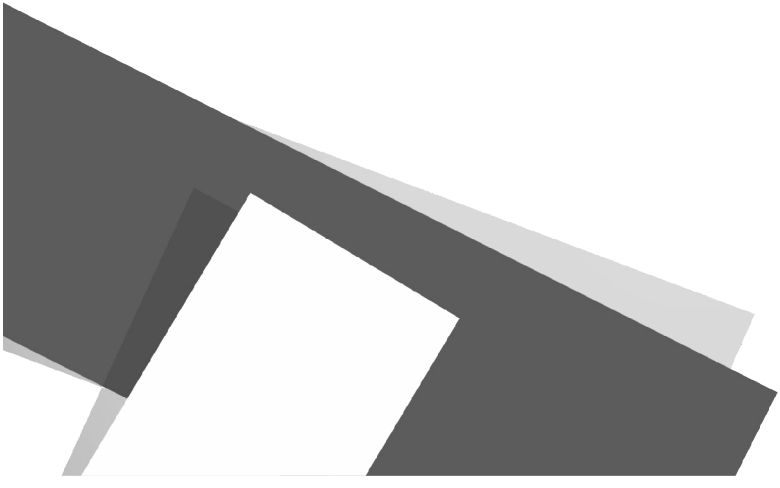
Contrary to conventional paramagnetic MRI agents, CEST compounds do not perturb the image contrast of anatomical images and can be turned on and off. CEST agents have been broadly classified in terms of containing paramagnetic metals (paraCEST) or not (diaCEST). Another classification is in terms of proton exchange, molecular exchange and compartmental exchange.

The current presentation, after an overview of basic principles, will focus on diaCEST. Currently, protons used for diaCEST include OH (hydroxyl, ~0-3ppm from water), NH2 (amine ~0-3 ppm from water), NH (amide, ~3-4ppm from water; imino, ~5-7ppm from water). The main compounds are carbohydrates (sugars), amino acids, peptides and proteins, and nucleic acids, which are natural bio-organic substances.

MRI is an insensitive method and, contrary to PET and optical approaches, the application of contrast agents often requires physiologically incompatible (micromolar-millimolar) concentrations. Unlike paramagnetic metallic contrast agents, diaCEST provides natural, non-metallic labels. As a consequence, this methodology has already allowed the use of many agents in vivo in animals, while endogenous markers such as cellular amino acids, peptides and sugar derivatives are even being studied in humans.

Recent data suggest that amide proton transfer (APT) may provide a biomarker for separating tumor recurrence from treatment necrosis in the brain.

Based on its non-invasive character, diaCEST is expected to be useful not only in the pre-clinical arena but also to revolutionize the rapid translation of contrast agents to the clinic. The field is evolving rapidly and many novel exogenous agents and endogenous markers are expected to be discovered in the near future.



PL 13

MAGNETIC RESONANCE OF POROUS MEDIA

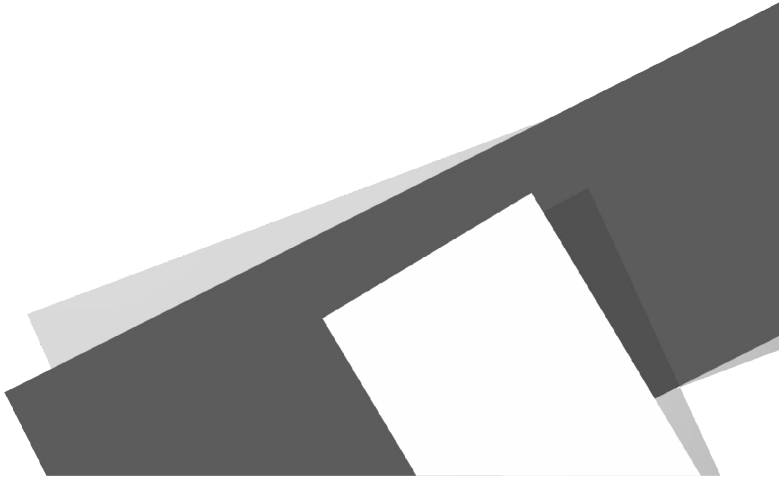
Yi-Qiao Song

Schlumberger-Doll Research and Mass General Hospital, MA USA

In recent years, NMR/MRI has become an important technique for characterization of a variety of porous media including inorganic materials and biological tissues. In particular, NMR/MRI measurement of relaxation and diffusion properties has found applications in petroleum exploration, material sciences, medical imaging, and other fields. Such an advance requires better understanding of relaxation and diffusion processes in the complex structure of these materials, as well as novel designs of MRI methods and technology.

This talk will outline the physics of the relaxation and diffusion processes in porous media and the recent MR development including multi-echo techniques, compressed sensing, 2d methods for diffusion and relaxation, in particular to study complex diffusion dynamics in porous media. I will also discuss several applications of these techniques in the study of polymer degradation, molecular composition, porosity in sedimentary rocks, food productions and biological tissues.

I will discuss a new concept of NMR hardware system with a fully broadband front-end electronics without using the conventional resonant circuit. This change to the MR front-end electronics enables a fully digital MR system with unparalleled flexibility and simplicity for multi-frequency MR, a transition analogous to the one from classic analog radios to modern digital receivers found in mobile electronics.



Plenary Lectures

PL 14

Zero- and Low-Field NMR Spectroscopy

Dmitry Budker[1,2]

[1]Department of Physics, University of California, Berkeley, [2]Nuclear Science Division, E. O. Lawrence Berkeley National Laboratory, Berkeley, CA 9430, USA

We will present an overview of the recent work on NMR spectroscopy based on measuring J-couplings at zero and low magnetic fields, including experiments on NMR entirely without magnets (not counting a low-field-pulse coil), where signal detection is accomplished with atomic magnetometers. The experiments have been performed by our group in collaboration with the group of Prof. Alexander Pines at the Department of Chemistry at UC Berkeley and the Materials Science Division of LBNL, and our various American and international collaborators. Up-to-date bibliography of this work can be found at the following web sites:

http://budker.berkeley.edu/PubList.html

http://waugh.cchem.berkeley.edu/publications.php .

ACKNOLEDGEMENTS: NSF, DOE

PL 15

NMR of Large Proteins: Molecular Chaperones

Charalampos G. Kalodimos

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Molecular chaperones are necessary for maintaining a functional proteome in the cell by preventing the aggregation of unfolded proteins and/or assisting with their folding. Despite the central importance of the binding of chaperones with unfolded substrates, the structural basis of their interaction remains poorly understood. The scarcity of structural data on complexes between chaperones and unfolded client proteins is primarily due to technical challenges originating in the dynamic nature of these complexes.

I will discuss how NMR spectroscopy can be used as an extremely powerful tool to determine the structural and dynamic basis for the recognition and interaction of unfolded proteins by molecular chaperones.

Parallel Session Lectures

PARALLEL SESSION LECTURES

PS 101

SYNERGY BETWEEN NMR, CRYO-EM AND LARGE-SCALE MD SIMULATIONS - NOVEL FINDINGS FOR HIV CAPSID FUNCTION

Angela M. Gronenborn

Department of Structural Biology, University of Pittsburgh School of Medicine…

Mature HIV-1 particles contain a conical-shaped capsid that encloses the viral RNA genome and performs essential functions in the virus life cycle. Previous structural analysis of two- and three-dimensional arrays provided a molecular model of the capsid protein (CA) hexamer and revealed three interfaces in the lattice. Using the high-resolution NMR structure of the CA C-terminal domain (CTD) dimer and in particular the unique interface identified, it was possible to reconstruct a model for a tubular assembly of CA protein that fit extremely well into the cryoEM density map. A novel CTD-CTD interface at the local three-fold axis in the cryoEM map was confirmed by mutagenesis to be essential for function. More recently, the cryo-EM structure of the tube was solved at 8 Å resolution and this cryo-EM structure allowed unambiguous modeling and refinement by large-scale molecular dynamics (MD) simulation, resulting in all-atom models for the hexamer-of-hexamer and pentamer-of-hexamer elements of spheroidal capsids. Furthermore, the 3D structure of a native HIV-1 core was determined by cryo-electron tomography (Cryo-ET), which in combination with MD simulations permitted the construction of a realistic all-atom model for the entire capsid, based on the 3D authentic core structure. In addition, interaction with the innate immune defense restriction factor TRIM5 was studied. TRIM5α recognizes the lattice of the retrovirus capsid through its B30.2 (PRY/SPRY) domain in a species-specific manner. Upon binding, TRIM5α induces premature disassembly of the viral capsid and activates the downstream innate immune response. We have determined the crystal structure of the rhesus TRIM5α PRY/SPRY domain that reveals essential features for capsid binding. Combined cryo-electron microscopy (cryo-EM) and biochemical data show that the monomeric rhesus TRIM5α PRY/SPRY, but not human TRIM5α PRY/SPRY, can bind to HIV-1 capsid protein assemblies, without causing disruption of the capsid. Our data suggests a model for how this factor disrupts the virion core and suggests that structural damage of the viral capsid by TRIM5 is likely one of the important components of the mechanism of HIV-1 restriction.

PS 102

A FLEXIBLE PARTNERSHIP WITH AMYLOGENIC PROTEINS

N.H. Meyer, SF Falsone, K Zangger

Institute of Chemistry, University of Graz

Protein misfolding into a toxic conformation and the extra- or intracellular accumulation of misfolded protein in large oligomeric structures - termed amyloid fibrils - coincides with the onset of numerous age-related neurodegenerative diseases like Alzheimers disease and Parkinsons disease. In spite of being structurally and functionally unrelated, amylogenic proteins can generally be converted into insoluble fibers with a distinct cross beta-sheet structure. Understanding the structural and dynamical principles underlying the pathways of amyloid formation can thus point to novel strategies in the clinical treatment of neurodegenerative diseases.

Recently, the evolutionary conserved protein class MOAG-4/SERF was identified to affect amyloid formation *in vivo* and it was thus proposed that MOAG-4/SERF provides a previously unexplored mechanism to regulate age-related proteotoxicity. Therefore, we initiated a study to reveal the molecular mechanisms of the interaction of MOAG-4/SERF with the amylogenic protein alpha synuclein. Here, we show that both proteins remain entirely disordered and no secondary structural content is formed in the transient complex. Moreover, we could pinpoint the interaction to 3 critical lysine sidechains of SERF within the interaction surface. Mutation of one of those residues to alanine drastically reduces binding affinity. A combination of SAXS and NMR experiments are used to derive a structural model of how SERF1a promotes fibril formation. To extract a maximum of information from NMR spectra we utilize instant homonuclear broadband decoupling, a technique we recently developed in our lab. For the first time we show how proton homonuclear broadband decoupling can impressively reduce spectral overlap, when applied to intrinsically disordered proteins.

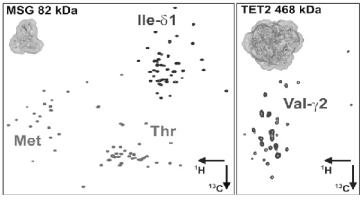
PARALLEL SESSION LECTURES

PS 103

SPECIFIC ISOTOPIC LABELLING OF METHYL GROUP: A TOOL BOX FOR THE DYNAMICS AND STRUCTURAL STUDIES OF LARGE PROTEINS AND COMPLEXES

G. Mas, R. Kerfah, P. Gans, I. Ayala, E. Crublet, M. Plevin, O. Hamelin and J. BoisbouvierStructural Biology Institute, CNRS/CEA /UJF - GRENOBLE – France. The NMR study of large proteins and complexes has been a real challenge for a long time. Recent developments in specific isotope labelling of methyl groups in a perdeuterated protein has significantly extended the frontier of liquid state NMR. In recent years, we have exploited metabolic pathways in E. coli and synthesized new isotope-labelled precursors to allow regio- or stereo-specific labelling of the methyl groups of Isoleucine (Ayala et al. Chem Comm 2012), Leucine and Valine (Gans et al. Angew. Chem. 2010), including the specific labelling of methyl groups of Valine without Leucine in order to reduce overlaps in very large protein assemblies (Mas et al. submitted).

Additionally, new protocols have been developed to extend and optimize specific labelling approaches to methyl groups of Methionine and Threonine residues (Hamelin et al. in preparation), as well as Alanine residues (Ayala et al. J. Biomol NMR 2009). With these new tools in hand, we can now label any combination of methyl groups in proteins reporting directly on the structure and dynamics of both the protein backbone and side chains extremities.



These advances extend the available labelling tools offering the possibility to study more and more challenging protein systems. These specific labelling strategies are particularly adapted to extract precise long-range NOE distance between remote probes separated by more than 10 E. Applications to the assignment and structural studies of proteins of 82 kDa and 468 kDa will be presented .

PS 104

SOLUTION STRUCTURE OF BUD 31, A YEAST PROTEIN CONTAINING A HIGHLY UNUSUAL METAL CLUSTER.

J.-C. Yang[1], A-M.M. van Roon[1], D. Mathieu[2], W. Bermel[2], K. Nagai[1] and D. Neuhaus[1].[1] *MRC Lab. of Molecular Biology, Cambridge, U.K.*
[2] *Bruker BioSpin GmbH, Karlsruhe, Germany.*

Bud31 is a 157-residue protein from yeast. It is thought to be involved in the splicing process, whereby coding portions of a pre-mRNA transcript are separated from non-coding portions and spliced together to form a continuous message, but its exact function has proved elusive. It contains a cysteine-rich region near the C-terminus and mass spectrometry shows that 3 zinc ions are bound, however there is no homology with known zinc finger structures and there are insufficient Cys residues for the zincs to be bound independently. Attempts to crystallize the protein were unsuccessful.

We have used NMR to determine the solution structure of Bud31, which revealed that its main novel feature is that it contains a highly unusual Cys_9Zn_3 zinc cluster. In order to determine this structure it was necessary to substitute the zinc ions with NMR-active ^{113}Cd and to use a variety of (^{113}Cd,^{1}H) heteronuclear correlation experiments to delineate the metal-binding topology. While such experiments have been used previously on very small protein domains, their use for moderately sized structures can be more difficult due to higher cadmium linewidths, and in the present case the work was only made feasible by employing a broadband cryoprobe capable of (^{113}Cd,^{1}H) double resonance experiments. The structure of Bud31 and its determination by NMR will be described, with particular attention to the novel metal cluster.

PS 105

Perturbation of neuronal signaling pathways by the rabies virus : Role of the PDZ domains

Maisonneuve[1,3], P., Terrien[1,3], E., Caillet[1,3], C., Lafage[2,3], M., Cordier[1,3], F., Préhaud[2,3], C., Buc[3], H., Delepierre[1,3], M., Lafon[2,3], M., **Wolff[1,3], N.**

1. Unité de RMN des Biomolécules-Département de Biologie Structurale et Chimie.
2. Unité de NeuroImmunologie Virale-Département de Virologie.
3. Institut Pasteur, Paris, France.

The human tyrosine phosphatase PTPN4 and the Ser/Thr kinase MAST2 are two enzymes expressed in neurons. While PTPN4 is an anti-apoptotic protein, MAST2 inhibits neurogenesis and neuroprotection. The PDZ domain of these two enzymes is specifically targeted by the cytoplasmic domain of the envelope glycoprotein (G protein) of the rabies virus (RABV) during neuron infection (Préhaud et al, 2010). We have solved the NMR structures of the complexes formed by MAST2-PDZ and PTPN4-PDZ with their respectives endogenous and viral ligands. As a result, the complexes formed by the PDZ of the two enzymes and their respective ligands are disrupted, triggering drastic effect on cell signaling and cell commitment either towards death or survival. By targeting MAST2-PDZ, the G protein of virulent RABV alters the intracellular trafficking of PTEN (Terrien et al., 2012) and promotes survival, whereas the G protein of attenuated RABV induces neuronal cell death by targeting PTPN4-PDZ (Babault et al., 2011). We recently demonstrated that the catalytic activity of PTPN4 is regulated by its PDZ domain and that the viral sequence interfered with this allosteric regulation.
We provided structural and biological evidences that the RABV G proteins act as competitors endowed with specificity and sufficient affinity in a vital cellular process. The disruption of critical endogenous protein-protein interactions by viral protein altered drastically intracellular protein trafficking and catalytic activity controlling the cellular homeostasis.

· Terrien E *et al.* (2012) Science Signal. 5(237):ra58. · Cordier F. et al. (2012) *JACS*. 134(50):20533-43.
· Babault *et al.* (2011) *Structure* **19**: 1518-1524. · Préhaud C *et al.* (2010) *Science Signal*. **3**(105)**:** ra5.

PS 106

TRACKING TISSUE MICROSTRUCTURE WITH DIFFUSION TENSOR IMAGING : CLINICAL APPLICATION IN BREAST CANCER DIAGNOSIS

E Furman–Haran[1], E Eyal[1], N Nissan[1], M Shapiro Feinberg[2], N. Weisenberg[2], D Grobgeld[1] and H.Degani[1]

Weizmann Institute of Science and Meir Medical Center, ISRAEL

Water diffusion coefficients in tissues are often anisotropic and can be described by a diffusion tensor. The diffusion process can be affected by contributions of flow, restriction by cell membranes and vessels' walls,, extracellular tortuosity and exchange between tissue compartments
The diffusion of water molecules in tissues with ductal /glandular components such as the breast, prostate and pancreas presents a particular example of restricted movement in well defined microstructures. Specifically, the functional breast tissue is composed of well defined 9-14 ductal/glandular tree systems. Blockage of the ducts by cancer cells predominantly affects the free diffusion parallel to the walls, reducing the diffusion coefficient in all directions, and the extent of anisotropy. We have applied an experimental DTI protocol at 3T to track the anisotropic diffusion in tissues. We used the twice refocused echo planar imaging sequence in order to minimize distortion of eddy-currents and further corrected geometric distortions that arise from B0 field inhomogeneity applying a field mapping technique. The DTI datasets were processed pixel by pixel by a proprietary fast software program using non linear regression according to Stejskal-Tanner equation calculating a symmetric tensor that was further diagnonalized by principal component analysis. The final output for each pixel included eigenvectors defining the diffusion direction in three orthogonal axes of an ellipsoid and their corresponding eigenvalues that quantify the diffusion coefficient in each direction. Vector maps and parametric maps of the prime diffusion coefficient and the maximal anisotropy index enabled tracking the ducts and detecting growth of cancer. Based on the scanning of 150 volunteers with malignant and benign lesions we found that the DTI detection and diagnostic efficiencies are high and similar to that of dynamic contrast enhanced-MRI. However, in highly fatty breasts, we encountered problems due to imperfect fat suppression and low S/N.
In conclusion, clinical application of parametric DTI is feasible and has the potential to become an important diagnostic method. However, improvements are required in hardware, pulse sequences and software to unravel the detailed tissue microstructure *in vivo*.

PS 107

HYPERPOLARIZED ^{129}Xe NMR-BASED SENSORS OF BIOLOGICAL EVENTS

Naoko Kotera[b], Céline Boutin[a], Estelle Léonce[a], Yves Boulard[a], Bernard Rousseau[b], Thierry Brotin[c], Jean-Pierre Dutasta[c], Patrick Berthault[a]

[a] *CEA Saclay, IRAMIS, SIS2M, UMR CEA/CNRS 3299, Laboratoire Structure et Dynamique par Résonance Magnétique, 91191 Gif sur Yvette, France*
[b] *CEA Saclay, SCBM, iBiTec-S, Building 547, PC # 108, 91191 Gif sur Yvette, France*
[c] *Laboratoire de Chimie, CNRS, Ecole Normale Supérieure de Lyon, 46 Allée d'Italie, 69364 Lyon Cedex 07, France*

Among the hyperpolarized noble gases used for sensitive MRI (^{3}He, ^{129}Xe, ^{83}Kr), xenon takes a special place. Indeed the large polarizability of its electron cloud makes that even tiny changes of its local environment induce significant modification of the NMR parameters, in particular the chemical shift. Such a property has been used to propose a molecular MRI approach based on xenon encapsulated in cage-molecules functionalized to reach defined biological targets.[1] Fast in-out xenon exchange gives an even higher sensitivity to the method by constant refreshment of the cage in hyperpolarized xenon.
Here we extend this concept to smart ^{129}Xe MRI probes where the chemical shift of caged xenon is influenced by the nature of the entity chelated by the functional group(s) of the cryptophane.
Two such sensors are studied: the first one enables simultaneous detection of several metal ions in low quantity thanks to a generic functional group,[2] the second one aims at the *in vitro* and *in vivo* imaging of recombinant 4Cys proteins *via* dual fluorescence/^{129}Xe MRI modality.[3]
The challenge for optimized use of these systems with hyperpolarized xenon is to perform fast imaging while keeping spectral discrimination between the signals of caged xenon. HyperCEST[4] combined to Echo-Planar Imaging[5] or other imaging scheme can be performed in this purpose and are an excellent way to achieve low detection threshold.

[1] M. M. Spence et al. *Proc. Natl Acad. Sci. USA* **2001**, *98*, 10654-10657.
[2] N. Kotera et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 4100-4103 and other manuscript submitted.
[3] Manuscript in preparation - [4] L. Schröder et al. *Science* **2006**, *314*, 446-449.
[5] M. Kunth et al. *Angew. Chem. Int. Ed.* **2012**, *51*, 8217-8220.

PS 108

NON INVASIVE DETECTION OF EARLY PRECURSOR LESIONS OF PANCREATIC CANCER USING HYPERPOLARIZED [1-^{13}C]PYRUVATE

Eva M Serrao, Mikko I Kettunen, Tiago B Rodrigues, Ferdia A Gallagher, David Tuveson, Kevin M Brindle

Cancer Research UK Cambridge Institute; Biochemistry Department, University of Cambridge

Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer-related deaths in the USA, with a five-year survival of less than 5%. Mortality improvement will only be possible by early diagnosis of pancreatic precursor lesions (i.e.PanIN), however this is still clinically challenging due to the lack of sensitive and specific biomarkers. Recently, real time metabolic imaging of tissues was made possible in humans by using hyperpolarized [1-^{13}C]pyruvate as a probe for ^{13}C magnetic resonance spectroscopy (^{13}C-MRS).The aim of this study was to identify metabolic signatures of the different stages of progression of PDA, using hyperpolarized [1-^{13}C]pyruvate in well a established genetically-engineered mouse model of pancreatic cancer. **Methods:** LSL-Kras$^{G12D/+}$-p48$^{Cre/+}$ (KC) mice (2-13 months old) with mPanIN lesions, LSL-Kras$^{G12/D+}$;LSL-Tpr53$^{R172H/+}$;Pdx-1-Cre (KPC) mice (3-6 months old) with spontaneous pancreatic cancer and control mice (age matched) were used. KPC and C57BL/6 mice were used as models of established tumor and normal pancreatic tissue, respectively. MRI was performed at 7T using a ^{13}C/^{1}H volume coil/20-mm diameter ^{13}C surface coil combination. A chemical shift image (TR 30ms; TE 1.5ms; FOV 40×40 mm; data matrix 32×32; flip angle 5°) was acquired from a single 4–8mm axial slice covering the pancreas 20 sec after injection of 0.3 mL of 75 mM hyperpolarized [1-^{13}C]pyruvate. [1-^{13}C]Pyruvate, [1-^{13}C]lactate (lac) and [1-^{13}C]alanine (ala) signal intensities in the pancreas were analyzed. High-resolution ^{1}H NMR spectra of pancreatic tissue extracts, collected from animals at different ages, were obtained at 14.1T (TR 12.5s) and Lac and ala concentrations calculated. Pancreatic tissues were evaluated histologically. **Results:** Progressive reduction of the [1-^{13}C]Ala/[1-^{13}C]Lac ratio was observed with increasing disease burden. No changes were observed in control mice.^{1}H NMR and histology results revealed and confirmed the observed Ala/Lac signature of cancer progression. **Conclusions:** [1-^{13}C]Pyruvate metabolism and the ratio of the subsequently formed [1-^{13}C]Ala/[1-^{13}C]Lac may form useful probes to detect and follow progression of panIN lesions, before any mass-forming lesion (tumor) can be detected by conventional imaging. This may offer an improved diagnostic tool in high-risk populations, such as patients with chronic pancreatitis or familial pancreatic cancer.

PS 109

^{17}O METHODS FOR DETERMINATION OF REGIONAL CEREBRAL METABOLIC RATE OF OXYGEN ($rCMR(O_2)$) IN THE HUMAN.

Daniel Fiat

UIC

Two methods used for the determination of $rCMR(O_2)$ in the human will be described:
1) Measurement of $H_2{}^{17}O$ in the brain and in the arterial blood and the measurement of Regional Cerebral Blood Flow (rCBF) by an independent method.
2) Utilizing ^{17}O MRI model that simulates the metabolic process of oxygen metabolism in body organs. The metabolic rate of body organs is determined by a least square fit of the model equations to the ^{17}O MRI experimental data of $H_2{}^{17}O$ concentrations in the body organs.
The theory, the experimental data and the analysis of the data will be presented.[1-7]

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PS 110

MR IMAGING AND SPECTROSCOPY IN RODENTS: FROM TRANSPORT AND METABOLISM TO CANCER TREATMENT AND NMR SIGNAL ENHANCEMENT

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Several examples of MRI and MRS applications will be presented. 1) Distribution, mobility and transport of water and their variation with age in the eye lenses of OXYS and Wistar rats have been addressed ex vivo. The age-related changes were pronounced, but the early cataract development in OXYS rats did not affect the diffusivity and distribution of water in the lens. 2) A comparative study of the airflow patterns and dust sedimentation in the nasal cavities of subterranean and terrestrial rodents provided valuable information on the protection mechanisms against nanoparticlulate aerosols developed by nature. In addition, the transport of nanoparitcles and ions from the nose to the brain along olfactory nerve axons in rodents was visualized. 3) The metabolic responses in the rodent brain (hippocampus) to administration of 2-deoxy-d-glucose (2DG) and lipopolysaccharide (LPS) were assessed using ^{1}H MRS in vivo. The inhibition of glycolytic pathway induced by 2DG was accompanied by the increase in the excitatory metabolites (glutamate, glutamine) and the reduction in the inhibitory neurotransmitter GABA and in N-acetylaspartate and choline. The pro-inflammatory inducer LPS has an opposite effect. The metabolic changes observed may be similar to those observed under neurodegenerative pathologies. 4) As an example of a novel anticancer theranostic agent, a vector-drug construct was developed by coupling the linoleic acid-modified branched polyethyleneimine with urocanic acid and further conjugating it with trifluorothymidine 5'-monophosphate (pTFT). The promising results on the ^{19}F MRI of the conjugate in vivo, the pH-sensitive release of the drug pTFT and its use in the treatment of murine carcinoma have been obtained. 5) Parahydrogen-induced polarization (PHIP) is one of the known ways to overcome sensitivity limitations in NMR. It is shown that both biphasic gas-liquid hydrogenations of gaseous substrates and heterogeneous fluid-solid hydrogenations over various types of heterogeneous catalysts allow one to cleanly separate the hyperpolarized products from the catalysts and to produce PHIP continuously. In addition, the use of nuclear spin isomers of molecules other than H_2 to produce hyperpolarization is demonstrated experimentally.
Grants 11-03-00248, 12-03-00403, 12-03-31386-mol (RFBR), 5.1.1 (RAS), 60, 61, 57, 122 (SB RAS), NSh-2429.2012.3, MK-4391.2013.3 and 11.G34.31.0045 are acknowledged.

PS 111

DONORS IN SILICON: ESR CLOCK TRANSITIONS AND HYPERPOLARISATION

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Electron and nuclear spins of donor atoms in silicon are promising candidates for representing quantum bits. Understanding and overcoming spin decoherence mechanisms in these materials is an important step in developing silicon-based quantum computers. Certain atoms possess spin transitions whose frequency has zero-first order magnetic field dependence (df/dB=0) around particular values of static magnetic field. These have been studied in trapped ions, for example, and used in frequency standards, and are hence often termed 'clock transitions'. We have performed pulsed ESR at such a clock transition (~7 GHz, 80 mT) for bismuth donors in silicon. Aside from allowing more accurate determination of the hyperfine coupling strength, we find such transitions are much more robust to various sources of decoherence (including instantaneous diffusion, and spectral diffusion from nuclear spins in the bulk, or flip-flops of neighbouring donors). At this clock transition, at 5 K, we measure electron spin coherence times up to about 3 seconds [1]. We expect the use of such clock transitions will be of additional importance for donor spins in nano-devices, such as those which have been recently used to perform ESR and NMR on a single donor atom in silicon [2]. Another important challenge for spin quantum bits is initialising them into pure states – in other words, hyperpolarizing them. We apply recently developed [3] optical pumping techniques to donors in silicon to show dramatic enhancements in the ESR signal intensity, suggesting a spin polarisation exceeding 90% (at 5 K, 340 mT).

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PS 112

POINT DEFECTS IN SIC AS A PROMISING BASIS FOR SINGLE-DEFECT RESONANCE SPECTROSCOPY WITH ROOM TEMPERATURE CONTROLLABLE SPIN QUANTUM STATES

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The unique quantum properties of the nitrogen–vacancy defect in diamond have motivated efforts to find defects with similar properties in wide-band-gap semiconductor silicon carbide (SiC), which can extend the functionality of such systems not available to the diamond. Optically induced spin polarization of the ground-state spin sublevels of a family of Si-vacancy related defects in silicon carbide has been shown to persist up to the room temperature. The continuous wave (cw) and pulse electron paramagnetic resonance (EPR) data show that the probed defects spin ensemble can be prepared in a long-lived coherent superposition of the spin states at room temperature [1]. Depending on the defect type, temperature, SiC polytype, and crystalline position, two opposite schemes have been observed for the optical alignment of the ground state spin sublevels population of the Si-vacancy related defects upon irradiation with unpolarized light. Optically detected magnetic resonance (ODMR) shows the possibility to manipulate of the ground state spin population by applying radiofrequency field. The unique properties of the defects make them a promising quantum system for single-defect and single-photon spectroscopy in the infrared region. These properties can be used to implement high-power masers and low-noise radio-frequency amplifiers with optical or electrical pumping.
Silicon carbide light-emitting diode is shown to be a perspective room temperature source of single photons and electrically driven alignment of the ground state spin sublevels with inverse population at room temperature. As these defects can potentially be generated at a low or even single defect level, electrically driven single photon source for quantum telecommunication and information processing can be realized [2].
SiC with highly developed device technologies is a very attractive material for practical applications. These altogether make the Si-vacancy related defects in SiC very favourable candidate for spintronics, quantum optics, quantum information processing, nanoscale magnetometry, biolabelling.

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PS 113

INCREASING SENSITIVITY OF ECHO DETECTED EPR EXPERIMENTS USING CPMG

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Pulsed EPR experiments are nowadays routinely used to study a variety of systems of interest ranging from solid state physics to structural biology. In particular we note the use of pulsed Electron-Nuclear Double Resonance (ENDOR) in metalloenzymes studies, and of Double Electron - Electron Resonance (DEER) experiments to determine distances between selected sites in bio-macromolecules (proteins, DNA). Pulse EPR experiments are usually performed at low temperatures, where relaxation times are longer and polarization is higher, to increase the Signal/Noise Ratio (SNR). When working with biological samples that are limited in amount and concentration, sensitivity becomes a limiting factor and EPR sequences used nowadays often require a significant accumulation time, up to 12 h or more, to reach sufficient SNR.
As practically all pulse EPR sequences are based on echo detection, a considerable increase in SNR can be obtained by replacing the single echo detection scheme with a train of echoes, generated by the Carr Purcell-Meiboom-Gill (CPMG) 1,2 pulse sequence as often done in NMR. Here we demonstrate this on Echo-Detected EPR, Davies and Mims ENDOR, DEER and ELDOR (electron-electron double resonance) detected NMR experiments combined with CPMG detection applied to a model nitroxide biradical and a Mn^{2+} complex. Collecting the transient signal and integrating the refocused echoes generated by the CPMG detection scheme allows SNR improvement in by a factor ranging from 1.6 to 6, at constant experimental time without significant signal distortion, depending on the radical and the sequence considered. This allows obtaining high fidelity signals with a significant time saving.

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PS 114

QUANTUM CONTROL OF HYBRID NUCLEAR-ELECTRONIC QUBITS

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The time required to flip either dilute electronic or nuclear spins in silicon is orders of magnitude shorter than their decoherence times. We proposed that qubit control could be speeded up and decoherence slowed down when the eigenstates approximate 50:50 superpositions of the electronic and nuclear spin states [1-3]. This hybrid regime can be accessed with bismuth dopants in silicon (Si:Bi) [4, 5] using a 4 GHz spectrometer because of the large hyperfine coupling of 1.475 GHz and the large nuclear spin of 9/2. Here we demonstrate this, achieving quantum control of hybrid nuclear-electronic states in just 32 ns: orders of magnitude shorter than experiments with nuclear states [6]. The coherence times of our states are five orders of magnitude longer than the manipulation times, reaching 4 ms, and are limited by the naturally-occurring ^{29}Si nuclear spin impurities.

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PS 115

EPR OF "BREATHING" MATERIALS WITH NITROXIDES

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Switchable magneto-active materials attract significant attention due to both fundamental interest and potential applications in spintronics. Among other techniques, EPR plays important role in studying spin states of these compounds and their dynamics under external stimuli. In this report we focus on two types of switchable compounds containing nitroxides. The first one, copper-nitroxide based molecular magnets $Cu(hfac)_2L^R$, represents an interesting type of thermo- and photo-switchable materials which exhibit reversible magneto-structural rearrangements with significant changes of the unit cell volume (and therefore are often called "breathing crystals"). The unusual type of magnetic switching between weakly- and strongly exchange-coupled states occurs in spin triads nitroxide-copper(II)-nitroxide. We overview experimental approaches developed for these systems, discuss general trends and focus on the characteristics of light-induced spin state switching and relaxation using CW X/Q-band and time-resolved EPR. The second type of "breathing" structures addressed in this work refers to the metal-organic framework (MOF) compound MIL-53(Al), which undergoes reversible temperature-induced structural transition between large-pore and narrow-pore crystalline states with a significant hysteresis. We report CW and pulse X/Q-band EPR study of MIL-53(Al) with adsorbed guest molecules of stable nitroxide (TEMPO). We have found that the mobility of nitroxides in nanochannels of MIL-53(Al) strongly depends on both temperature and crystalline state of the MOF. In addition, guest-host interactions of TEMPO with OH groups of the framework have been investigated. The "breathing" mode of MIL-53(Al) is suppressed at high concentrations of guest molecules, therefore application of many analytical methods in this mode is impossible. High sensitivity of EPR in conjunction with low concentrations of spin probes makes it an indispensable tool to study guest-host interactions in MIL-53(Al) and other structurally flexible MOFs. This work was supported by RFBR (11-03-00158, 12-03-33010, 12-03-31329) and RF President grant (MK-1662.2012.3).

PS 116

INTERNAL MOBILITY IN PROTEINS FROM THE ESTABLISHED PERSPECTIVE OF RESTRICTED MOTIONS

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We developed in recent years the slowly relaxing local structure (SRLS) approach for NMR relaxation in proteins. SRLS is a two-body (protein and probe) coupled-rotator theory where the probe executes restricted local motion. When the two rotators are timescale separated (decoupled), the main features of the standard single-body theories for treating restricted motions are recovered. The generic description consists of time correlation functions (TCFs), i.e., sums of weighted exponentials (eigenmodes), with coefficients containing the kinetic, structural and geometric information inherent in the experimental data. There is ample evidence that the theory has to allow for general tensorial properties to exact this information insightfully. This implies a multi-eigenmode scenario. We select the axiality of the local probe diffusion as an example of low tensor symmetry (typical probes are at least axially symmetric). In the presence of strong axial local ordering potentials distinct eigenmodes for the global motion, and the two components of the local motion, prevail. Weaker axial potentials mix the local motional components. When the timescale separation is not large, mode-mixing due to dynamical coupling also occurs. We assess the concerted motions as correlated or anti-correlated. It is shown that discrepancies observed recently between squared generalized order parameters from the model-free (MF) method, and a 1.2 s long molecular dynamics (MD) trajectory, can be ascribed to oversimplified geometric features in MF. It is suggested to compare mesoscopic SRLS TCFs with their atomistic MF counterparts. One can adopt a viewpoint where the TCF comprises two (or three) eigenmodes. However, in such cases, constructs/composites act as descriptors of protein dynamics.

PS 117

MOLECULAR RECOGNITION THROUGH CONCERTED BACKBONE AND SIDE CHAIN MOTION

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Motion is involved in a large number of protein functions. For ubiquitin, residual dipolar coupling (RDC) derived ensembles have suggested it recognizes binding partners via conformational selection through motions occurring within the supra-t_c (> 4 ns) range [1]. Subsequent relaxation dispersion (RD) studies identified microsecond fluctuations in backbone [2,3]. However, it has not been clear if these motions are independent or collective, and what role side chains play. To address those questions, we have conducted an in-depth RD analysis of the backbone and side chain methyl groups using recently developed high power R_{1r} experiments [3]. In these RD experiments, not only microsecond fluctuations in the side chains have been observed but also motions in the side chain and the backbone have a common time scale. This result suggests that ubiquitin undergoes collective motion involving both the backbone and side chains. The presence of microsecond side chain motion agrees with previous RDC measurements of methyl groups within ubiquitin [4]. In addition, long-range correlated motions [5] across b-strands are characterized by cross-correlated relaxation experiments. Finally, progress made towards the fitting of a four state recognition model of Dsk2-UBA with ubiquitin will be discussed.

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PS 118

INTERESTING FEATURES OF SOLVENTS IN THE SUGAR-BASED ORGANOGELS REVEALED BY NMR: DIFFUSIVE DIFFRACTION PHENOMENON AND DISPERSION OF THE SPIN-LATTICE RELAXATION TIME

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Sugar-based compounds are representative of LMOGs that have the ability to gel various organic solvents, at very low concentrations, through the creation of three-dimensional networks, which entrapped the solvent molecules. The network is formed by self-assemble of gelator aggregates via noncovalent interactions. The resulting supramolecular gels have attracted much interest because of their unique properties and potential applications as new soft materials. In this presentation we report two interesting features of solvents in the matrix of selected sugar-based gels. First, concerns the solvent-gelator interactions yet open question in gels form by LMOGs. We present experimental evidence by NMR relaxometry that solvent-gelator interactions occur in methyl-4,6-*O*-benzylidene-*α*-D-glucopyranoside gel with toluene – Fig. A. The second interesting phenomenon is the diffusive diffraction pattern observed of toluene protons in a gel matrix of methyl-4,6-O-(*p*-nitrobenzylidene)-α-D-glucopyranoside by PGSE NMR – Fig. B. This phenomenon is rarely observed in a real porous material.

Fig. A

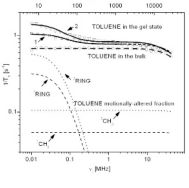
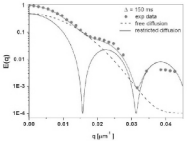


Fig. B



Acknowledgments
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PARALLEL SESSION LECTURES

PS 119

HIGH POLARIZATION OF NUCLEAR SPINS MEDIATED BY NANOPARTICLES AT MILLIKELVIN TEMPERATURES

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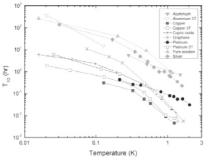

High levels of nuclear spin polarization can be achieved, in principle, by exposure to low temperature and high magnetic field, but the long time required for the polarization to approach thermal equilibrium poses a serious drawback unless a suitable relaxation agent can be found. ^{1}H T_1 measurements at 2.45T of 50:50 water/glycerol containing 2M [1-^{13}C] sodium acetate and 1M sodium phosphate reveal that copper, cupric oxide and platinum nanoparticles are highly effective relaxation enhancers at millikelvin temperatures, whereas aluminium and silver nanoparticles are ineffective (see figure). At 9.74T, with 1 part copper nanoparticles (by volume) to 8 parts solution, the estimated half-time ($T_{1/2}$) for ^{13}C to reach equilibrium polarization was about 40 hours at 19mK; this was only 3 times as long as at 770mK. For comparison, the ^{13}C $T_{1/2}$ value measured in the presence of aluminium nanoparticles was at least one year. In further experiments at 14T and 15mK, with a 1:4 volume ratio of copper nanoparticles to solution, the ^{13}C $T_{1/2}$ value for growth towards the equilibrium polarization of 23% was estimated to be about 60 hours. The relaxation mechanism may involve the conduction electrons although other findings suggest additional or alternative mechanisms, possibly involving the remarkable magnetic properties that many nanoparticulate materials (including copper and cupric oxide) display. Magnetic impurities may also play a role. We conclude that this methodology will enable us to generate and store large-scale quantities of highly polarized materials. With further developments, including the use of polarization transfer techniques and simultaneous polarization of many samples, outputs of ten or more samples per day should be feasible.

PS 120

ENSEMBLE-RESTRAINED MD SIMULATIONS: ACCURATE STRUCTURE LEADS TO ACCURATE DYNAMICS

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Currently, the best existing MD force fields cannot accurately reproduce the global free-energy minimum which realizes the experimental protein structure. As a result, long MD trajectories tend to drift away from the starting coordinates (e.g. crystallographic structures). To address this problem, we have devised a new simulation strategy aimed at protein crystals. An MD simulation of protein crystal is essentially an ensemble simulation involving multiple protein molecules in a crystal unit cell (or a block of unit cells). To ensure that average protein coordinates remain correct during the simulation, we introduced crystallography-based restraints into the MD protocol. Since these restraints are aimed at the ensemble-average structure they have only minimal impact on conformational dynamics of the individual protein molecules. So long as the average structure remains reasonable, the proteins move in a native-like fashion as dictated by the original force field. To validate this approach we have used the data from solid-state NMR spectroscopy, which is the orthogonal experimental technique uniquely sensitive to protein local dynamics. The new method has been tested on human ubiquitin and SH3 domain from chicken α-spectrin. The ensemble-restrained MD simulations produced lower crystallographic *R* factors than conventional simulations; they also led to more accurate predictions for solid-state chemical shifts and backbone order parameters. Taken together, these results suggest that the presented trajectories may be among the most realistic protein MD simulations ever reported. In this context the ensemble restraints based on high-resolution crystallographic data can be viewed as protein-specific empirical corrections to the standard force fields.

PARALLEL SESSION LECTURES

PS 121

DISSOLUTION DNP METHODS FOR CHEMISTRY

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Nuclear magnetic resonance (NMR) spectroscopy is traditionally an important technique for the elucidation of molecular structure and reaction mechanisms in Chemistry. Here, we present new developments for bringing the substantial signal enhancements obtained from dissolution dynamic nuclear polarization (D-DNP) to bear in these applications. In combination with high-resolution NMR, D-DNP enables detection of small amounts of substance, rapid C-13 NMR spectroscopy even at natural isotope abundance, and time-resolved observation of chemical reactions. A living polymerization reaction is observed in-situ, by incorporation of hyperpolarized styrene monomers. The hyperpolarization translates to the selective signal enhancement of the active, anionic site. C-13 NMR resonances with characteristic carbanionic chemical shifts are observed. These resonances can be correlated to the chemical shifts of the unreacted styrene using an NMR experiment with selective inversion. Further, the time course of signal intensities for these peaks can be used to elucidate the kinetics of the reaction. The strategy of incorporation of hyperpolarized monomer units may prove interesting for the study of other polymerization reactions, as it circumvents relaxation losses that can occur in direct D-DNP with larger molecules. In a broader sense, the application of D-DNP to the elucidation of molecular structure is often hindered by the need for two-dimensional chemical shift correlations. A new method based on multiple NMR scans using a flow cell is presented for the acquisition of Hadamard correlation spectra. This method complements other available chemical shift correlation techniques for hyperpolarized samples. Hadamard spectroscopy is compatible with many types of correlation experiments, but when applied with D-DNP, scan-to-scan variations in signal intensities present a challenge to the reconstruction of spectra. We demonstrate that this challenge can be overcome by using a maximum entropy based method for data processing.

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MATRIX-FREE DYNAMIC NUCLEAR POLARIZATION ENABLES SUPRAMOLECULAR STRUCTURAL STUDIES ON BIOSOLIDS AT NATURAL ^{13}C ABUNDANCE

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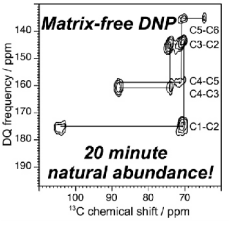
Magic angle spinning dynamic nuclear polarization (MAS-DNP) has been proven to be a powerful technique to enhance the sensitivity of solid-state NMR. It has great potential to tackle challenging tasks such as rapid natural-abundance ^{13}C–^{13}C correlation NMR, hyperdimensional NMR and microscale NMR in solids. However, MAS-DNP currently faces the obstacles of line-broadening and reduction of effective sample weight owing to the use of frozen DNP-matrices which uniformly distribute polarizing agents around the sample of interest. Therefore, the aforementioned challenging applications have not been previously reported despite the tremendous success of development of MAS-DNP.

In order to successfully overcome these problems and to fully exploit acquired DNP enhancements, we have recently demonstrated matrix-free DNP [1]. This utilizes a binding affinity of polarizing agents, clearly evidenced during "on-cell" MAS-DNP studies that we recently performed [2]. We will present very encouraging results where only 20 minutes were sufficient to record natural-abundance 2D ^{13}C–^{13}C correlation experiments on cellulose (see Figure) [1]. Furthermore, technologically interesting self-assembled peptide nanotubes were also studied in this manner [3] - which would have required two "Saturn-years" using conventional solid-state NMR. Importantly, intermolecular constraints were obtained for the peptide nanotubes, which demonstrates the feasibility of supramolecular structure determination of such nano-assemblies at natural isotopic abundance.



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PS 123

DYNAMIC NUCLEAR POLARIZATION SURFACE ENHANCED NMR SPECTROSCOPY: THE DESIGN OF NEW BIRADICALS ENABLES APPLICATION TO CHALLENGING MATERIALS

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NMR spectroscopy (often in conjunction with diffraction methods) is the method of choice for characterizing surfaces whenever possible, but the detection limit of NMR is far too low to allow many modern materials to be examined. Because it provides dramatic sensitivity enhancement, solid-state Dynamic Nuclear Polarization (DNP) NMR is currently emerging as a powerful tool to study samples previously inaccessible to NMR. We have recently shown how DNP could be used to selectively enhance the NMR signals from surfaces in a wide range of materials (DNP SENS). While signal enhancements of between 20 and 50 are routinely obtained at 9.4 T and 100 K (for ^{29}Si, ^{13}C, or ^{27}Al nuclei after cross-polarization from protons), these enhancement factors are still far from the predicted maximum values. Much effort is currently devoted to the development of ever more efficient polarizing agents. We will present a series of bTbK derivatives suitable for high-field DNP enhanced solid-state NMR spectroscopy. These biradicals differ by the functional groups at the vicinal position of the two nitroxides. We establish clear relationships between the DNP efficiencies of these new radicals and (i) their molecular weight, (ii) the number of methyl groups and (iii) their electron spin relaxation rates. In particular, a new radical is introduced, that yields proton enhancement of over 200 at 9.4 T and 100 K in bulk solution as well as in mesoporous materials. The temperature and spinning speed dependence of the DNP enhancement factors is also discussed for this new family of polarizing agents. These new polarizing agents are demonstrated through the application of DNP SENS to the characterization of periodic mesoporous organosilicates (POM). The rapid acquisition of high quality natural abundance 1D ^{13}C, ^{15}N, and ^{29}Si, and 2D ^{1}H-^{13}C and ^{1}H-^{29}Si DNP solid-state NMR spectra was essential to distinguish outer and inner layers in these porous materials and to monitor their surface functionalization.

PS 124

NEW WAYS FOR REVEALING UNOBSERVABLE NUCLEAR SPIN ORDER AND HYPERPOLARIZATION

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In this work we consider nuclear spin orders which cannot be observed directly in NMR spectra, and discuss new ways for revealing these orders via chemical or physical means. A typical example of such unobservable order is the nuclear singlet obtained in parahydrogen molecules. It is well-known that in order to reveal the singlet order in parahydrogen enriched gas using NMR, the high symmetry of parahydrogen molecules has to be destroyed and parahydrogen-induced polarization (PHIP) [1] can be observed as a result. Commonly, catalytic hydrogenation is employed for the symmetry breaking, and the polarization signals are detected for the reaction products, but other approaches also exist [2]. Herein, we suggest using organic molecules, the so-called “molecular tweezers” (MT), which can reversibly bind molecular hydrogen [3] to reveal parahydrogen spin order. Experimental data supported this idea as PHIP signals were observed in ^{1}H NMR spectra of QCATH2 [4] MT molecules every time after saturating its solution with parahydrogen. Symmetrical molecules other than molecular hydrogen may also have nuclear spin order which is relatively long-lived, but directly unobservable in NMR. For instance, contrary to molecular hydrogen having two spin isomers (ortho and para), ethylene has four spin isomers in the ground molecular state [5]. In our study, we show that unobservable nuclear spin order of ethylene can be produced in acetylene hydrogenation with parahydrogen, and thereafter, revealed in the electrophilic addition reaction of product ethylene. It is also found that ethylene spin order can be observed if ethylene molecules are placed in liquid crystalline media.

Acknowledgements: The grants 11-03-00248-a, 12-03-00403-a, 12-03-31386-mol_a (RFBR), 5.1.1 (RAS), 60, 61, 57, 122 (SB RAS), NSh-2429.2012.3, MK-4391.2013.3 and 11.G34.31.0045 are acknowledged. We thank Dr. K. Chernichenko and Prof. T. Repo (University of Helsinki, Finland) for providing MT samples.

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PARALLEL SESSION LECTURES

PS 125

SOME MORE OR LESS SUCCESSFUL ATTEMPTS AT IMPROVING DISSOLUTION DNP

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Since its invention ten years ago, dissolution DNP has become a method of choice to hyperpolarize low-gamma nuclear spins with long T_1's in view of performing *in-vivo* metabolic imaging and a variety of enhanced *in-vitro* experiments. Regrettably, the method has not significantly evolved and still relies, in most experiments, on the direct polarization of low gamma nuclear spins such as ^{13}C at low temperatures and moderate fields (typically T = 1 K and B_0 = 3.35 T) by saturation of the unpaired electrons of polarizing agents with narrow ESR-lines such as trityl radicals. We have wandered off the beaten tracks and attempted different approaches, some of which could possibly improve the technique. (**1**) We have shifted the focus from direct polarization of low gamma nuclei to direct polarization of protons, which can be enhanced quite effectively by saturating polarizing agents with broad ESR-lines such as TEMPOL. (**2**) We have implemented $^1H \rightarrow {}^{13}C$ cross polarization (CP), resulting in a net improvement in both ^{13}C polarization levels and build-up rates. (**3**) We investigated CP-DNP at a higher field B_0 = 6.7 T where direct ^{13}C polarization tends to suffer from prohibitively long build-up times. To our surprise we observed fast build-up rates for protons to unprecedented polarization levels $P(^1H) > 90\%$, close to unity. In this context, we have reported recently a polarization level $P(^{13}C) > 70\%$ achieved in less that 20 minutes. (**4**) We have made this method compatible with rapid dissolution and transfer to a liquid solution. (**5**) This has been combined with scavenging of TEMPOL by vitamin C. (**6**) The solution has been transferred through a 0.8 T magnetic tunnel. (**7**) Measurements at even higher fields such as B_0 = 9.4 T are currently being attempted, and preliminary results are encouraging. Some other approaches that may turn out to be less promising will also be presented, such as (**8**) thermal mixing in low field or (**9**) dissolution of samples containing hyperpolarized protons, followed by polarization transfer to ^{13}C in solution state.

PS 126

NUCLEAR MAGNETIC RESONANCE IN SEMICONDUCTOR QUANTUM DOTS

Alexander Tartakovskii

Department of Physics and Astronomy, University of Sheffield

Recent fast advances in device fabrication and development of sophisticated experimental techniques enabled major breakthroughs in applications of semiconductor nano-structures for control of single spins and generation of non-classical light. This research has been largely driven by the prospects for novel applications in quantum computation and quantum cryptography, for which III-V semiconductor quantum dots (QDs) present a very versatile quantum system suitable for both electrical and optical addressing. It has been found that both single spin manipulation and generation of entangled photons in a dot requires understanding and control of the magnetic environment in the dot formed by 10^4-10^5 nuclear spins of the lattice. Here we report on direct detection and fast manipulation of nuclear spins in individual quantum dots using optically detected nuclear magnetic resonance (NMR).

We show that by using pulsed radiofrequency (rf) excitation it is possible to manipulate on the microsecond timescale large Overhauser fields on the order of 0.5 T experienced by a single electron spin in a single quantum dot. Such fast manipulation of nuclear spins with short rf pulses is only possible in strain-free QDs, where quadrupole interaction is relatively weak. However, broadly studied self-assembled QDs are strongly strained. NMR techniques can provide non-invasive structural analysis, but have been restricted to strain-free semiconductor nanostructures because of the significant strain-induced quadrupole broadening of the NMR spectra. We have developed optically detected NMR spectroscopy method that can be used to analyse individual strained quantum dots. Our approach uses continuous-wave broadband radiofrequency excitation with a specially designed spectral pattern and can probe individual strained nanostructures containing only 10^4-10^5 quadrupole nuclear spins. With this technique, we are able to measure the strain distribution and chemical composition of a quantum dot in the volume occupied by the single confined electron. The applications of these new techniques are not limited to single QDs only and also extend far beyond conducting the structural analysis of nano-structures.

PS 127

NANOSTRUCTURED MATERIALS: AN ARIADNE'S THREAD TO UNDERSTAND DYNAMIC NUCLEAR POLARIZATION

O. Lafon[a], A. S. Lilly Thankamony[a], D. Lee[b], T. Kobayashi[e], D. Carnevale[c], H. Takahashi[b], V. Vitzthum[c], I. Slowing[e], M. Bardet[b], H. Vezin[a], M. Pruski[e], G. De Paëpe[b], G. Bodenhausen[c,d], J.-P. Amoureux[a]

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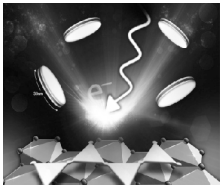

We demonstrate that Dynamic Nuclear Polarization (DNP) can enhance the sensitivity of solid-state NMR experiments on nanostructured materials, including mesoporous alumina[1], functionalized mesoporous silica loaded with surfactant[2] and dispersed inorganic nanoparticles.[3] Sensitivity enhancements of 1-2 orders of magnitude compared to conventional NMR enable an easy detection of surface and defect sites.[1-4] In particular, Al-Al proximities near the interfaces of mesoporous alumina are probed using DNP-enhanced ^{27}Al-^{27}Al dipolar recoupling.[1]
Besides, DNP experiments on nanostructured materials turn out to be a fruitful approach to understand several DNP effects. We show that DNP direct excitation enhances the signal of nuclei near unpaired electrons, whereas DNP cross-polarization (CP) from ^{1}H is able to enhance NMR signals of nuclei located at 100's nm from the radicals.[2,3] We also systematically analyze the different contributions (microwave irradiation, paramagnetic effects, presence of frozen solvent, temperature) to sensitivity enhancement of DNP ^{13}C and ^{29}Si CP-MAS experiments of functionalized mesoporous silica.[4]

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PS 128

INVESTIGATION OF THE QUANTUM ROTATION OF A WATER MOLECULE ENCAPSULATED INTO A FULLERENE CAGE BY SOLID STATE NMR.

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School of Chemistry University of Southampton, SO17 1BJ, UK

Endohedral fullerenes are supra-molecular complexes in which an atom or a small molecule is inserted into a fullerene cage. Such materials are ideal for the study of the physical and chemical property of the trapped molecules due to the homogeneity, stability and rigidity of the confinement.
Here we report a solid state NMR study of a water molecule inside the fullerene C_{60}. The ^{1}H MAS spectra display clear signature of anisotropic interactions despite the icosahedral symmetry of the confinement. The chemical shift tensor and the residual dipolar coupling have been determined combining NMR methods and numerical simulation. The origin of such anisotropies is related to a symmetry breaking of the water environment.
NMR at cryogenic temperatures reveals that the ortho-water ground state has a lifted degeneracy in good agreement with infrared and neutron scattering studies.
We report direct observation of the spin conversion between the ortho and para-H_2O spin isomers. The kinetics of the spin conversion has been studied as function of temperature and cage filling factor. We show that for high filling factors the conversion is driven by a combination of a first order and a second order process while at low filling factor (<70%) only the first order process is present . We link the first order process to the effect of spin-rotation and the second-order process to inter-molecular dipolar interactions between ortho-molecules. The possibility to observe conversion induced polarization effects on the nuclear spin is discussed.

PS 129

TOWARDS ROOM TEMPERATURE DNP AND LOW FIELD NMR USING NITROGEN-VACANCY CENTRES IN DIAMOND

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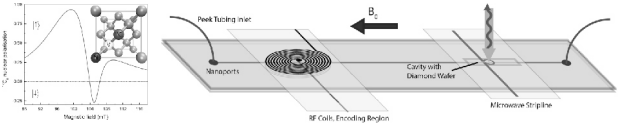
NMR is non-invasive, chemically specific and can be used for micron resolution imaging. However, present NMR and MRI systems suffer from poor sensitivity, poor portability and high cost. Here we focus on using point defects in diamond, nitrogen-vacancy (NV) centres, as polarising agents for low and high field NMR and also as magnetometers for low field NMR applications. For using NV centres as a polarising source, we demonstrate high polarisation of nuclei near optically polarised NV centres in diamond near the ground state level anti-crossing, 100mT. We find that the 13C nuclei in the first shell are polarised in a pattern that depends sensitively on the magnetic field. Controlled polarisation of surrounding nuclei could potentially be used for enhancing the sensitivity of NMR via polarisation transfer methods. For using NV centres as sensitive magnetometers, we fabricate microfluidic devices integrated with diamond wafers for remote detection NMR experiments at low field. We flow 2T pre-polarised water to a microfluidic chip, encode the nuclear spins and subsequently detect nuclear magnetization using an ensemble of NV centres at 5mT. We measure a sensitivity of 3.44nT/Hz and detect a 94Hz, 600pT simulated NMR signal. Using NV magnetometers at low field can in principle be significantly more sensitive than inductive methods, with a substantial sensitivity gain from present sensitivities. A low field NMR device that can both hyperpolarise samples and detect them with high sensitivity would be a significant advance in the development of cheap, affordable and portable NMR systems.



PS 130

ROOM TEMPERATURE NANOSCALE MAGNETIC SENSING BASED ON SINGLE ELECLTRON SPIN IN DIAMOND

Fazhan Shi, Jiangfeng Du

Hefei National Laboratory for Physics Sciences at Microscale and Department of Modern Physics, University of Science and Technology of China

Current spin-magnetic resonance spectrometers are based on the principle of ensemble detection and the test object is an ensemble sample containing billions of identical spins. However, at room temperature NMR at nano-scale is still a huge challenge.
To achieve the scientific goal, we choose single spins in solids based on NV defect center in diamond - (NV) as the sensitivity magnetic probe. The single NV spin can be easily visualized, polarized and detected with a confocal microscope. Ultra-long spin coherence time for such qubits, even at room temperature, enables it is ultra-sensitivity to external magnetic noise with characteristic frequency. Instead of traditional electrical defect, weak magnetic signals generated by the nano-scale spin system is mapped to coherent state phase, so as to realize high sensitivity signal detection.

PARALLEL SESSION LECTURES

PS 131

HETERONUCLEAR SPIN DECOUPLING IN SOLID-STATE NMR

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Heteronuclear spin decoupling is very important in solid-state NMR experiments. The pulse sequence employed in decoupling determines the spectral resolution and magnitude of coherence decay times (T_2') in sequences with spin-echo blocks. We will present here outline of pulse schemes that can be applied for a wide range of magic-angle spinning frequencies, their properties with respect to resolution and T_2' values, and their dependence on various experimental parameters. We will also look into the commonality of some of the phase-modulated decoupling schemes.

PS 132

COMBINATION OF J-EDITED AND CORRELATION SPECTROSCOPIES WITHIN A MULTI-DIMENSIONAL SPATIAL FREQUENCY ENCODING

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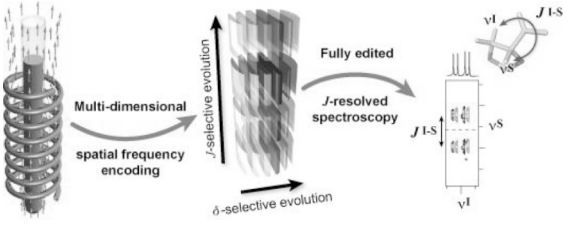

Most of the developments that have been made over the recent years in the field of so-called "Fast NMR" have focused on accelerating the acquisition of multidimensional data, sometimes with spectacular results. Despite the considerable progress accomplished in that field, the whole experimental and analytical process that leads to the extraction of proton-proton couplings remains however a hard and time-consuming task for chemists. One reason is the complexity and the amount of information that is made available in state-of-the-art experiments, even for small or medium sized molecules. There is currently no general NMR experiment that can give access, on a single spectrum, to a fully edited –and assignable- measurement of a whole proton coupling network.
We present here an experiment, based on a multi-dimensional frequency encoding of the NMR sample, that can help the chemists to reach this goal. The concept behind this sequence is original in sofar as it proposes to combine in a tailored fashion the different spin evolutions that contribute to the fine structure of each correlation, by coding their evolution separately along different gradient axes. It yields 2D spectra in which the whole proton network appears as a series of fully resolved doublets, triplets or quartets that can be straightforwardly assigned. We will show that using the resulting spectrum, it becomes easy to assign and measure straightforwardly all the proton chemical shifts and scalar couplings in model compounds. Despite being less sensitive, this approach is very powerful, using a conventional liquid spectrometer, due to its simplicity and efficiency.

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PS 133

SOLID-STATE PROTON NMR OF PARAMAGNETIC METAL COMPLEXES: DANTE SPIN ECHOES FOR SELECTIVE EXCITATION IN INHOMOGENEOUSLY-BROADENED LINES

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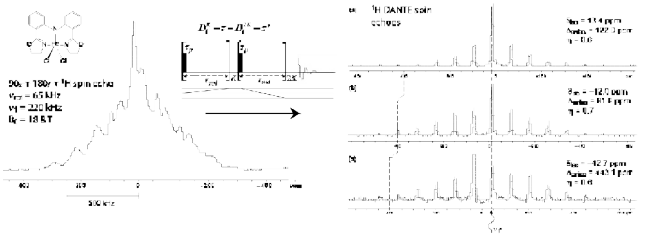
A paramagnetic Fe(III) complex is investigated by means of solid-state proton NMR at 18.8 T (800 MHz) using magic-angle spinning (MAS) at 65 kHz. The use of spin echoes refocused by combs of rotor-synchronized pulses in the manner of 'Delays Alternating with Nutation for Tailored Excitation' (DANTE) allows one to characterize different chemical environments that severely overlap in conventional MAS spectra. Such sequences combine two apparently contradictory features: an overall bandwidth exceeding several MHz, and very selective irradiation within inhomogeneously-broadened sidebands of a few kHz width. The properties of individual sites can be correlated to distances between various protons and the paramagnetic center.



PS 134

NMR STUDY OF THE EFFECT OF DILUTION ON ISOTROPIC BICELLES

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Bicelles are popular model membranes used in the study of membrane protein (MP) interactions and structure by NMR. However, experimental constraints may require their use at high dilutions at which their morphology is unclear. In this work, ^{31}P NMR spectroscopy has been employed to better understand the composition and morphology at strong dilutions of isotropic bicelles with *q* ratios (*q* = [DMPC]/[DHPC]) below 2. As the concentration is lowered, the concentration of free DHPC remains constant, in complete analogy with a critical micelle concentration (CMC), and the proportion of DHPC in the bicelles decreases thus modifying their morphology. The critical bicellar concentration or *CBC*, analogous to CMC, was calculated by successive dilutions following the method of Glover *et al.* (2001). It was shown to decrease with increasing *q* ratios between 0.15 to 0.75 and then to stay constant at a value of 6 mM up to the maximum studied *q* ratio of 2. Taking into account the free DHPC, the effective *q* ratios (q^*) were calculated. It appears that the q^* values (and therefore the bicelle morphology) are constant for total phospholipid concentrations above 80 mM. Below this concentration threshold, q^* starts to increase significantly, especially for high *q* ratios. For the more diluted concentrations, between 2 and 25mM and for *q* ratios above 0.75, samples become cloudy as DHPC-impoverished bicelles form large vesicles. The CBC variation with *q* ratio together with complementary FTIR measurements will be used to discuss the miscibility properties of the two bicelle-forming lipids.

Glover et al. (2001). Structural evaluation of phospholipid bicelles for solution-state studies of membrane-associated biomolecules. Biophysical journal, 81, 21632171.

PS 135

HOW TO SQUEEZE MORE SIGNAL INTENSITIES OUT OF NUCLEAR SPINS IN SOLIDS ... MEHR LICHT!

K. Takegoshi

Kyoto University, Japan

Under the constraints of using an rf-coil for detection and not relying on the gain by low temperature and electron spins (I mean under the conventional experimental conditions), there are not many ways to appreciably increase the sensitivity of NMR signals of what commercial NMR machines can provide. In my talk, I will discuss some examples of our struggling for better sensitivity in these years. For example, solid-state NMR people like me have been envying a cryo-probe for liquid-state NMR. Because we want to rotate the sample rapidly at the magic angle, it was difficult to make a cryo MAS probe. Recently, we made several cryo-coil MAS probes and enjoy sensitivity enhancement of factors 3~5 [1]. For adjustment of the magic angle, we developed a gadget called as an X0-shim coil [2], which enables us to adjust the angle precisely without tears. The signals at the "exact" magic angle get sharper than those observed at the spinning angle adjusted manually using the conventional gear system. In data acquisition, we have not used the range of the AD converter fully during acquisition as the fid signal decays. By increasing the receiver gain concomitantly to digitize the fid signal using the full range of the AD converter from the top of fid to its end , we can dig out an exceedingly small signal usually buried in noise [3]. Regrettably, we have discarded all individual fid signals during accumulation and satisfied with using just its summation. Can we apply statistical analysis to these individual fid and obtain some additional information? The answer is, of course, YES [4] (otherwise I did not ask). Like an old COCONOESY experiment, we can utilize so-far discarded magnetizations/coherences [5]. Such Green techniques may be a good excuse to buy/make a double-receiver system, which we used to examine covariance data processing for HETCOR [6].

[1] Mizuno et al., to be published in future I hope.
[2] Matsunaga et al., to be submitted soon.
[3] Takeda&Takegoshi, JMR 208 (2011) 305.
[4] Fukazawa&Takegoshi, PCCP 12 (2010) 11225; Fukazawa et al., JMR 211 (2011) 52.
[5] Fukuchi&Takegoshi, SSNMR 34 (2008) 151; Fukuchi et al., JMR 194 (2008) 300.
[6] Takeda et al., PCCP 14 (2012) 9715.

PS 136

GATE DYNAMICS REGULATE THE CATALYTIC ACTIVITY OF UBIQUITINATION ENZYMES

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The synthesis of polyubiquitin chains on cellular proteins is a fundamental regulatory pathway for numerous biological processes. A key chemical step in ubiquitination is the reaction of a lysine amino group with a thioester group on an activated ubiquitin conjugation enzyme, to form an amide bond between ubiquitin and the target protein. We combined measurements of enzyme kinetics, main chain NMR relaxation, and X-ray crystallographic studies with molecular dynamics simulations to demonstrate that nanosecond timescale motions of a loop adjacent to the active site function as a gating mechanism to regulate the catalytic activity of ubiquitin conjugation enzymes. The remarkable rate enhancement achieved by these enzymes requires that their biological activity be precisely controlled; this is accomplished in large part by the loop gating mechanism, which is driven by fast protein dynamics. Mutagenesis of crucial loop residues to glycine demonstrates that loop dynamics serve to balance the rates of gate opening and closure, a balance that precisely tunes the reaction to determine the catalytic efficiency of ubiqutination enzymes and the ensuing biological outcomes.

PS 137

STRUCTURAL AND DYNAMIC INSIGHTS INTO SUBSTRATE BINDING AND CATALYSIS IN HUMAN LIPOCALIN PROSTAGLANDIN D SYNTHASE

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Lipocalin-Prostaglandin D synthase converts Prostaglandin-H2 (PGH2) to Prostaglandin-D2 (PGD2), an important inflammatory and signaling mediator. It also binds biliverdin, all-trans retinoic acid and bilirubin, acting as lipophilic compound carrier. We employed structural, biophysical and biochemical approaches to address the mechanistic aspects of substrate entry, catalysis and product exit. The structure of human L-PGDS was solved in a complex with a substrate analog and in ligand-free-form. Catalytic Cys 65 thiol group is found in two different conformations, each making a distinct hydrogen bond network to neighboring residues, elucidating the mechanism of the cysteine nucleophile activation. Ligand electron density was observed in the active site demarking substrate-binding regions, but did not allow unambiguous fitting of the substrate. To further understand ligand binding, we used NMR spectroscopy to map the ligand binding sites and to show the dynamics of protein-substrate and protein-product interactions. A model for ligand binding at the catalytic site is proposed, showing a second binding site involved in ligand exit and entry. NMR chemical shift perturbations and NMR resonance line-widths alterations (observed as changes of intensity in 2D cross-peaks in [1H,15N]-TROSY) for residues at Ω helix, E-F loop and G-H loop besides the catalytic sites indicating involvement of these residues in ligand entry/egress.

PS 138

TIME-RESOLVED NMR STUDIES OF THE LIGHT-ACTIVATED STATES OF RHODOPSINS

Jochen Stehle, Deep Chatterjee, Krishna Saxena, Frank Scholz, Judith Klein-Seetharaman, Josef Wachtveitl, Harald Schwalbe

Institute of Org. Chemistry and Chemical Biology, Institute of Phys. Chemistry, Center for Biomolecular Magnetic Resonance, Johann Wolfgang Goethe-University Frankfurt

Rhodopsin is the major membrane protein of the rod outer segment (ROS), a highly ordered array of hundreds of discs that are enclosed by a separate plasma membrane, in vertebrate retina. As a visual photoreceptor it contains a photoreactive retinal chromophore that is covalently attached to the protein and is converted into a receptor agonist by light.
We here present NMR structural studies yielding information on the transient light-activated state of rhodospin using light-triggered time-resolved NMR spectroscopy. We have obtained both selectively and uniformly isotope-labeled samples of rhodopsin in a high level mammalian expression system (HEK293) of the bovine opsin gene yielding mg quantities of functional protein with all posttranslational modifications for NMR studies. We further discuss interaction of rhodopsin with peptides derived from arrestin transducin to obtain information about protein-protein interactions involved in the signalling cascade.

PS 139

INTEGRATED STRUCTURAL BIOLOGY SHOWS HOW SCARCE SEQUENCE ELEMENTS CONTROL THE FUNCTION OF SINGLE ß-THYMOSIN/WH2 DOMAINS IN ACTIN ASSEMBLY

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β-thymosin (ßT) and WH2 domains are found as single or repeated units in a large number of multi-domain actin-binding proteins involved in developmental processes, synaptic plasticity, polarized cell migration or intracellualr pathogen infections. They are archetypes of small intrinsically disordered actin-binding modules with highly variable sequences of 25-55 residues. In the last years, multi-domain protein organizations with ßT/WH2 domains have emerged as multifunctional regulators of actin-assembly dynamics. They display significant sequence variability associated with versatile regulations of actin assembly in motile processes. Understanding the structure-function relationship governing their versatile or multiple functions remain extremely challenging.
Here we reveal the structural and dynamics determinants, in their basic 1:1 stoichiometric complexes with actin, which govern either the assembly inhibition by sequestering actin monomers (Thymosin-ß4) or the motility enhancement by directing polarized filament assembly (Ciboulot ßT or WASP/WAVE WH2 domains).
To reach this goal, we combined mutational, functional, and structural analysis by X-ray crystallography, SAXS and NMR in a typical integrated structural biology approach. Based on the examples of Thymosin-ß4, Ciboulot and chimeras and on their complexes with monomeric actin, we show that functionally different ßT/WH2 domains do not target alternative actin binding sites but rather differ by alternative dynamics of their C-terminal half interactions with G-actin pointed face. The detailed NMR analysis of the dynamics of the free chimeras using 15N relaxation, residual dipolar couplings and relaxation dispersion experiments demonstrates specific individual behaviors, which bring insight into the folding upon binding mechanisms of these peptides. We show how the structural plasticity of WH2 domains is partially conserved and functional in their G-actin bound state. At physiological ionic strength the local interaction dynamics are primarily controlled by strong electrostatic interactions of a single residue along their sequence. These results open fascinating perspectives for elucidating the functions of ßT/WH2 domains in other modular proteins and enlighten how intrinsic structural disorder can lead to a novel mode of functional versatility.

PS 140

ORDER IN DISORDER – ATOMIC RESOLUTION STUDIES OF UNSTRUCTURED PROTEINS

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NMR represents the ultimate tool for studies of unstructured or partially disordered proteins at the atomic resolution. In principle, intrinsically disordered proteins can be assigned using a standard set of triple-resonance NMR experiments applied to ^{13}C, ^{15}N-labelled samples. However, combination of the structural disorder with a high incidence of sequential repeats often results in spectra with severely overlapped peaks, impossible to assign by the traditional approach. The lecture will review recent developments from our lab to significantly shorten time needed for thorough description of unstructured or partially disordered proteins. To facilitate the atomic resolution studies, we have designed a suite of high-dimensional (4D-5D) NMR experiments, which combines ^{13}C-direct detection, non-uniform sampling, and non-standard data processing procedures to substantially enhance the attainable resolution. The power of the developed methodology is documented on studies and disorder characterization of 20 kDa delta subunit of RNA polymerase unique for gram-positive bacteria, 12.8kDa intrinsically disordered WIPs protein having a high content of proline residues (26%) in the sequence, and 49.2 kDa microtubule-associated protein 2c.

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PARALLEL SESSION LECTURES

PS 141

NMR IN HIGH MAGNETIC FIELDS AND THE PUZZLE OF HIGH TEMPERATURE SUPERCONDUCTIVITY

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Despite a quarter century of intense research, the origin of high-T_c superconductivity in copper-oxides remains a mystery and arguably one of the greatest challenges in condensed matter physics.

In this talk, I shall describe how recent experiments, in particular NMR experiments (1,2), in high magnetic fields (> 30 Tesla) have led to tremendous progress in the understanding of the electronic properties of these superconductors.
Specifically, ^{63}Cu and ^{17}O NMR experiments in the archetypal superconductor $YBa_2Cu_3O_y$ have revealed the existence of charge order (a state where the electronic density becomes spatially modulated). This charge-density-wave only sets in once a strong magnetic field has sufficiently weakened superconductivity, thus providing a direct evidence of close competition between these two, nearly-degenerate, electronic states.
Although some forms of charge order have been known for several years in some of these high-T_c cuprates, this discovery in a cleaner superconductor like $YBa_2Cu_3O_y$ gives a new twist to the field as it suggests that this phenomenon is actually generic and that it underlies some of their most mysterious properties including, possibly, superconductivity itself.

(1) Wu, T. *et al*. Magnetic-field-induced charge-stripe order in the high temperature superconductor $YBa_2Cu_3O_y$. *Nature* **477**, 191–194 (2011).
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PS 142

UNCONVENTIONAL SUPERCONDUCTIVITY OF ALKALI-DOPED FULLERENES

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The superconductivity of the alkali-doped fullerenes (A_3C_{60}, A = alkali metal) has been so far discussed within the standard theory of superconductivity developed by Bardeen, Cooper and Shrieffer (BCS), even-thought, they exhibited relatively high critical temperatures (up to T_c = 32 K). However, after our recent high-pressure measurements on Cs_3C_{60} such description became questionable. We have shown that the superconducting phase of A_3C_{60}, in fact, borders the antiferromagnetic insulating (AFI) phase, commonly observed for high-temperature superconductors like cuprates or pnictides [1,2]. In addition, we also increased the maximal T_c to 38 K. To investigate this peculiar superconductivity close to the border with AFI state we employed nuclear magnetic resonance (NMR) technique on $Cs_{3-x}Rb_xC_{60}$ and on Cs_3C_{60} at various high pressures. Our results could not be correctly explained either by the standard BCS or the extended BCS that includes electron-electron repulsion interaction - the Migdal-Eliashberg theory. Far better agreement is obtained by the Dynamical Mean Field Theory (DMFT), which correctly treats the electron-electron correlations and the Jahn-Teller active intramolecuar phonons on equal footing [3]. Due to similarity with other unconventional superconductors these results could also be relevant to other unconventional high-temperature superconductors.

[1] Y. Takabayashi, *et al*., *Science* **323**, 1585 (2009).
[2] A.Y. Ganin, A. Potočnik, *et al*., *Nature* **466**, 221 (2010).
[3] M. Capone *et al*., *Rev. Mod. Phys.* **81**, 943 (2009).

PS 143

ANOMALOUS BEHAVIOUR OF $1/T_2$ IN THE IRON-BASED SUPERCONDUCTORS

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Nuclear magnetic resonance has proven since decades to be one of the best tools to investigate the microscopic properties of superconductors. It allows to probe the symmetry and magnitude of the superconducting order parameter, the spin state of Cooper pairs, the flux lines lattice and the normal state excitations which can be involved in the pairing mechanism. Most of these information are derived from $1/T_1$ and paramagnetic shift measurements which allow to access both the dynamic and the static spin susceptibility, respectively. In the high temperature superconductors (HTcSC) it has been found that also the echo decay time $1/T_2$ could provide additional information on the local spin susceptibility. Typically, besides the standard contribution from the direct nuclear dipole-dipole interaction, one observes an additional indirect contribution to the echo decay involving the electron spins. On the other hand, in the iron-based superconductors quite a different scenario is observed. ^{75}As NMR measurements in the normal state of $Ba_2(Fe_{1-x}TM_x)_2As_2$ (TM = Rh or Co) superconductors show that $1/T_2$ is about 5 times smaller expected from the direct dipole-dipole interaction, which is quite surprising since no nuclear diffusive dynamic is at work. Moreover, on approaching the superconducting transition Tc from above one observes a progressive increase of $1/T_2$ leading to a peak in the superconducting phase, even in compounds without any long range magnetic order. That peak, was initially ascribed to the slowing down of the vortex dynamics [1,2], however the recent observation that $1/T_2$ starts to increase already above Tc appears to question that interpretation. Moreover, the comparison of the echo decay after Hahn echo and CPMG pulse sequences clearly evidences that a very low frequency dynamic is present. Here we discuss the possible mechanisms underlying that very slow dynamics and the modification in the behavior of $1/T_2$ upon spanning the whole phase diagram of $Ba_2(Fe_{1-x}TM_x)_2As_2$, from the underdoped to the overdoped compounds. Finally the effect of an external dc current on T_2 for $T<Tc$ is discussed in the light of the flux lines lattice dynamics.

[1] L. Bossoni et *al.*, Phys. Rev. B **85**, 104525 (2012);
[2] S. Oh et *al.,* Phys. Rev. B **83**, 214501 (2011);

PS 144

HIGH-FIELD ESR AND NMR SPECTROSCOPY OF THE NOVEL LOW-DIMENSIONAL QUANTUM MAGNET $BaAg_2Cu[VO_4]_2$

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The complex transition-metal (TM) oxide $BaAg_2Cu[VO_4]_2$ (BACVO) belongs to a new material class of low-dimensional quantum magnets where, depending on subtle steric details and the type of the TM ion, either frustrated triangular 2D spin lattices or a network of 1D spin chains can be realized [1]. DFT band structure calculations, quantum Monte-Carlo simulations, and high-field magnetization measurements suggest that the physics of BACVO is determined by a superposition of ferromagnetic and antiferromagnetic uniform spin-1/2 chains with nearest neighbour exchange couplings of $J_{FM} = -19K$ and $J_{AFM} = 9.5K$ [2].
Here we report an experimental study of BACVO by high-field ESR and NMR spectroscopies, which probe the local magnetic properties and in particular address an interplay between the structure and the exchange coupling between the Heisenberg quantum spins s = 1/2 associated with the TM Cu(II) ions. A comparative analysis of the Cu(II) ESR and ^{51}V NMR spectra enables to identify non-equivalent V sites which suggests a non-equivalency of the VO_4 structural fragments mediating the superexchange between the Cu ions in the chains. By analysing the frequency-, magnetic field and temperature dependence of the Cu(II) ESR signals and the temperature dependence of the ^{51}V Knight shifts and the T_1 relaxation rates we determine the distribution of local magnetic fields arising due to the onset of the spin correlations in the chains at low temperatures. Indeed, our data support the scenario of the tunable ferro- and antiferromagnetic coupling in the Cu spin-1/2 chains driven by the specific tilting of the VO_4 exchange mediators and by this put forward the $BaAg_2Cu[VO_4]_2$ as a new model system for studies of low-dimensional Heisenberg quantum magnetism.

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PS 145

SPIN DYNAMICS IN THE SPIN-1/2 TRIANGULAR-LATTICE ANTIFERROMAGNET CS_2CUBR_4

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Magnetic excitations in Cs_2CuBr_4, a spin-1/2 spatially anisotropic antiferromagnet (AF) with a distorted triangular lattice, are probed by means of high-field electron spin resonance (ESR) spectroscopy. We show that the high-energy spin dynamics of this material is determined by short-range-order spin correlations (presumably of 1-dimensioanl nature), which are stable against the magnetic order down to well below T_N. Such a behavior is consistent with the spin-liquid picture, revealed previously in the isostructural compound Cs_2CuCl_4 and some other quasi-1D systems. On the other hand, the observation of a substantial zero-field gap illuminates the important role of frustrated interchain interactions, making Cs_2CuBr_4 an excellent model system to study effects of frustration and dimensionality in spin-1/2 triangular-lattice Heisenberg AFs. In addition, we report on ESR measurements of Cs_2CuBr_4 in pulsed magnetic fields up to H_{sat} ~ 30 T and above. In the magnetically saturated phase quantum fluctuations are fully suppressed and the spin dynamics is determined by ordinary spin waves (magnons); this behavior can be described semi-classically. This allows us to accurately determine spin-Hamiltonian parameters for Cs_2CuBr_4.

This work was partly supported by the DFG and EuroMagNET (EU contract No. 228043).

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MONITORING ENZYMATIC CONVERSION OF DNP-ENHANCED SUBSTRATES BY INDIRECT DETECTION

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Dynamic Nuclear Polarization (DNP) combined with low temperature operation offers enhancements of NMR sensitivity reaching several orders of magnitude. Rapid dissolution of substrates polarized in this way has allowed monitoring of rapid enzymatic conversions, several of which are finding application to *in vivo* diagnosis and treatment monitoring of disease. Applications have, however, been dominated by direct observation of ^{13}C sites in products coming from a small number of metabolic substrates, primarily pyruvic acid. Here we explore potential applications to other nuclei (^{15}N) and to ^{13}C systems where additional information may be gleaned from conversion of deuterated sites to protonated sites. The studies were initially motivated by the need to prolong storage of polarization on normally protonated ^{15}N sites by deuteration. Subsequently, additional enhancement in sensitivity was achieved by indirect detection through protons as deuterons were replaced by protons from solvent. Appropriate pulse sequences have been developed and applied to detection of ^{15}N glutamine. Prolonging polarization storage by deuteration applies equally well to ^{13}C sites and there are many systems in which protons are reintroduced on enzymatic conversion. Patterns of incorporation in these systems provide additional mechanistic detail. This is illustrated in observation of conversion of deuterated, $^{13}C3$ pyruvic acid to alanine by alanine transaminase. Preliminary extension to 2D detection in more complex systems will also be described.

PARALLEL SESSION LECTURES

PS 147

NANOLITER HR-MACS NMR METABOLIC PROFILING OF BIOPSY AND MICROORGANISM

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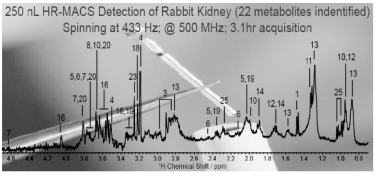
HR-MAS NMR spectroscopy combined with chemometric has now emerged as a powerful methodology for metabolomics NMR of biopsies, and has led to many important disease diagnosis and environmental assessments.[1] However, the required tissue quantity is of the order of 10 mg. Such large quantity can compromise the metabolic evaluation because of the high degree of tissue heterogeneity. Moreover, the tissue availability is often a limitation and prevent from the possibility of multi-clinical diagnostic analyses. Such large quantity also renders great challenges for localization analysis.

A spinning nanoliter MAS detector (Magic-Angle Coil Spinning MACS) has been introduced to enhance sensitivity for mass-limited sample NMR detections.[2,3] MACS can wirelessly coupled to commercial HR-MAS probes without modifications, making it easy to adapt. Here, we present a *refined* MACS, High-Resolution (HR)-MACS, that further improves the spectral resolution without scarifying the sensitivity enhancement, rendering for nanoliter biopsy MAS study.[4] With HR-MACS, we show the capability of obtaining enhanced sensitivity (6-7 folds) and high resolution (1-2 ppb) results for nanoliter biopsies and microorganisms such as *Caenorhabditis elegans* nematodes in a reasonable acquisition time span, allowing for an in-depth metabolic profiling. We also demonstrate a double-resonance $^{1}H\{^{13}C\}$ HR-MACS experiment to metabolomics study. HR-MACS detections can be considered less sample destructive analysis compare to the bulk sample HR-MAS study, owing to the slow sample spinning and small sample size acquisitions minimizing the centrifugal force exerts onto the biopsy. The HR-MACS detector presents possibility of NMR applications to metabolomics study of nanoliter biopsies, making the concept of needle NMR biopsy more realistic.



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FLUORINATED CARBOHYDRATES AS LECTIN LIGANDS: DISSECTING GLYCAN-CYANOVIRIN INTERACTIONS BY ^{19}F-NMR

Elena Matei[1]; Anja Glinschert[2]; Angela Simona Infantino[2]; Stefan Oscarson[2]; Angela M. Gronenborn[1]

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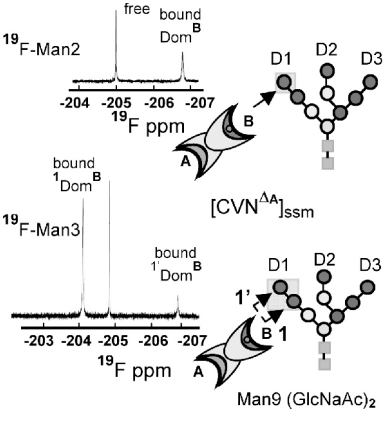
We will present a versatile and sensitive fluorine NMR approach that exploits the ^{19}F nucleus of ^{19}F-labeled carbohydrates as a sensor to study glycan binding to lectins. Our approach will be illustrated with the 11 kDa Cyanovirin-N, a mannose binding anti-HIV lectin. Two different oligomannose sugars that posses single F atoms in one mannose ring are utilized. Binding was studied by ^{19}F-NMR spectroscopy of the ligand and ^{1}H-^{15}N HSQC NMR spectroscopy of the protein. The NMR data agree well with those obtained from the equivalent reciprocal and direct ITC titrations.

Our study shows that strategic design of fluorinated ligands and 19F-NMR holds great promise for easy and fast identification of glycan binding as well as for their use in reporting structural and/or electronic perturbations that ensue upon interaction with a cognate lectin.



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DECIPHERING CANCER METABOLISM FOR DRUG DISCOVERY USING NMR

Ulrich Guenther, John Carrigan, Katarzyna Koczula, Christian Ludwig, Farhat Khanim, Chris Bunce

University of Birmingham

Metabolomics has gained significant ground in Biomedicine, including the field of cancer research. This is in part because metabolomics observes a phenotypical end point, reflecting small changes in various pathways linked to metabolism. Applications range from typical diagnostics to drug discovery. While the potential diagnostic value is increasingly accepted, the use of metabolomics to understand the mechanisms of drug actions has been realized only more recently. Such developments are very closely linked to the fact that the aspect of altered metabolism in cancer has been rediscovered. Although known since Otto Warburg's research in the 1920s, metabolism has been neglected for a ling time.
Now we find altered metabolism in different places of metabolic processing, and we learn how metabolism is affected by drugs. This has been studied in AML and CML (acute and chronic myeloid leukemia) cells, where we observe unforeseen changes in metabolism following a new drug combination. In AML cells a pronounced metabolic effect arises from the generation of high levels of reactive oxygen species, which chemically modify several metabolites.
For this metabolic analysis we combined metabolomics with isotopic tracer based metabolic flux analysis, using ^{13}C-labelled metabolic precursors. In this context NMR has a significant advantage as it can observe site-specific label incorporation, revealing unforeseen effects, in particular in Krebs' cycle metabolites. Using metabolic fluxes open new avenues to decipher mechanisms of metabolism.

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PROBING HEPARIN STRUCTURE THROUGH NMR MEASUREMENTS OF EXCHANGEABLE PROTONS

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University of California - Riverside

NMR measurements of exchangeable NH and OH protons of the biologically important carbohydrates heparin and heparan sulfate (HS) will be described. These biopolymers are members of a special class of nitrogen containing polysaccharides called glycosaminoglycans (GAGs). Our group determined experimental parameters for detection of the ^{1}H and ^{15}N resonances of the sulfamate ($NHSO_3^-$) groups of the *N*-sulfoglucosamine residues of heparin and HS and demonstrated the sensitivity of the chemical shifts to local structure. ^{1}H and ^{15}N chemical shift assignments were performed for intact and unfractionated heparin, HS, and the low molecular weight heparin enoxaparin by comparison of HSQC correlations measured for isolated oligosaccharides with defined structures and intact heparin subjected to specific chemical modifications. The exchange properties of the HSQC correlations of enoxaparin were also evaluated as a function of pH confirming the identity of the 3-*O*-sulfated GlcNS residues. Two-dimensional HSQC-TOCSY spectra provided additional information confirming the assignments of the discrete cross peaks observed in the [^{1}H, ^{15}N] HSQC spectra of enoxaparin and related GAG samples. Evaluation of the exchange kinetics of the sulfamate group protons with the bulk water allows exploration of the relationship between primary and secondary structure in heparin oligosaccharides and led to identification of the first solution state hydrogen bond between a sulfamate group NH proton and the adjacent 3-*O*-sulfate moiety of the heparin drug Arixtra. Molecular dynamics simulations supported the presence of this NH hydrogen bond and predicted additional hydrogen bonds involving the Arixtra hydroxyl protons prompting us to seek experimental evidence for their existence in isolated oligosaccharides of known structures. Hydrogen bonds involving selected Arixtra hydroxyl groups were probed experimentally by measuring temperature coefficients, chemical shift differences and NOE initial build-up rates, which taken together can indicate protection from solvent exchange, usually in the form of a hydrogen bond. Oligosaccharides structurally similar to Arixtra were also examined to determine the effects of subtle structural changes on hydroxyl group hydrogen bonds.

PARALLEL SESSION LECTURES

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A UNIFIED CONFORMATIONAL SELECTION AND INDUCED FIT APPROACH TO THE MODELLING OF PROTEIN-PEPTIDE INTERACTIONS

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Protein-protein interactions are vital for all cellular mechanisms. Among those, peptide-mediated interactions contribute up to 40% of all protein-protein interactions. Due to their important role in regulatory and signalling processes, peptides are in many cases implicated in diseases. They are also known to block protein-protein interactions, which makes them interesting leads for protein drug design.
Despite the large amount of structural data available, it remains very challenging to predict protein-peptide interactions by homology modelling. This leaves biomolecular docking as one of the few amenable computational technique to predict these interactions. Yet, the intrinsic flexibility of peptides is a major obstacle for any modelling technique. Docking methods are sensitive to conformational changes and generally require high-resolution structures and/or knowledge of the bound form to perform well. However, it is possible to narrow down the uncertainty by using information to drive the search, allowing concentrating on further refining the conformation of the peptide at the interface. We have developed HADDOCK (http://haddock.science.uu.nl), a flexible information-driven docking program that distinguishes itself from others by the use of experimental and/or bioinformatics data to drive the modelling process [1]. HADDOCK has been widely applied to model 3D structures of protein-protein and protein-nucleic acids complexes and has also demonstrated strong performance in CAPRI, a blind experiment for the prediction of biomolecular interactions. We will present recent results on the application of HADDOCK to protein-peptide modelling using a benchmark of 103 complexes [2]. In the absence of information on the bound form of the peptide, we achieve near-native predictions for ~80% of the benchmark by combining the concepts of conformational selection and induced fit into our flexible docking approach.
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PS 152

FIRST-PRINCIPLES ANALYSIS OF NMR ISOTOPE SHIFTS IN HEAVY-ELEMENT SYSTEMS

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The comparison of *in vacuo* calculated and experimental temperature dependence of the absolute NMR shielding or chemical shift is impractical if the experiments are carried out in the solution phase, due to the large solvent effects. Therefore, only the rarely available low-pressure gas experiments provide reasonable data for such investigations [1]. The NMR isotope shifts, on the other hand, are almost solvent-independent and depend on the same potential energy and property surfaces as the rovibrational effects of absolute shieldings. Therefore, they provide an excellent platform for testing many different aspects of first principles theoretical modeling involving electron correlation treatment as well as the description of relativistic effects and the motion of the nuclei. Here, we discuss quantum-mechanical studies of the effects of thermal rovibrational motion in heavy-element molecules with relativistic effects for the heavy ($^{129/131}$Xe and ^{77}Se) and light (^{19}F and ^{13}C) nuclear shieldings in the linear XeF_2 [2,3] and CSe_2 [4,5,6] molecules. The first comprehensive quantum-mechanical treatments of finite-temperature effects [3,5,6] in heavy-element compounds are able to provide quantitative agreement with the experimental secondary isotope effects on nuclear shielding [2,4]. This is achieved by combining piecewise approximation of high-level *ab initio* nonrelativistic and DFT relativistic shielding surfaces together with a high-quality potential energy surface, both at the basis-set limit.
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PS 153

DE NOVO STRUCTURE PREDICTION OF PROTEINS USING SOLVENT PRES

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Characterization of structure and dynamics of medium to large biomolecules and bio-molecular complexes by NMR spectroscopy is hampered by increasing overlap and severe broadening of NMR signals. As a consequence, the number of available NMR data is often sparse and new approaches providing complementary NMR data are needed. Paramagnetic relaxation enhancements obtained from inert and soluble paramagnetic probes (solvent PREs) provide detailed quantitative information about the solvent-accessibility of NMR-active nuclei. Solvent PREs can be easily measured without modification of the biomolecule, are sensitive for molecular structure and dynamics and are therefore becoming increasingly powerful for the study of biomolecules and their complexes in solution. Here we present our approaches for *de novo* structure prediction of proteins using solvent PREs. *De novo* protein structure prediction by computational approaches has been shown recently to provide accurate structural models, but is typically limited to small proteins. To overcome the current limitations we included solvent PREs in the *de novo* protein structure prediction program ROSETTA. As long as chemical shift assignments are available, solvent PRE data can be easily obtained without the need of modifying the sample. Solvent PREs provide quantitative information about the distance of spins to the protein surface. Thus, solvent PREs are excellent indicators of the quality of structural models. In our approach, solvent PREs are used i) directly in the Monte-Carlo based protein folding algorithm and ii) to re-score the final models. In order to keep computational loads low, an efficient method for back-calculating solvent PRE data of a given structure was implemented and incorporated into the ROSETTA framework. We applied our protocol to a benchmark of small- to large-size proteins and show that direct refinement against solvent PRE data improves structural accuracy and precision significantly. We show that solvent PREs provide a new class of restraints in *de novo* structure prediction programs that are easily accessible and applicable to any kind of protein. In particular for challenging systems and in cases when only sparse data is available, our approach promises significant time-savings and significantly improved quality of *de novo* structure predictions based on NMR data.

PS 154

ADVANCES IN COMPUTATIONAL MODELLING OF MACROMOLECULAR ASSEMBLIES USING LIMITED NMR DATA

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Macromolecular assemblies play key roles in a variety of cellular functions including protein homeostasis, cell signalling and pathogen-host interactions. Structural studies of such high molecular weight protein complexes by NMR are inherently limited by low signal-to-noise ratios, spectral overlap and further complicated by the presence of both spectral and structural ambiguity in the interpretation of the identified NMR cross-peaks. Recent advances in Rosetta modelling methodologies have enabled for the atomic-detail modelling of monomeric proteins up to 25kDa using a very limited set of NMR data as a means to limit conformational sampling in relevant regions of the energy landscape. Here, we present a generalized framework to model oligomeric protein assemblies using NMR data combined with datasets from complementary techniques (SAXS, cryoEM) in a hybrid approach. We apply these tools to determine a complete atomic model of the *Salmonella typhimurium* type-III secretion system needle using a combination of solid-state NMR, sparse ^{13}C labeling protocols, and Rosetta modelling [1]. We find that the 80-residue subunits form a right-handed helical assembly with roughly 11 subunits per two turns similar to the flagellar filament of *Salmonella typhimurium.* Finally, we present an application of the new approach to model the eye lens chaperone alphaB-crystallin 24mer cage using solid-state NMR and mass spectrometry data.

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PS 155

THEORETICAL EPR SPECTROSCOPY OF OPEN-SHELL TRANSITION METAL COMPLEXES WITH STRONG SPIN ORBIT COUPLING

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The majority of molecules have orbitally nondegenerate ground states. The molecular electric and magnetic properties are well described by standard models of electronic structure theory, e.g. perturbation or linear response theory. These methods have found very widespread use in the community of quantum chemistry users. The ORCA program developed in our group features many such methods for the calculation of optical and magnetic properties of transition metal complexes. There is, however, a significant class of molecules with orbitally (nearly) degenerate ground states where the established methods do not work. Here, a more careful treatment of the leading relativistic effects is necessary in order to correctly predict their spectroscopic properties and obtain molecular level electronic structure insight. This is not possible on the basis of density functional theory (DFT). Recently efficient methods based on multireference wavefunction theory have been implemented into ORCA that allow such calculations on large molecules. Their use will be demonstrated by a recent study that deals with the electronic structure of the only low-molecular weight catalyst known to be capable of reducing dinitrogen to ammonia.

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NMR IN MOLECULAR SYSTEMS BIOLOGY: FROM STRUCTURES TO FUNCTION

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NMR spectroscopy, with its multiple approaches and applications, has a unique role in describing functional biological processes at atomic level and in a cellular context. NMR is indeed suitable not only to characterize the structure and dynamics of biomolecules but, even more importantly, to describe functional events still maintaining atomic resolution. Furthermore, along a functional process, most interactions are transient in nature. Among these, there are the metal transfer processes, in which metal transfer, from metal transporters to the final recipient proteins, occurs through a series of protein-protein interactions so that the metal ion is transferred from one protein to the next[1,2]. These transfer processes are determined by metal affinity gradients among the various proteins, with kinetic factors contributing to the selectivity of the processes[3]. Key processes for Life are also those responsible for the biogenesis and transport of iron-sulfur clusters. The latter systems contain paramagnetic ions which dramatically affect the NMR spectra. The power of NMR approaches to describe cellular pathways at atomic resolution will be presented for a few pathways responsible for copper trafficking in the cell and for the biogenesis of iron-sulfur proteins. New major advancements in in-cell NMR[4] and in the characterization of highly paramagnetic systems[5] will be discussed. The results will be presented and discussed within an integrated approach where, from single structures to protein complexes, the processes are described in their cellular context within a molecular perspective.

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PS 157

PARAMAGNETIC NMR (PNMR): RECENT DEVELOPMENTS ALONG THE BORDERLINE OF THEORY AND EXPERIMENTS

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NMR relaxation in paramagnetic systems is potentially very informative, but the underlying theory is relatively complicated. In this presentation, we shall discuss some recent developments of importance for future applications.
Understanding the mechanisms of electron and nuclear spin relaxation is necessary for developing new MRI contrast agents. In the case of Gd(III) complexes, the experimental NMR relaxation dispersion (NMRD) data are commonly interpreted assuming that the electron spin relaxation is governed by the zero-field splitting (ZFS) interaction, modulated by molecular reorientation as well as a faster internal dynamic process. We shall present a combined investigation of NMRD and ESR lineshapes indicating that this approach indeed can interpret relaxation processes covering frequency range from 10 kHz to hundreds of GHz [1].
Nitroxide radicals are used in ESR, DNP and pNMR. We are going to present recent results for ^{1}H NMRD in viscous solutions containing nitroxides with either ^{14}N or ^{15}N [2]. The differences between the two types of nitrogen species reflect the quantum mechanics determined by the nuclear spin quantum numbers, I=1 for ^{14}N and I=1/2 for ^{15}N.
One way to improve theoretical understanding of spin relaxation processes in pNMR is to combine quantum chemistry and molecular dynamics. This avenue has previously been pursued for example for aqueous Ni(II) ion [3,4]. One of the issues which need to be settled before moving further along this line is the selection of appropriate quantum chemical methods. Recent data [5] for Ni(II) complexes will be presented and discussed.

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PS 158

APPLICATION OF ROTATION-SYNCHRONIZED DANTE TO PARAMAGNETIC SOLIDS UNDER MAS

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A large number of spinning sidebands appearing in magic-angle spinning (MAS) NMR spectra of paramagnetic solids, such as Li_2MnO_3 and $LiFePO_4$ used for the positive electrode in a lithium ion battery, can be used to obtain structural information through evaluation of an electron–nuclear dipolar interaction. In this study, we examine applicability of rotation-synchronized Delays Alternating with Nutation for Tailored Excitation (rs-DANTE) [1] to a crowded sideband spectrum spreading over a few 100 kHz by the paramagnetic interaction [2]. It is shown that rs-DANTE can be used to excite ^{6}Li spinning sideband manifolds of the three crystallographic Li sites in a MAS spectrum of ^{6}Li-enriched Li_2MnO_3. Each sideband pattern can be described by the paramagnetic anisotropies evaluated by taking the electron–^{6}Li dipolar interactions into account. We show that since the distance range of the electron-nuclear dipolar interaction is much larger than that of short-range interactions that determine the isotropic shift, the calculated sideband patterns is less sensitive to local structural variation. This is useful for grouping the signals at the same crystallographic site but shifted by the local interaction. The nuclei at different crystallographic sites generally bear different sideband patterns. Hence by evaluating the paramagnetic anisotropy from atomic coordinates, it is possible to assign the signal and determine its isotropic shift. It is therefore possible by this approach to obtain both isotropic and anisotropic shift information. Further effects of structural disorder in Li_2MnO_3 on the isotropic shift and the sideband pattern are discussed.

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AN OPTIMAL PARAMAGNETIC RELAXATION AGENT FOR NMR SPECTROSCOPY OF INTRINSICALLY DISORDERED PROTEINS

Nur Alia Oktaviani, Young-Ho Lee, Rik P Megens, Djurre H de Jong, Renee Otten, Ruud M Scheek, Takahisa Ikegami, Frans A.A. Mulder

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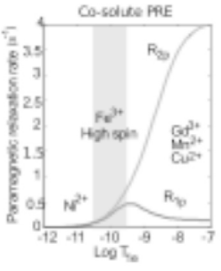
Time and sensitivity gains afforded by paramagnetic co-solutes are of general interest in NMR spectroscopy, and are particularly important for the rapid and sensitive study of aggregation-prone (unfolded) proteins. Previously, Gd3+ and Ni2+ chelates have been applied for this purpose, but these produce line broadening or are only mildly effective. Herein we review the characteristics that the optimal relaxation agent for (protein) NMR spectroscopy should possess, and, based on theoretical considerations, identify a promising neutral high-spin iron(III) complex. This chelate produces the most effective co-solute PRE reported to date, without producing concomitant line broadening. As a consequence, we are able to reduce NMR recording times for intrinsically disordered proteins five-fold. The compound is also suited for proton-less NMR spectroscopy.



PS 160

UNCONVENTIONAL EPR INVESTIGATIONS OF MOLECULAR NANOMAGNETS

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We have used a number of unconventional methods to record electron paramagnetic resonance spectra of molecular nanomagnets. These systems have been proposed for applications in data storage, quantum information processing and magnetic cooling. From a fundamental point of view their main interest lies in the fact that they are complex, yet well-defined mesoscopic systems. This allows an in-depth and microscopic understanding of their electronic and quantum mechanical properties.
We have studied crystal field excitations in lanthanide-based single-ion magnets. These molecules currently receive an extraordinary amount of attention, because they show slow spin dynamics, with very high apparent energy barriers towards relaxation of the magnetic moment. We elucidate the electronic structure and the spin relaxation mechanism.
We have also investigated alternative detection methods for EPR, where we have measured the magnetic torque under microwave irradiation. This allows the study of small samples of a size that is currently very challenging in non-resonant high-frequency EPR.
Finally, we have observed spin-forbidden transitions in the well-known V_{15} nanomagnet. Through comprehensive theoretical analysis, we have identified the mechanisms allowing these transitions.

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AMYLOID AGGREGATES AND LARGE SOLUBLE PROTEIN COMPLEXES

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Perdeuteration and back-substitution of exchangeable protons in microcrystalline proteins in combination with recrystallization from D2O containing buffers reduces 1H, 1H dipolar interactions such that amide proton line widths on the order of 20 Hz are obtained (Chevelkov et al., 2006). Aliphatic protons are either accessible via specifically protonated precursors or by using low amounts of H2O in the bacterial growth medium (Asami et al., 2010). This labeling scheme is applied to amyloid aggregates like fibrils formed by the Alzheimer's disease ß-amyloid peptide (Aß) (Linser et al., 2011). We present data on solid-state NMR studies of drug induced Aβ aggregates focussing in particular on the interactions between Aβ and the polyphenolic green tea compound epigallocatechin-gallate (EGCG). We show that MAS solid-state NMR techniques are applicable for the structural characterization of large soluble protein complexes (Mainz et al., 2009), in case the tumbling correlation time exceeds the rotor period. Experimental results are presented for the small heat shock protein αB crystallin (600 kDa) as well as for the 20S proteasome core particle in complex with its 11S activator (1.1 MDa).

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PS 162

STRUCTURE AND DYNAMICS OF SOLID-STATE BIOMOLECULES: NOVEL METHODS TO PROBE SHORT AND LONG RANGE ^{13}C-^{13}C PROXIMITIES (UP TO 100 PM) AND TO MEASURE ^{1}H-^{15}N AND ^{1}H-^{13}C DIPOLAR COUPLINGS

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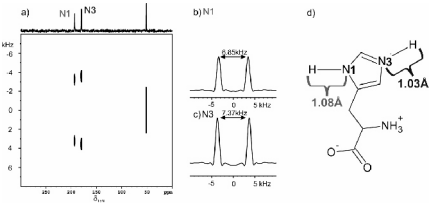
We introduce three novel solid-state NMR methods useful for the study of bio-molecules:
1) SHA+ and CORD are ^{1}H-driven ^{13}C-^{13}C recoupling sequences. They are much more efficient than all previously published methods. They can be applied either in a broad-band way,[1,2] or as frequency-selective method to observe long-distance contacts, up to ca. 100 pm, between two ^{13}C sites.[3]
2) CP-VC (CP with Variable Contact-time) is very efficient at ultra-fast MAS to measure accurately the C-H and N-H distances, and to analyze the dynamics of bio-molecules.[4] This 2D experiment can even be performed in ^{13}C or ^{15}N natural abundance. The method presents a large scaling factor (0.71) allowing a more accurate determination of dipolar coupling, especially for CH_3 or NH_3 moieties. This experiment allows distinguishing small difference in hydrogen bond lengths.



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PARALLEL SESSION LECTURES

PS 163

MULTI-PHASE NMR – A VALUABLE TOOL FOR STRUCTURAL ANALYSIS OF POLYSACCHARIDES

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NMR spectroscopy is one of the few analytical tools that allows for analysis of samples in the liquid, the gel and the solid phase, but NMR can actually be used these phases simultaneously. This asset is highly relevant for detailed structural analysis of polysaccharides as their interactions with water – or water and enzymes. Such suspensions are real and complex multiphase systems and they are relevant in both food and biofuel production. In this context the food hydrocolloids comprise eg. starch, pectin and dietary fibers, whereas cellulose and plant stems are relevant in relation to bioethanol.

Presently the main focus is native and enzymatically treated starches with emphasis on the impact of enzymatic treatment on the water compatibility.

We demonstrate that by combining ^{13}C single-pulse (SP) MAS and cross-polarization (CP) MAS experiments the total sample as well as the immobile part of the sample can be fully characterized during hydration. Previously a similar approach has been used for characterization of pectins and modified celluloses [1,2]. For potato starch a similar approach is used to analyse the phosphorous sites by ^{31}P NMR. Assignments are supported by 2D high-resolution (HR) MAS [3] and liquid-state NMR experiments on very dilute samples in either the gel or liquid state.

Using this approach it was demonstrated that

- the distribution of C1 chemical shifts were sensitive towards enzymatic treatment
- the main chain and the branch points in starch exhibit different hydration properties
- pH-insensitive phosphorous sites were present in potato starch
- torsion angles of the glycosidic linkages in gelatinized starches were averages of the one observed in crystalline A- and B-type structures

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PS 164

SITE-SPECIFIC PROTEIN DYNAMICS IN THE SOLID STATE PROBED BY ^{13}C AND ^{15}N RELAXATION MEASUREMENTS EMPLOYING MAGIC ANGLE SPINNING FREQUENCIES UP TO 100 KHZ

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Slow nanosecond-millisecond motions play a crucial role in many biophysical processes, including enzymatic catalysis, ligand binding, signalling and protein folding. Recent progress in the fast magic angle spinning technology enabled solid-state NMR relaxation measurements for characterizing these notoriously difficult to access motions. Spinning at 60-100 kHz, while suppressing spin diffusion and dipolar dephasing, provides excellent resolution and superior sensitivity through ^{1}H detection with or without perdeuteration. For example, 100 kHz MAS makes practical study of just ~30 nmol protonated protein (see Fig. 1 for a region from a ^{1}H-^{13}C 2D obtained on of fully protonated GB1 at 850 MHz ^{1}H Larmor frequency). New probe technology allows us to introduce site-specific ^{13}C spin-lattice relaxation in the rotating frame (R_ρ) as a probe of picosecond-millisecond protein motions (see Figure 2 for ^{13}C' $R_{1\rho}$ rates obtained at 850 MHz on protein GB1). We use ^{13}C R_1 and R_ρ rates together with ^{15}N R_1 and R_ρ measured in fully-protonated GB1 to investigate time scales, amplitudes and anisotropy of backbone and side chain motions in this protein.

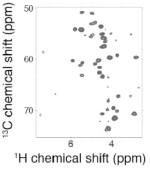

Figure 1

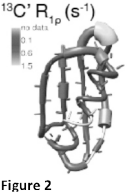

Figure 2

PS 165

STRUCTURE AND DYNAMICS OF MICROTUBULE-ASSOCIATED AND HIV-1 PROTEIN ASSEMBLIES: METHODS AND APPLICATIONS

Si Yan[1], Guangjin Hou[1,3], Yun Han[1,3], Christopher L. Suiter[1,3], In-Ja L. Byeon[2,3], Jinwoo Ahn[2,3], Charles D. Schwieters[4], Shubbir Ahmed[5], John C. Williams[4], Angela M. Gronenborn[2,3], and Tatyana Polenova[1,3]

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[2]Department of Structural Biology, University of Pittsburgh School of Medicine, 1051 Biomedical Science Tower 3, 3501 Fifth Ave., Pittsburgh, PA 15261, United States
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[5]Department of Molecular Medicine, Beckman Research Institute of City of Hope, 1500 East Duarte Road, Duarte, CA 91010, United States

In this talk, I will present recent progress made in our laboratory on the development and application of solid-state NMR spectroscopy for structural and dynamics characterization of: i) HIV-1 assemblies of CA and Gag proteins; and ii) microtubule-associated protein assemblies.
Microtubules (MTs) and their associated proteins (MAPs) play important roles in vesicle and organelle transport, cell motility and cell division. Dynactin multisubunit assembly is the activator of the cytoplasmic microtubule-based dynein retrograde motor complex. CAP-Gly microtubule binding domain of dynactin's p150^{Glued} subunit is critical for the regulation of dynein's motility. Mutations in the CAP-Gly domain are associated with neurological disorders, but the mechanism by which the CAP-Gly domain recognizes microtubules remains largely unknown, particularly at the atomic level. I will present the 3D structure of CAP-Gly determined by MAS NMR and the insights gained into structural and dynamic basis of CAP-Gly's biological function and interaction with its binding partners and microtubules.
HIV-1 capsid proteins (CA) assemble into cone-like structures and enclose the viral RNA genome together with a small complement of proteins during viral maturation. CA exhibits structural polymorphism and can assemble into various morphologies, such as cones, tubes, and spheres. I will present our recent results delineating conformational dynamics in assembled CA by a hybrid MAS NMR/MD simulations approach. To study the above assemblies, we have focused our efforts on establishing MAS-based methodologies that overcome sensitivity and resolution challenges, including experiments for measurements of distance restraints and for recoupling of anisotropic interactions under fast MAS frequencies (40 kHz and above) and nonuniform sampling protocols. An overview of these methods will be presented.

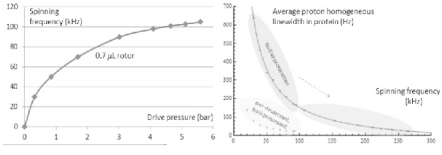
PS 166

SPINNING BEYOND 250 kHz

Andres Oss, Andres Reinhod, Tiit Tuherm, Jaan Past, Tiit Anupõld, Ago Samoson, Vipin Agarwal, Susanne Penzel, Beat Meier

NMR Institute & Tallinn University of Technology, Estonia, ETH Zürich, Switzerland

New rotor bio-VT probe technology[1] allows for sensible sample amounts and meaninful spinning frequencies over several orders of magnitude. Not only “high-resolution” magnets and amplifiers, but also numerous aspects of experiment philosophy can/should be adopted to study of solid or viscous structures as the mechanical spinning approaches effect of thermal tumbling.



We studied homogeneous linewidth of two different proton spin systems, a basic 13C, 15N uniformly labelled ubiquitin (ca 680 structural protons) and per-deuterated and fully backprotonated with ca 140 protons in the same volume. Overlap of the N-H 2D spectra confirmed structural, and with that dynamic-relaxation identity of the two molecular systems, except proton spin dilution. Integral 13C and 15N decoupled protein proton Hahn-echo relaxation data were measured over 10-100 kHz spinning range at a constant 8C, water signal was suppressed by the CP-15N -CP filter. Extrapolation of the dilute proton relaxation data generates effective spinning rates over 250 kHz projected to the dense proton system. The results allow for speculation on scaling properties of the multispin system against sample spinning rate in proteins and more generally, solid state, also induce further progress in MAS technology . [1] *www.nmri.eu*

PARALLEL SESSION LECTURES

PS 167

DEVELOPMENTS OF MULTI-EXTREME HIGH FIELD ESR SYSTEM AND ITS APPLICATION TO HONEYCOMB LATTICE ANTIFERROMAGNETH

Ohta, S. Okubo, T. Sakurai, E. Ohmichi, W. Zhang, T. Shimokawa, T. Ueda, N. Onishi, M. Azuma, Y. Shimakawa

Kobe University, Kobe, Hyogo 657-8501, Japan
Kyoto University, Uji, Kyoto 611-0011, Japan

Multi-extreme high field ESR is a powerful means to study electronic states in condensed matter. Compared to the conventional X-band (9.5 GHz) ESR, it has several advantages, such as the high spectral resolution, the observation beyond the zero field gap or the magnetic phase transition. Our muti-extreme high field ESR can cover the magnetic field region up to 55 T, the frequency region from 0.03 to 7 THz, the temperature region from 1.8 to 300 K, and the pressure region up to 2 GPa [1]. Moreover, we have also developed a micro-cantilever ESR [2] and a SQUID ESR [3]. A micro-cantilever ESR enables the measurement of micro-meter size single crystal and achieved the sensitivity of 10^9 spins/G and the highest frequency up to 0.37 THz. On the other hand, a SQUID ESR enables the absolute intensity measurement through the magnetization measurement using SQUID magnetometer. As an application of our multi-extreme high field ESR, the results of $Bi_3Mn_4O_{12}(NO_3)$ [4], which is considered as a honeycomb lattice antiferromagnet with spin frustration, will be presented. At 1.8 K, resonances, whose frequency-field relation corresponds to antiferromagnetic resonance (AFMR) with an easy-plane type anisotropy and a Dzyaloshinsky-Moriya (DM) interaction, have been observed above the field-induced magnetic order (FIMO) phase transition at 6 T. The D term of the DM interaction is estimated as 1.3 K from the AFMR analysis, and the direction of the D term is suggested to be along the c-axis, which is perpendicular to the honeycomb plane. Possible origin of FIMO phase will be discussed.

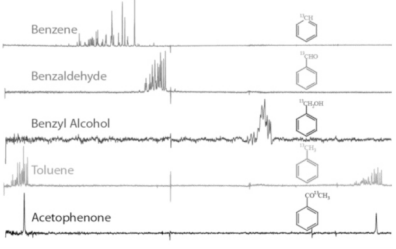
[1] H. Ohta *et al*., J. Low Temp. Phys. **170** (2013) 511.
[2] H. Ohta *et al*. AIP Conf. Proceedings **850** (2006) 1643; E. Ohmichi *et al*., Rev. Sci. Instrum. **79** (2008) 103903; E. Ohmichi *et al*., Rev. Sci. Instrum. **80** (2009) 013904; H. Ohta and E. Ohmichi, Appl. Mag. Res. **37** (2010) 881; E. Ohmichi *et al*., J. Low Temp. Phys. **159** (2010) 276; Y. Tokuda *et al*., J. Phys.: Conf. Series **400** (2012) 032103; E. Ohmichi *et al*., J. Mag. Res. **227** (2013) 9. [3] T. Sakurai *et al*., J. Phys.: Conf. Series **334** (2011) 012058; T. Sakurai *et al*., J. Mag. Res. **223** (2012) 41.
[4] S. Okubo *et al*., Phys. Rev. B **86** (2012) 140401(R).

PS 168

DEVELOPMENTS IN ZERO-FIELD NMR FOR CHEMICAL ANALYSIS

John W. Blanchard,[1,2] Micah P. Ledbetter,[2] Thomas Theis,[1,2] Mark C. Butler,[1,2] Tobias F. Sjolander,[1,2] Vikram S. Bajaj,[1,2] Dmitry Budker,[1,2] and Alexander Pines[1,2]

[1]*Lawrence Berkeley National Laboratory, Berkeley, CA*
[2]*University of California, Berkeley, CA*



Zero-field nuclear magnetic resonance (ZF-NMR) spectroscopy does not require large, immobile, and expensive superconducting magnets. Even at low frequencies, atomic magnetometers sensitively detect NMR signals, in this case arising entirely from electron-mediated scalar couplings (*J*-couplings) in heteronuclear spin systems. These *J*-couplings reveal valuable information about electron density, molecular connectivity, and bond orientation, and can thus be used for fingerprinting and chemical structure determination. We report the acquisition and interpretation of nuclear magnetic resonance *J*-spectra at zero magnetic field for a series of benzene derivatives, demonstrating the analytical capabilities of ZF-NMR.[1] We record linewidths as narrow as 11 mHz, a resolution sufficient for the precise determination of long-range *J*-couplings.

We will also discuss our recent introduction of residual dipolar couplings in ZF-NMR using strain-induced alignment in polymer gels. This technique increases the chemical and orientational information available in ZF-NMR spectra, expanding the potential applicability of ZF-NMR for chemical analysis.

1) J.W. Blanchard, et al., *J. Am. Chem. Soc.*, **2013**, *135*(9), pp 3607-3612

J.W.B., T.T., M.C.B., T.F.J., V.S.B., and A.P. were supported by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. M.P.L. and D.B. were supported by the National Science Foundation under award #CHE-0957655. J.W.B. is also supported by a National Science Foundation Graduate Research Fellowship under Grant No. DGE-1106400.

PARALLEL SESSION LECTURES

PS 169

TOWARDS PARA-WATER IN BULK

Nicola Salvi[1], Daniele Mammoli[1], Jonas Milani[1], Roberto Buratto[1], Akansha Sehgal[2], Aurélien Bornet[1], Takuya F. Segawa[1], Sami Jannin[1], Philippe Pelupessy[2] and Geoffrey Bodenhausen[1,2]

1 Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne, Batochime, 1015 Lausanne, Switzerland.
2 Département de Chimie, Ecole Normale Supérieure, 24 Rue Lhomond, 75231, Paris Cedex 05, France.

Para-water is an analogue of *para*-hydrogen, where the two proton spins are in a singlet state that is antisymmetric under permutation. Like *para*-hydrogen, *para*-water should have a very long lifetime, if exchange can be slowed down. If the high-temperature approximation is fulfilled, ordinary water contains 25% *para*- and 75% *ortho*-water. The two forms can be separated in molecular beams, and *para*-water can be prepared by inducing suitable transitions if it is trapped in C60 cages.
We shall outline methods that should allow one to prepare *para*-water in bulk by adapting procedures for dissolution dynamic nuclear polarization. Frozen dilute solutions of H_2O in DMSO-d6 contain isolated H_2pairs. The symmetry of the two protons can be lifted either by intermolecular dipole-dipole interactions or by the anisotropy of their chemical shifts. Using DNP at 6.7 T and 1.2 K, one can achieve a proton spin temperature in the vicinity of 30 mK, where the high-temperature approximation is violated, so that the ground state that belongs to the triplet manifold is strongly populated, while the singlet state is partly depleted. After rapid dissolution with an aprotic solvent, it should be possible to obtain liquid water in a long-lived state (actually an excess of *ortho*- over *para*-water), provided it remains diluted in an aprotic solvent.
We have studied the exchange rate and hence the lifetime of HDO diluted in aprotic solvents. We have proved that a suitable choice of the solvent allows one to slow down the exchange process by several orders of magnitude, so that sufficiently isolated water molecules can exist as a molecular entity in solution for several seconds. We have also studied the exchange rate as a function of pH by combining Meiboom's oxygen-17 experiments in natural abundance with proton decoupling. The excess of *ortho*- over *para*-water, which is the hallmark of long-lived water, can be revealed by performing chemical reactions, in the style of the ALTADENA and PASADENA experiments. We have carried out kinetic studies to test addition reactions adequate to 'reveal' the existence of long-lived water.

PS 170

A PORTABLE PERMANENT-MAGNET ANALYZER FOR HIGH-RESOLUTION ^{1}H MAS NMR SPECTROSCOPY

Dimitrios SAKELLARIOU

Commissariat à l'Energie Atomique et aux Energies Alternatives

NMR is nowadays an indispensable tool for chemists and biochemists. The applications cover areas such as organic synthesis, nano-materials, proteins and bio-molecular complexes, surfaces and porous media. MRI has also impacted medicine providing anatomical and functional information. There are however many other areas, where Magnetic Resonance is absent mainly due to the constraints of the technique, mainly because of the instrumentation.

We will present recent advances in magnet design that allow us to build miniaturized compact and portable NMR and MRI systems using permanent magnets. The recent development of NMR using low-cost permanent magnets has so far been limited to low resolution relaxation studies or high resolution on liquid samples. We introduce here a high-resolution, low-field Magic Angle Spinning (MAS) NMR instrumentation based on low-cost a ~0.9 T portable permanent magnet and a 7mm MAS probe for liquids and solids. We show dramatic enhancement from susceptibility and field inhomogeneity averaging and how this can be used for studying complex chemical structures such as the lipidic composition of seeds and rocks without any sample preparation.

These developments open the door to low-cost industrial applications since dedicated instruments can now be designed and offer the best information to price ratio. Furthermore, compact magnets can be easily spun rapidly offering novel possibilities for spin dynamics manipulations in cases where the sample, object or subject cannot be rotated at the magic angle. Some ideas and preliminary Magic Angle Field Spinning magnet designs will also be presented and discussed.

PARALLEL SESSION LECTURES

PS 171

HYDROGEN DYNAMICS IN ZR- AND HF-BASED ALLOYS STUDIED BY ^{2}D NMR

Hae Jin Kim[1], Anton Gradišek[1,2], Andraž Kocjan[2], Sang Moon Lee[1], Janez Dolinšek[2]

1. Korea Basic Science Institute, Daejon 305-333, Korea
2. Josef Stefan Institute, 1000 Ljubljana, Slovenia

Zr-based alloys are known to exhibit favourable hydrogen storage properties, with high hydrogen absorption capacity and moderate hydrogen desorption temperatures. In the last couple of years, Hf-based alloys have also attracted interest due to even lower desorption temperatures, making them attractive materials for practical applications. Here, we present a study of deuteron dynamics in a series of Ti-Hf-Ni and Ti-Zr-Ni alloys with crystalline, quasicrystalline, and amorphous structures. Since deuterium nucleus exhibits a strong quadrupolar but a weak dipolar moment, the ^{2}D NMR spectra and spin-lattice relaxation provides an important insight into the local chemical environments of deuteron nuclei. Deuteron dynamics can be viewed as classical hopping of nuclei between the interstitial sites (an Arrhenius-like process) and can be attributed an activation energy for over-the-barrier hopping. At low temperatures, the relaxation is dominated by the interaction with the conducting electrons in the metal - we can therefore obtain the electron density at the Fermi level.

PS 172

POLYMER COMPOSITE MATERIALS –STRUCTURE, DYNAMICS AND INTERFACES

Ute Böhme, Anastasia Vyalikh, Ulrich Scheler

Leibniz-Institut für Polymerforschung Dresden e.V.

For the enhancement of mechanical, electrical and thermal properties polymer materials are composites with inorganic fillers. The present study focuses on the investigation of the interaction between the polymer and the filler. Steps of modification required to enhance the compatibility of the polar filler with the non-polar polymer are monitored using ^{27}Al solid-state NMR, demonstrating the partial transformation from octahedral to tetrahedral aluminium during the modification and the subsequent reconstitution. ^{1}H T_{1rho} detected with chemical shift resolution shows changes in the local mobility as a result of the modification and incorporation into the polymer separately for the polymer and the filler.
Dedicated solid-state NMR experiments enable the investigation of material at the interface. They are based on either a selection of the magnetization at the interface by a trans-interface transfer of magnetization using cross polarization or by spectral selection of interfacial species. Thin polymer films are studied utilizing the high sensitivity of proton NMR. Spectral resolution obtained by CRAMPS detection in relaxation experiments is crucial for the interpretation of data. Thus solvent signals are separated from polymer signals in swelling experiments. For the comparison between bulk material and thin films T_{1rho}probing kilohertz motion is most effective and robust.
For the in-situ characterization of the polymer dynamics under external mechanical stress a low-field Halbach magnet is placed in a stretching apparatus for stress-strain experiments. NMR relaxation and double quantum intensities as a measure of residual dipolar couplings and thus a measure of dynamic order parameters are determined as a function of the applied external mechanical stress. Significant effects of the filler on the polymer relaxation and the response to mechanical load have been observed. As a result of the mechanical stress the chain mobility is reduced and T_2 becomes shorter. T_2 measured in CPMG provides fastest experiments for time-resolved studies. Under constant extension polymer chains in semicrystalline materials reorganize and relaxation times extend to the values of the nascent material.

PS 173

COMPLEX MAGNETISM OF $MnCO_3$ SMALL HOLLOW NANOSPHERES: FROM AFM/FM TO SPIN GLASS

Magdalena Wencka

Institute of Molecular Physics, Polish Academy of Sciences, ul. Smoluchowskiego 17, PL-60179 Poznan, Poland

Canted antiferromagnetism on a nanodimensional spherical surface geometry was investigated on manganese carbonate $MnCO_3$ small hollow nanospheres of mean diameter 7.0 ± 0.3 nm and shell thickness of 0.7 nm, by performing magnetic measurements (SQUID and EPR) and specific heat study, in comparison to the bulk form of the same material. Contrary to the expectation that small magnetic nanoparticles become superparamagnetic, the phase transition to the canted antiferromagnetic (AFM) state in the $MnCO_3$ hollow nanospheres is preserved and retains, at a qualitative level, all the features of the canted AFM state of the bulk material. At a quantitative level, some significant differences between the hollow nanospheres and the bulk were observed, which can all be explained by the weakened interspin interactions in the hollow nanospheres due to reduced atomic coordination by the neighboring atoms. This makes the canted AFM structure of the hollow nanospheres more soft and fragile with respect to external forces like the magnetic field, as compared to the rigid and robust structure of the bulk material. A Curie-type paramagnetic component in the magnetization coexisting with the FM and AFM components was observed only for hollow nanospheres. This suggests the presence of a fraction of Mn atoms on the nanospheres with so strongly reduced coordination by the neighboring atoms that they behave like isolated paramagnetic centers. Such Mn sites are associated with defects in the $MnCO_3$ structure. The presence of defects is supported by the relatively large width of the XRD lines in the spectrum and by the appearance of hyperfine structure for Mn^{+2} in EPR spectra below 16 K. The nanospheres exhibit lower decomposition temperature (558 K) as compared to the bulk powder (530 K), evidenced from high-temperature EPR and DTA experiments.

J. B. Lee, W. G. Hong, H. J. Kim, Z. Jagličić, S. Jazbec, M. Wencka, A. Jelen, J. Dolinšek: Canted antiferromagnetism on nanodimentional spherical surface geometry: The case of $MnCO_3$ small hollow nanospheres, *Phys. Rev. B* **86** (2012) 224407

PS 174

DYNAMIC CORRELATIONS IN IONIC CONDUCTOR CU_2HGI_4 PROBED BY NMR

Damjan Pelc, Igor Marković, and Miroslav Požek

University of Zagreb, Faculty of Science, Department of Physics, Zagreb, Croatia

Solid state ionic conductivity is of immense technical importance, mainly because of battery technology. Yet it is not always clear what makes a substance undergo a transition from insulator to ionic conductor, and how the mobile ions manage to travel around the lattice relatively undisturbed. Here we discuss the ionic transport in Cu_2HgI_4, a well known fast ion conductor that undergoes an insulator-conductor transition at 344 K. NMR measurements provide ion-selective insight into dynamics, and we show [1] that conduction is, surprisingly, mediated by the heavy Hg^{2+} ions, rather than much lighter and smaller Cu^{+}. Although disordering of the Cu sublattice occurs at the transition, copper ions seem to remain static, while mercury motion is evident from a strong narrowing of the ^{199}Hg NMR line. However, conductivity calculated from NMR linewidths shows a systematic discrepancy from DC conductivity, so frequency-dependent nonlinear conductivity measurements were used as a probe for dynamical heterogeneity. They reveal an 'intermediate' characteristic correlation timescale t_{corr} ~1 ms. The existence of dynamical correlations is confirmed by stimulated echo NMR measurements [2,3], where the fraction of ions trapped after t_{corr} can be quantitatively determined.

To explain the observations, we propose a simple model requiring the existence of two kinds of particles, slow and fast [4]. A simulation based on the model shows islands of trapped fast particles forming and dissolving in time, with a characteristic timescale similar to the one observed experimentally.

The observed dynamical cooperativity can be compared with recent studies of arrested and glassy materials [5,6], thus opening an unexpected connection between seemingly unrelated fields of physics.

[1] D. Pelc, I. Marković, M. Požek, *Phys. Rev. Lett.* **109**, 095902 (2012) - [2] E. L. Hahn, *Phys. Rev.* **80**, 580 (1950);
[3] A. Heuer et al., *Phys. Rev. Lett.* **75**, 2851 (1995) - [4] D. Čapeta and D. K. Sunko, *Phys. Rev. B* **74**, 220201(R) (2006).
[5] C. Crauste-Thibierge et al., *Phys. Rev. Lett.* **104**, 165703 (2010) - [6] A. Duri and L. Cipelletti, *Europhys. Lett.* **76**, 972 (2006).

PS 175

NMR AS A TOOL FOR CHECKING THE STRUCTURAL MODELS OF COMPLEX METALLIC ALLOYS AND QUASICRYSTALS

M. Klanjšek, P. Jeglič, M. Bobnar, S. Vrtnik, S. Jazbec, J. Dolinšek

Jožef Stefan Institute, Ljubljana, Slovenia
EN-FIST Centre of Excellence, Ljubljana, Slovenia

In terms of structural complexity, intermetallic systems range from simple cubic metals like PdGa [1] to complex metallic alloys (CMAs) with large unit cells, the ultimate limit being non-periodic quasicrystals with infinitely large unit cells [2,3]. In CMAs, the atoms are locally arranged in well-defined clusters of polyhedral order, while their large unit cells provide translational periodicity of the lattice. This results in two competing length scales, one defined by the unit-cell parameters and the other by the cluster substructure, which affects the physical properties of CMAs. Because of their large unit cells, the structural determination of CMAs is a formidable task and the structural models of long-known CMAs are still being refined. While the diffraction methods provide an indispendible tool for structural determination, nuclear magnetic resonance (NMR) offers complementary structural information on the atomic scale. In particular, as the NMR-active nuclei with nonzero quadrupole moment are coupled to the local electric field gradient, which crucially depends on the local atomic arrangement, the resulting NMR spectra carry a structural information. This information is most readily obtained from the rotation patterns of the NMR spectra in single-crystalline samples, allowing a direct access to the number of crystallographically inequivalent lattice sites and to the local symmetry of the crystalline lattice. We will demonstrate this procedure on a number of intermetallic systems of increasing complexity: (i) a simple cubic metal PdGa with 8 atoms in a unit cell [1] and its orthorhombic cousin Pd_2Ga, (ii) a cubic V-$Al_5Cu_6Mg_2$ of intermediate structural complexity, with 39 atoms in a unit cell [4], (iii) a family of CMAs comprising $Al_{13}Co_4$ and its derivatives, with 102 atoms in a unit cell [5], (iv) icosahedral Al-Pd-Mn and Al-Cu-Fe quasicrystals [2], and (v) a decagonal Al-Co-Ni quasicrystal [3].

[1] M. Klanjšek et al., J. Phys.: Condens. Matter **24**, 085703 (2012). - [2] M. Klanjšek and J. Dolinšek, Phil. Mag. **86**, 413 (2006).
[3] M. Bobnar et al., Phys. Rev. B **85**, 024205 (2012). - [4] M. Klanjšek et al., Intermetallics **39**, 50 (2013).

PS 176

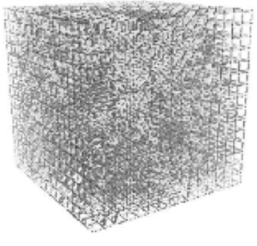
NMR DISTANCE MEASUREMENTS, ALONG WITH MOLECULAR SIMULATIONS, ACT AS A BLUNT INSTRUMENT FOR ASCERTAINING LINKER DISTRIBUTIONS IN METAL-ORGANIC FRAMEWORKS

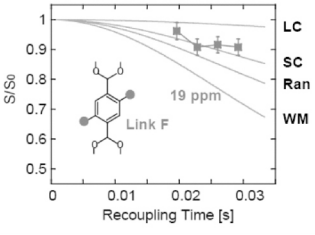
Jeffrey A. Reimer

University of California at Berkeley - USA

Solid-state NMR distance measurements, in conjunction with molecular simulations, are used to uncover the heterogeneous mesoscale spatial apportionment of functional groups in a series of multivariate metal-organic frameworks (MTV-MOF-5). Our analysis reveals that these methods easily discern between random, well mixed, and various cluster forms of functional group apportionments. Characterization of gas sorption properties of these mesoscale arrangements shows that this apportionment matters.

Xueqian Kong, Hexiang Deng, Fangyong Yan, Jihan Kim, Joseph A. Swisher, Berend Smit, and Omar M. Yaghi are co-authors of this work that is supported by the Center for Gas Separations Relevant to Clean Energy Technologies, an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under award DE-SC0001015.





PS 177

EFFICIENT CO-CA TRANSFER IN DEUTERATED AND PROTONATED PROTEINS BY BAND-SELECTIVE HOMONUCLEAR CROSS-POLARIZATION.

Veniamin Shevelkov, Chaowei Shi, Karin Giller, Stefan Becker, Adam Lange

Max Planck Institute for Biophysical Chemistry, Göttingen, Germany

We will present a method for robust and efficient band-selective magnetization transfer between CO and CA spins in solid state MAS NMR by dipolar-based homonuclear cross polarization (1). The approach is designed for moderate magic-angle spinning rates (ca. 20 kHz) and high external magnetic fields (ca. 800 MHz) where the isotropic chemical shift difference of CO and CA considerably exceeds the spinning rate. The most efficient recoupling is achieved when the sum of effective radio-frequency fields on CO and CA resonances equals two times the spinning rate and yields up to 30% and 50% of magnetization transfer for protonated and deuterated proteins, respectively. This method has been implemented for COCA correlation spectroscopy in deuterated PrgI needles (2). More recently we have adapted the approach for N[CO]CA and CA(i)[NCO]CA(i-1) 2D interresidual correlation experiments for resonance assignment of protonated ubiquitin.

(1) Shevelkov et.al., JMR, 2013

(3) Loquet et.al., Nature, 2012

PS 178

SENSITIVITY ENHANCEMENT IN SOLID-STATE NMR AT VERY FAST MAGIC-ANGLE SAMPLE SPINNING BY RFDR MIXING

Yue Qi Ye, Charlotte Martineau, Francis Taulelle, Yusuke Nishiyama

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Univ. Versailles, Versailles Cedex, France

Sensitivity enhancement in solid-state NMR at very fast magic-angle sample spinning (MAS) is demonstrated. ^{1}H nuclei at moderate MAS or static conditions shows uniform T_1 relaxation time due to rapid ^{1}H-^{1}H spin diffusion. Thus the efficient repetition time, which is 1.3 times longer than T_1, is uniform for all the ^{1}H nuclei. On the other hand, the very fast MAS, which now reaches 110 kHz [1], decrease the ^{1}H-^{1}H spin diffusion rate due to the partial suppression of ^{1}H-^{1}H dipolar interactions. This results in different ^{1}H T_1 relaxation times for each ^{1}H nuclei. Although some ^{1}H nuclei reach thermal equilibrium very rapidly, the other might take much longer; we should wait for recovering the magnetization with longest ^{1}H T_1 relaxation time. Otherwise, the signal intensity with long ^{1}H T_1 will be decreased and even be suppressed. Here we show reintroduction of ^{1}H-^{1}H dipolar interaction at very fast MAS efficiently shortens the optimal repetition time. We have applied RFDR (Radio Frequency driven Dipolar Recoupling) during repetition delay to enhance ^{1}H-^{1}H spin diffusion [2,3]. This allows us to shorter repetition time and improves the signal to noise ratio (S/N) per unit time. We have demonstrated the effect of RFDR during repetition delay on ^{1}H DQMAS, ^{1}H EXSY, and ^{1}H-^{13}C HETCOR. This method also improves sensitivity in ^{13}C CPMAS spectra.

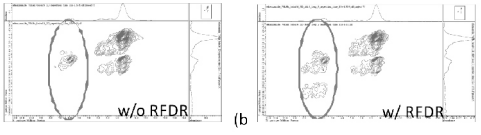

Figure ^{1}H DQMAS spectra of ethenzamide without (a) and with (b) RFDR during repetition delay.

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PS 179

WATERCONTROL: THE NEXT GENERATION SOLVENT SIGNAL SUPPRESSION IN NMR

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Efficient and selective solvent signal suppression is essential for many NMR experiments. In spite of their ease of setup and high efficiency, all the current frequency differentiation based solvent signal suppression techniques not only generate 'null' regions but also unquantifiable suppression process. The newly developed WaterControl technique contains a standard stimulated echo sequence, two selective π pulses for inverting only the resonances (e.g., most resonances of interest) in the inversion bands of the selective π pulses, and a train of pulsed magnetic field gradients for diffusion based attenuation and coherence selection. The new technique affords the collection of the inverted and non-inverted resonances in one scan and therefore a quantifiable and extremely selective solvent signal control/suppression without any loss of the contiguous-to- solvent and under-solvent resonances.

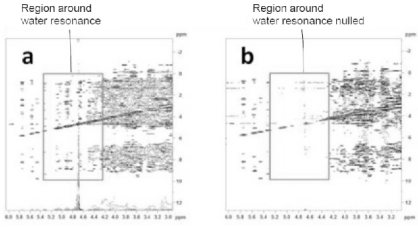

Figure 1. ^{1}H 500 MHz 2D NOESY NMR spectra of a 2 mM aqueous (90% H_2O and 10% D_2O) lysozyme sample using WaterControl (a) and 'W5'-WATERGATE (b) based water signal suppression.

To demonstrate the outstanding performance of the WaterControl technique, 2D water-suppressed NOESY NMR experiments were performed on a 2 mM aqueous lysozyme sample (Figure 1). As shown in Figure 1, there is no 'null' region in the NOESY-WaterControl spectrum (a) while a 1ppm (F2) wide 'null' region is clearly observable in the NOESY-'W5'-WATERGATE spectrum (b). **Reference:** 1. Zheng & Price, Prog Nucl Magn Reson Spectrosc, 56 (2010) 267; 2. Piotto et al., J Biomol NMR, 2 (1992) 661; 3. Liu et al., J Magn Reson, 132 (1998) 125.

PS 180

ALLOSTERIC REGULATION OF THE SARCOPLASMIC RETICULUM Ca^{2+}-ATPASE BY PHOSPHOLAMBAN AND SARCOLIPIN USING SOLID-STATE NMR SPECTROSCOPY

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The membrane protein complexes between the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and phospholamban (PLN) or sarcolipin (SLN) control Ca^{2+} transport in cardiomyocytes, thereby modulating cardiac muscle contractility. Both PLN and SLN are phosphorylated upon b-adrenergic-stimulated phosphorylation and up-regulate the ATPase via an unknown mechanism. Using solid-state NMR spectroscopy, we mapped the interactions between SERCA and both PLN and SLN in membrane bilayers. We found that the allosteric regulation of the ATPase depends on the conformational equilibria of these two endogenous regulators that maintain SERCA's apparent Ca^{2+} affinity within a physiological window. Here, we present new regulatory models for both SLN and PLN that represent a paradigm-shift in our understanding of SERCA function. Our data suggests new strategies for designing innovative therapeutic approaches to enhance cardiac muscle contractility.

PARALLEL SESSION LECTURES

PS 181

IN-CELL NMR SPECTROSCOPY OF LARGER PROTEINS AND G-QUADRUPLEXES

DOETSCH VOLKER[1], Haensel-Hertsch Robert[1], Foldynova-Trantirkova Silvie[2], Trantirek Lukas[2], Loehr Frank[1], Luh Laura[1], Corbeski Ivan[1]

[1] Goethe University, Frankfurt, [2]Masaryk University, Brno

The telomeric G-overhang is the 3' single stranded protrusion of double stranded telomeres and consists of repeating d(TTAGGG)n elements. These elements form G-quadruplexes, which however under different in vitro conditions can adopt several different conformations. In order to investigate which of these conformations is the biologically relevant conformation we have injected quadruplexes of different length into Xenopus oocytes or investigated them in oocytes extracts. These investigations revealed that G4 units coexist in two conformations, the hybrid-2 and the 2-tetrad antiparallel basket.
In addition, we have used in-cell NMR to investigate the behavior of Pin-1, a peptidyl-prolyl isomerase and show that the protein uses its WW domain to nonspecifically investigate other proteins as potential substrates. This non-specific interaction can be blocked by phosphorylation in the WW domain.

PS 182

REAL TIME MONITORING OF IN VIVO MULTI-PHOSPHORYLATION EVENTS IN AN INTRINSICALLY DISORDERED PROTEIN

Miquel Pons, Irene Amata, Mariano Maffei, Ana Igea, Marina Gay, Marta Vilaseca, and Angel R. Nebreda

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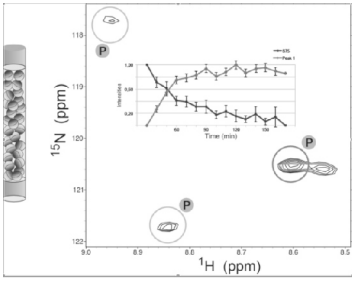
The intrinsically disordered Unique domain of c-Src is part of a newly discovered regulation element of this important oncogene (1). The unique domain acts as a sensor of an intracellular network of kinases and phosphatases and is reversibly phosphorylated at several serine residues. Phosphorylation results in modulation of key interactions of this domain with lipids and other proteins.
The favourable dynamics of intrinsically disordered domains facilitate their observation by NMR in living cells or cell extracts containing the soluble components of the cell cytoplasm. Phosphorylation causes a very large shift of the NH signals in HSQC spectra (2).
Fast acquisition strategies allow the observation of the phosphorylation of several sites in real-time and to measure the individual kinetics. Assignment of the phosphorylation sites was achieved by a combination of NMR, mass-spectrometry and biochemical methods.
Perturbation of the system by kinase inhibitors reveal that the actual phosphorylation pattern observed depends on a complex interplay between kinases and phosphatases, whose activity is itself determined by their phosphorylation state.



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PARALLEL SESSION LECTURES

PS 183

IN-CELL DYNAMICS, INTERACTIONS AND STRUCTURAL FEATURES OF THE HUMAN AMYLOIDOGENIC PROTEIN ALPHA-SYNUCLEIN.

Theillet F.X, Bekei B, Rose HM, Binolfi A, Selenko P

Leibniz Institute of Molecular Pharmacology (FMP Berlin)

Aggregation of intrinsically disordered proteins (IDPs) is a common phenomenon in human amyloid diseases. Intracellular aggregation of alpha-synuclein (aSyn) in dopaminergic neurons of the *substantia nigra* for example, is directly linked to Parkinson's disease. Evidently, the structural properties of aSyn, and cell-type specific changes thereof are important denominators in the pathophysiology of this aggregation process. How exactly the intracellular environment of dopaminergic neurons promotes aSyn aggregation remains unknown.

Here, we present high-resolution in-cell NMR data on aSyn in five different mammalian cell types, also including *substantia nigra* dopaminergic neurons. We have employed novel in-cell NMR techniques (in-cell NMR relaxation and in-cell PRE measurements) to analyze the intracellular behaviour(s) of aSyn. Here, we describe *in vivo* local conformations and dynamics, global compaction, and we map aSyn regions where transient cellular unspecific interactions occur. We have further established an exhaustive set of comparative *in vitro* NMR data in conditions mimicking physical properties of the intracellular space (i.e. macromolecular crowding, viscosity, etc…). These data enable us to dissect physical and biological contributions to the *in vivo* characteristics of aSyn. This sheds new lights on conformational and dynamic properties of IDPs inside cells.

PS 184

XENON XENON BASED HYPER-CEST-MRI OF CRYPTOPHANE LABELED CELLS

Stefan Klippel[1,2], Jörg Döpfert[1], Jabadurai Jayapaul[1], Martin Kunth[1], Federica Rossella[1], Matthias Schnurr[1], Christopher Witte[1], Christian Freund[2], Leif Schröder[1]

[1]*Leibniz-Institut für Molekulare Pharmakologie, ERC Project BiosensorImaging, Berlin, Germany*
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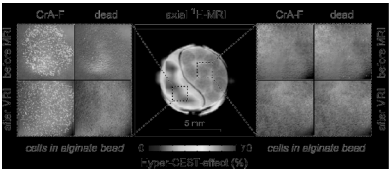
Motivation and Concept

Conventional contrast agents for cell labeling based on changes in proton spin relaxivity often suffer from limited differentiation between background signal and labeled areas. *In vivo* detection of such agents at low concentrations is challenging.

Here we demonstrate the capability of a hyperpolarized (hp) ^{129}Xe contrast agent to act as an unspecific cell tracker while avoiding the background signal problem. The signal of hp-xenon is targeted by trapping xenon atoms in fluorescein-coupled cryptophane cages that are internalized by cells [1]. Furthermore the amount of this biosensor required for detection is dramatically reduced using indirect Hyper-CEST detection schemes [2].



Imaging of a Cell-Internalized, Dual Mode Biosensor

We show that the cellular uptake of a cryptophane-fluorescein conjugate (CrA-F) leads to a clear downfield shift of entrapped xenon compared to free sensor. Hyper-CEST imaging allows the selective detection of the internalized sensor at low micromolar concentrations. To perform extended cell experiments we designed an NMR/MRI compatible packed-bed bioreactor that enables Hyper-CEST-MRI experiments at physiological temperature and high cell densities. The setup opens the way for the optimization of targeted biosensors, multiplexing applications, and of MRI read-out schemes necessary to bridge the challenging gap for translation to *in vivo* studies.

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PARALLEL SESSION LECTURES

PS 185

NON-INVASIVE IN CELL DETERMINATION OF NAD+/NADH RATIOS USING HYPERPOLARIZED GLUCOSE SHOW LARGE VARIATIONS IN METABOLIC PHENOTYPES

Mathilde Lerche[1], Caspar Elo Christensen[1], Magnus Karlsson[1], Pernille Rose Jensen[1], Jakob Rahr Winther[2]

[1]*Albeda Research, Copenhagen, Denmark,*
[2]*Depeartment of Biology, Copenhagen University, Copenhagen, Denmark*

Introduction: Cytosolic free NAD^+/NADH ratio is fundamentally important in maintaining cellular redox homeostasis and it can be generally considered as a cellular metabolic readout. The balance between the oxidized form (NAD^+) and the reduced form (NADH) is critical for the cell's proper function and ultimately, for its survival. Free NAD^+/NADH ratios can be determined from product/substrate ratios of suitable near-equilibrium redox reactions[1]. One such reaction is the conversion of cytosolic pyruvate to lactate governed by the enzyme lactate dehydrogenase (LDH) according to the reaction Pyruvate + NADH + H^+ ↔ Lactate + NAD^+. Owing to the very fast equilibrium obtained with this enzymatic reaction it is possible to use the ratio between pyruvate and lactate to calculate the cytosolic free NAD^+/NADH ratio.

Results: Hyperpolarized [$^{13}C_6$-d_7]glucose is infused to two different types of living cancer cells in suspension where it is metabolized through 10 enzymatic reactions in the glycolysis[2]. The signals from the glycolytic end products hyperpolarized 1-^{13}C pyruvate and 1-^{13}C lactate are followed over 1 minute, Figure 1A. The dynamic data are used as input for a model of the glycolysis. The model allows calculation of rate constants and concentrations from which the cytosolic free NAD^+/NADH ratio can be extracted using the K_{eq}=1.1*10^{-11}, Figure 1B.

Conclusion: We have developed a robust method for non-invasively determining the cytosolic free NAD^+/NADH ratio in cells using hyperpolarized glucose. The determined cytosolic free NAD^+/NADH ratio is four times higher in the prostate cancer cells than in the breast cancer cells. A difference that correlate to the distinct glycolytic phenotypes of these two types of cancer cells.

Williamson et al. (1967) Biochem. J. 103: 514-526.
Meier, S. et al. (2011) Mol. Biosyst. 7:2834-2836.

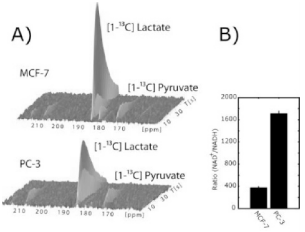

Figure 1. A) Hyperpolarized [$^{13}C_6$-d_7]glucose metabolism in MCF-7 and PC-3 cells. B) NAD^+/NADH ratios obtained from fitted [1-^{13}C]lactate and [1-^{13}C]pyruvate concentrations in the two cell types.

PS 186

WATER, LIGHT, AND … EPR SPECTROSCOPY

Vasili Petrouleas

NCSR "Demokritos"

Water is the most abundant/most unusual, substance of the earth's crust. Endowed with unique properties, it is vital for biological reactions inside living organisms, but also hosts life in the oceans rivers and lakes, it is a universal solvent carrying nutrients up to the top of the tallest trees, it is a source of atmospheric oxygen, ... Many of the unique properties of water are due to its ability to form hydrogen bonds.

Sunlight is the result of nuclear-fusion at the core of sun. The high-energy γ-rays created loose most of their energy during their trip to the surface, and end up in the visible region of the spectrum, just right for the needs of our planet. Sun's irradiation illuminates, warms up the planet, drives the recycling of matter, drives Photosynthesis...

These two unlike substances, water and light, meet at the chloroplasts, to perform a ubiquitous reaction: the splitting (oxidation) of water and evolution of oxygen:

$2H_2O + 4\ photons \rightarrow O_2 + 4H^+ + 4e^-$

This deceptively simple reaction requires the cooperation of light-absorbing pigments with redox cofactors in a membrane-bound protein-complex called water-plastoquinone oxidoreductase, or simply Photosystem II (PSII). Light activates electron transfer from water to plastoquinone via a central light-sensitive chlorophyll assembly, P_{680}, and a sequence of redox cofactors. Water oxidation reactions are catalyzed by a Mn_4O_5Ca complex undergoing four sequential oxidative transitions. EPR spectroscopy has had invaluable contributions to the identification and the understanding of the role of the various cofactors. Without its help the light reactions of Photosynthesis would probably be still in the … dark. The presentation will introduce the basics of photosynthesis, and of PSII, outline some of the EPR contributions in a non-systematic way, and will be concluded by brief reference to the recent advances in the 3-d mapping of PSII and the progress to understand the mechanism of water oxidation.

PS 187

NEW DEVELOPMENTS IN HIGH SENSITIVITY PULSE DIPOLAR ESR AND PROTEIN STRUCTURE DETERMINATION

Jack H. Freed

ACERT - National Biomedical Center for Advanced ESR Technology, Department of Chemistry and Chemical Biology,Cornell University, Ithaca, New York, USA

The importance of and requirements for high-sensitivity Pulse Dipolar ESR Spectroscopy (PDS) in measurements of distances in the 1 to 10 nm. range will be discussed. As projects in the determination of complex proteins structures become more challenging, increasing the sensitivity and accuracy is required. The relative virtues of double electron-electron resonance (DEER) and Double Quantum Coherence (DQC)-ESR in meeting these objectives is compared. It is shown how micromolar sensitivity available at ACERT is valuable both for dilute solutions and for accurate data analysis. The need for longer dipolar evolution times to measure longer distances in protein complexes is effectively addressed by the new technique of 5-pulse DEER, which can almost double such times as well as generally enhance the SNR.(1) The application of such developments to protein structure determination is demonstrated for the cases of an integral membrane protein complex and for locating how a substrate penetrates into the active site of an enzyme. In the former, the sodium and aspartate symporter from Pyrococcus hokoshii, GLtPh which forms a trimer complex, has each protomer transporter function by alternating between outward-facing and inward-facing states in the membrane. We show that the conformational ensemble of protomers samples both states with nearly equal probabilities, indicative of comparable energies, and independently of each other both in liposomes and detergent.(2) In the case of substrate binding, we located (to $2\sigma < 2$Å accuracy) how a lysolecithin spin-labeled on the polar-end, interacts with soybean seed lipoxygenase-1.(3) The general approach we developed could be used to locate other flexible molecules in macromolecular complexes.

ACKNOWLEDGEMENTS: Co-workers: Peter Borbat, Elka Georgieva, Betty Gaffney, and Olga Boudker. Support from NIH/NIGMS Grant P41GM103521 and NIH/NIBIB Grant R01EB003150.

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PS 188

EXTRACTION OF GEOMETRICAL PARAMETERS FROM EPR/PELDOR DATA AND THEIR USE FOR THE VERIFICATION OF MOLECULAR STRUCTURE OBTAINED BY NMR

A. Marko[a], C. Grytz[a], S. Kazemi[a], P. Güntert[a], S.T. Sigurdsson[b] and T.F. Prisner[a]

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[b] *Science Institute, University of Iceland, Dunhaga 3, 107 Reykjavik, Iceland*

PELDOR is frequently used to study the structure and dynamics of complex bio-macromolecule on nanometre scale. Since most of proteins and nucleic acids are diamagnetic, spin labels with flexible linkers are introduced into the bio-molecules in order to perform PELDOR experiments successfully. For such system the algorithm to extract distance distribution between paramagnetic centres from the experimental data have been developed based on the assumption that all possible mutual orientations of spin labels are equally probable. However, in recent years a growing number of PELDOR experiments have been carried out on the systems with the restricted mobility of spin labels.[1] Determination of the ensemble of molecular structure that fit experimental PELDOR data acquired at multiple mw-frequencies and magnetic fields has proven to be an non trivial task, especially, when no information about molecule under study is available.[2] To solve this problem we have simulated time traces data base for biradicals with all relative nitroxide orientations and inter-spin distances in the experimentally accessible range.[3] Further, a fitting algorithm searched for an optimal combination of presimulated PELDOR time traces that reconstructs experimental data. In this way a set of inter-spin distances and relative nitroxide orientations attached to a bio molecule has been found. Alternatively the relative orientations of the rigid spin labels and corresponding distances can be obtained from the analysis of NMR data. The comparison of both results offers a possibility to verify long range molecular configuration predicted by NMR methods that employ short range constraints. To demonstrate this, the structure of DNA molecule with two double-stranded stems and a bulge in the centre have been calculated by CYANA program. The analysis of the obtained result has shown a significant mismatch between the orientations of rigidly attached C spin labels predicted by NMR and PELDOR. In order to avoid this discrepancy new restraints are incorporated into the calculation of the NMR structure of the studied molecule.

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PS 189

CRYOGENIC RECEIVER FOR LOW TEMPERATURE ESR MEASUREMENTS

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TU Dortmund University

Low temperature ESR measurements profit from the increase of the Boltzmann factor to generate larger signals than room-temperature measurements. The lower sample temperature can also lead to saturation, and therefore reduced signal. Independent of the sample, it is possible to increase the signal to noise ratio by cooling not only the sample, but also the relevant components of the detector and thereby reduce the thermal noise that limits the sensitivity. This approach has been used successfully in “cold probes” for NMR, but very little in ESR. Here, we demonstrate the power of this approach. Our setup combines a cryogenic preamplifier with a magnetically shielded circulator working at cryogenic temperatures. A compact receiver design fitting into the variable temperature insert of the cryostat (Qxford Spectromag) greatly reduces additional noise from the passive components of the receiver between the resonator and the cryogenic preamplifier. Our receiver reaches at the physical temperature of 20K an input noise density level of 0.4 $nV/Hz^{1/2}$. This exceeds the thermal noise density at this temperature by a factor of 1.7. At low microwave power, the input noise density closely follows the temperature dependence of a cooled 50 W resistor over the whole measurement range from 20K up to room temperature.
To minimize the influence of the microwave source noise, we use in our setup a low quality factor (~40) but high microwave efficiency (~1.6$mT/W^{1/2}$) planar microresonator. Its very efficient conversion of the microwave power into magnetic field permits experiments with very low power levels, typically ranging from a few µW down to fractions of nW. This reduces the influence of noise from the microwave source.
Apart from quantitative measurements of signal and noise as a function of temperature with a standard DPPH test sample, we checked the performance of our receiver by measuring spectra of a ~35 nl $Cu:Ni(dtc)_2$ sample and a NiFe hydrogenase sample at several temperatures from 190 K down to 12 K. The obtained spectra had SNR ~1300 for the $Cu:Ni(dtc)_2$ sample and ~300 for the NiFe hydrogenase. These results demonstrate the suitability of our cryogenic receiver, combined with the planar microresonator, for EPR measurements on small samples at low temperatures.

PS 190

ACCURATE EXTRACTION OF MULTIPLE PULSE EPR DISTANCES IN HOMO-OLIGOMERIC SYSTEMS

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Nanometre distance measurements by pulsed electron−electron double resonance (PELDOR)[1,2] spectroscopy have become an increasingly important tool in structural biology. The theory of the experiment is well-defined for systems containing two nitroxide spin-labels (spin pairs) and we will present examples showing how dimer formation can be efficiently monitored by PELDOR. However, recently experiments have been reported on homo-oligomeric membrane proteins consisting of up to eight spin-labelled monomers[3]. It has been demonstrated that the presence of more than two spins leads to artefacts in data analysis[4], which can be efficiently suppressed for up to four or five spins by power-scaling the experimental data[5].
In this contribution we will revisit PELDOR experiments on the heptameric MscS[6] and the octameric Wza[3] proteins and evaluate the precision of distance information derived by (i) neglecting multi-spin effects, by suppressing them via (ii) power-scaling[5] or(iii) the experimental reduction of the pump pulse excitation bandwidth or (iv) by model-based forward calculation of the time domain data retaining multi-spin effects[7].

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PS 191

CO-TRANSLATIONAL PROTEIN FOLDING ON THE RIBOSOME: USING NMR SPECTROSCOPY TO PROVIDE STRUCTURE AND DYNAMICS OF RIBOSOME-NASCENT CHAINS

John Christodoulou

Institute of Structural and Molecular Biology, University College London, London, United Kingdom

The folding processes of nascent chains are intricately linked to their chain elongation, which occurs in a vectorial manner as the N-terminal part of the nascent chain emerges from the ribosome [1]. The use of NMR spectroscopy on ribosomes and ribosome nascent-chain complexes (RNCs) is providing detailed structural insights of the conformations of protein chains while they are being created on the ribosome. By producing *in-vivo* derived RNCs in which the nascent polypeptide is selectively labelled, our recent work has allowed us to use NMR follow, at a residue-specific level, the co-translational folding processes of proteins Recent strides towards a detailed understanding of the relationship between biosynthesis and folding will be discussed.

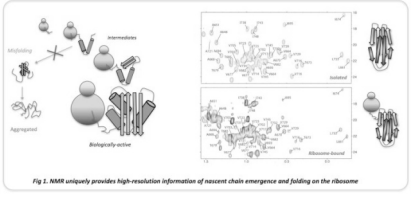

Fig 1. NMR uniquely provides high-resolution information of nascent chain emergence and folding on the ribosome

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PS 192

THE HETEROGENEOUS STRUCTURE OF E7 FROM HPV16 REVEALED BY NMR SPECTROSCOPY

Eduardo Calçada, Isabella C. Felli, Tomáš Hošek and Roberta Pierattelli

CERM and Department of Chemistry "Ugo Schiff", University of Florence, Italy

The importance of local flexibility in determining the function of proteins has been recognized long ago and also widely scrutinized. If the extent of local flexibility is taken to its extreme conditions it leads to completely random coil behaviour of a polypeptide chain, indicated *as intrinsic disorder*, through a wide variety of intermediate cases both in terms of extent of mobility or in terms of protein stretches involved. Intrinsically disordered proteins (IDPs) possess peculiar properties with respect to well folded ones. Many examples of IDPs appeared in the literature showing how their structural plasticity and intrinsic flexibility can be key features to enable them to interact with a variety of different partners and to adapt to different conditions. These properties provide functional advantages to IDPs enabling them to play key roles in many regulatory processes and their function has also been related to several diseases. IDPs seems to be extensively used by viruses to infect healthy cells since, in virtue of their small genomes able to code only a limited number of proteins, they need economic ways to interfere with the host. This is the case of human papilloma virus (HPV). The E7 protein from HPV plays a key role in the viral infection and it is a target of high biomedical interest. To date no high resolution information is available on the full protein but other biophysical techniques established the presence in the protein of a largely unstructured region as well as of a folded one. We present here the NMR characterization of the entire E7 from HPV16, one of the most dangerous variants of the virus. The protein results very heterogeneous in terms of structural and dynamic properties with a highly flexible N-terminal module and a more structured, yet not well-behaving, C-terminal part. This opens the way to study at the molecular level interactions and post-translational modifications of the protein to unravel functional details that may be linked to its highly oncogenic potential.

PS 193

FAST CONFORMATIONAL EXCHANGE GOVERNS ELECTRON FLUX EFFICIENCY THROUGH A MULTIDOMAIN DIFLAVIN REDUCTASE

Frances Oriane[1], Fatemi Fataneh[1], Pompon Denis[2,3,4], Guittet Eric[1], Sizun Christina[1], Perez Javier[5], Truan Gilles[2,3,4], Lescop Ewen[1]

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The NADPH cytochrome P450 reductase (CPR) is a multi-domain protein composed of the FAD- and FMN-binding domains covalently bound by a short flexible linker. In the CPR, FAD receives electrons from NADPH and transmits them to FMN prior to the final transfer to acceptors. The requirement for the shuttling of the FMN-domain between the FAD-domain and the final acceptor during the catalytic cycle was soon accepted after the unveiling of the crystal structure of the CPR. Yet there is only limited evidence for such large-scale domain motions. Our previous NMR study on this 70kDa protein demonstrated that the closed conformation observed in the crystal structure is predominant in solution. However, we now show using a combination of NMR ^{15}N relaxation and chemical shifts analysis and SAXS techniques that the CPR exists as a rapid equilibrium between the closed state (L) and a new highly flexible state (U) and that the relative populations p_U/p_L of the two states can be controlled at will. We also show that the CPR activity measured the on cytochrome c reduction rate is directly correlated to the $p_U p_L$ product quantity. We rationalize the observations by providing a mechanism of enzyme catalysis coupling the elementary chemical steps, including electron transfers, and the rapid conformational equilibrium between two competent states. To conclude, the sampling of mutually exclusive competent conformations at rates faster than chemical reactions may represent an alternative to substrate-induced conformational change to resolve conflicting structural requirements during enzymatic catalytic cycle for optimal turnover.

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DETAILED CHARACTERIZATION OF THE MEMBRANE-INTERACTION OF THE TOR FATC DOMAIN BY NMR, ORIENTED CD SPECTROSCOPY AND MD SIMULATIONS

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The target of rapamycin (mTOR) is a conserved 280 kDa serine/threonine protein kinase involved in the control of cell growth in response to a variety of factors such as nutrients, growth factors, the cellular energy state of the cells and stress (osmotic stress, hypoxia). The C-terminal FATC domain occurs in all PIKKs in combination with the FAT domain and was shown to be indispensable for TOR function. Based on NMR-monitored binding studies, the FATC domain can interact with different membrane-mimetics, such as differently composed neutral and negatively charged micelles as well as neutral bicelles. The three-dimensional structures of the micelle-immersed oxidized and reduced forms of y1fatc (PDB-id 1kio and 1kit) (2) are similar to that of the free oxidized state (PDB-id 1w1n) (1). Based on an estimate for the Kd from the NMR diffusion data, the oxidized form has a slightly higher affinity for DPC micelles than the reduced form (2). An initial model of the membrane-binding was based on the binding surface derived from a NMR-titration with DPC (dodecylphosphochline) (2). In order to better understand the importance of different residues in the y1fatc membrane anchor for the interaction with different membrane-mimetics and to better define the immersion depth and orientation, we analyzed in more detail the interaction of wild type y1fatc and a large array of mutants with different membrane-mimetics by NMR and CD spectroscopy as well as MD simulations. Based on ^{1}H-^{15}N-HSQC experiments, y1fatc mutant proteins were tested for their ability to interact with DPC, micelles, DihepPC/DMPC bicelles or DMPC liposomes. The immersion depth was defined by paramagnetic relaxation enhancement (PRE) using DPC micelles containing doxyl-labeled stearic acid. To better interpret the PRE data, MD simulations of the respective micellar systems with and without protein were performed. The orientation of the single helix in lipid bilayers was analyzed by oriented circular dichroism (OCD) spectroscopy.

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PARALLEL SESSION LECTURES

PS 195

^{13}C RELAXATION DISPERSION EXPERIMENTS FOR AROMATIC SIDE CHAINS IN PROTEINS

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Aromatic residues form a versatile subgroup within the 20 amino-acid residues commonly found in proteins. They are prevalent in protein binding interfaces, where especially Tyr and Trp contribute a significant fraction of the binding free energy. His and Tyr, and to some extent also Trp, play important roles in enzyme catalysis. Aromatic residues contribute a significant part (roughly 25% of the volume) of the hydrophobic core, where they are typically involved in specific aromatic-aromatic pair interactions, or clusters of three or more aromatic residues, which show a clear preference for a pairwise edge-to-centroid (orthogonal) orientation. Despite the dense packing of protein interiors, Phe and Tyr residues undergo frequent 180° rotations ('ring flips') of the c_2 dihedral angle, i.e. around the C^b–C^g–C^z axis, as was well established in landmark studies on protein dynamics by early pioneers in the field of protein NMR spectroscopy. We have recently introduced a set of longitudinal- and transverse-relaxation optimized (L-TROSY) pulse sequences for ^{13}C relaxation experiments, including R_1, R_2, [^{1}H]-^{13}C *NOE*, and CPMG dispersion. Here we use the ^{13}C L-TROSY-CPMG experiment to study conformational exchange of aromatic side-chains in BPTI. We show that the relaxation of the 'TROSY spin state' can be affected by strong coupling between the ^{13}C-attached proton and its vicinal proton neighbor, leading to anomalous relaxation dispersion profiles. We demonstrate that the observed relaxation dispersions can be analyzed to determine the ring-flip rate for cases that are normally intractable because they are characterized by slow flip rates (on the order of 1–100 s^{-1}) and the *absence of chemical shift differences* of the monitored ^{13}C spin between the two sides of the ring. Furthermore, the ^{13}C L-TROSY-CPMG experiment also detects conventional relaxation dispersions due to modulation of the ^{13}C chemical shift that arise as a consequence of either ring flips or reorientations of the aromatic ring relative to its surroundings. The finding of quite slow ring flips for aromatic residues that exhibit single and non-broadened peaks in both the ^{1}H and ^{13}C dimensions of the NMR spectrum raises the possibility that aromatic ring flips in many cases might be slower than previously anticipated.

PS 196

NMR METHODOLOGIES IN CULTURAL HERITAGE

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There is an always growing understanding that the characterization of the state of conservation, the knowledge of the causes of degradation of materials, the development of new methods and materials aimed at lengthening the life time of the artifacts, are mandatory in the correct safeguard of Cultural Heritage. The monitoring and investigation of artifacts is of help in preventing or delaying the degradation. In recent years Nuclear Magnetic Resonance (NMR) techniques have been increasingly applied to investigate, characterize and monitor objects of interest for Cultural Heritage [1-3]. Actually NMR is not confined to a few specific applications, but its use can be successfully extended to a wide number of different issues regarding Cultural Heritage. A breakthrough has surely been the development of portable NMR instrumentation. These devices can be applied directly on large objects such as *frescoes*, monuments, and in general any buildings fully preserving the integrity and the dimension of the object under investigation. The measured NMR parameters are important to establish the state of degradation of objects, to evaluate the performances of consolidation and water repellent treatments on porous materials, to monitor the detachment of the painted layer from the plaster, to quantitatively map the dampness in wall paintings. A further development of portable NMR devices is the availability of sensors to produce hydrogen driven NMR stratigraphy with microscopic spatial resolution for investigating the layer structure of artifacts with micrometric resolution. Cases of NMR application to monitor in situ the state of degradation of artifacts and the effectiveness of restoration treatments will be shown to illustrate the potentialities of portable NMR devices in the Cultural Heritage field.

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PS 197

INTERNAL FIELD ^{59}CO NMR FOR FISCHER-TROPSCH HETEROGENEOUS CATALYSTS CHARACTERIZATION

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Supported metallic cobalt particles are widely used as catalysts of Fischer-Tropsch (F-T) synthesis. Revealing the structure allows preparing highly productive and selective cobalt F-T catalysts. Nowadays, various techniques are utilized for the structural characterization. One of them is internal field ^{59}Co NMR that gives unique information about both structural and magnetic features. First, information concerning local magnetic fields distribution can be derived and, second, it helps to attribute some peaks in spectra to definite cobalt stacking such as magnetic single- and multidomain face-centered cubic (fcc) and hexagonal close-packed Co metal.
Correction of the NMR spectra to account for domain-walls hyperfine-fields oscillations allows for a quantitative analysis of the metal particles magnetic and structural domains. From the distribution of local magnetic fields, metallic Co single- and multidomain face-centered cubic structures, metallic Co hexagonal close-packed structures, stacking faults and strong Co-Al interaction representing Co-Al alloy can be distinguished and quantified.
This information is highly pertinent to rationalize differences in CO conversion efficiencies depending on the preparation mode of the catalysts. As an example, we will discuss the differences between metallic Co dispersed on δ-alumina and on γ–alumina. On δ-alumina, cobalt particles consisted predominantly of single-domain fcc cobalt with small amounts of stacking faults. On γ–alumina, multi-domain fcc structures were present indicating the existence of cobalt particles with size of more than 50 nm. We will thus show that despite the theoretical difficulties and care needed for their interpretation, zero field ^{59}Co NMR spectra are able to provide unique information relative to the dispersion and structure of cobalt metallic particles in Fischer-Tropsch heterogeneous catalysts which is crucial information to rationalize their catalytic activity.
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PS 198

ON-LINE PROCESS AND REACTION MONITORING BY LOW-FIELD NMR SPECTROSCOPY

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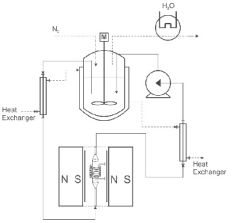

Fig. 1Reaction monitoring by MR-NMR spectroscopy in a by-pass system

On-line analysis of dynamic processes like chemical reactions by NMR allows detailed insight into mechanisms and kinetics, also in a production environment. Especially permanent magnet based low-field NMR (1-85 MHz ^{1}H-frequency) provides a suitable instrumental basis[]. Apart from feasibility, some more detailed considerations are mandatory to achieve applicability for quantitative online measurements of processes and reactions in by-pass systems[,]. Apart from temperature and pressure issues, data acquisition and processing known from high-field NMR cannot be transferred directly but have to be adapted to the special requirements of MR-NMR. A high-temperature, high pressure flow-through probe was designed with good properties regarding materials, fluid mechanical and NMR properties such as sensitivity, selectivity and time resolution at 20 MHz. First promising results were achieved on an esterification reaction at different temperatures, to prove the capability of low-field NMR spectroscopy for on-line monitoring of a chemical reaction.

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PARALLEL SESSION LECTURES

PS 199

COMPACT STRAY-FIELD NMR OF MOISTURE: FRESCOES AND POLYETHYLENE

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The simplest NMR device is a stray-field magnet equipped with a surface coil to measure signal from objects in the vicinity of the magnet and the coil. This concept is used in well-logging tools and the NMR-MOUSE®. Such devices are well suited to analyze soft matter including moisture in porous media following the principles of slice-selective excitation known from magnetic resonance imaging. From the NMR signal of moisture in confined environment, two properties can be derived, moisture content in terms of signal amplitude and moisture mobility in terms of the translational self-diffusion coefficient. Both quantities are comparatively easy to measure with the Profile NMR-MOUSE, which exhibits a well-defined gradient field in a thin slice at a fixed distance from the sensor surface and is suitable for 1D depth profiling. For simple systems relaxation times and diffusion coefficients are quantified from fitting model functions to the experimental data. More complex systems are analyzed in terms of distributions of relaxation times and diffusion coefficients. We have studied frescoes, fresco fragments and fresco models in terms of depth profiles of moisture content. These profiles reveal the pore structure of the mortar layers, which carry the fresco painting. Different schools of crafting frescoes have been identified by chemometric methods in a set of profiles collected from the ancient city of Herculaneum, Imperium Romanum, and verified with profiles from modern frescoes produced in the historic style. A study in the Salone Nero at Herculaneum further showed, that differences in diffusion coefficient of wall moisture correlate with different conservation treatments. The same approach employed to study frescoes has been followed to study the uptake of solvents by semi-crystalline polymer materials. In particular, *n*-hexane and toluene are solvents that ingress into the amorphous domains. The crystalline domains confine their diffusion. The long-time diffusion coefficient explores the tortuosity limit known from fluid diffusion in porous media. The tortuosity correlates with the crystallinity of the polyethylene material and accordingly with the state of aging, as demonstrated by example of a tortuosity depth profile of an aged polyethylene pipe section.

PS 200

Applications of MRI in Imaging Fluid Flows and Reaction Engineering

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This presentation will consider two aspects of our recent work: (i) the application of compressed sensing and Bayesian techniques in imaging fluid flows; and (ii) the implementation of a high temperature, high pressure reactor for MRI studies of catalytic processes *in situ*.

Compressed Sensing (CS) and Bayesian Methods in MRI CS and Bayesian MR offer the opportunity to substantially speed up Magnetic Resonance (MR) data acquisition. We have been particularly interested in exploiting this to achieve greater temporal resolution in imaging both gas and liquid fluid flows. Applications in fixed-bed reactors, single-phase pipe flow and gas-liquid bubbly flows will be given. Imaging of three-dimensional flow fields at timescales of <4 ms is now possible. These approaches also offer opportunities for industrial applications in low-field MR.

MRI at High Temperature and Pressure

An *in situ* reactor has recently been commissioned which enables us to study adsorption and heterogeneous catalytic processes up to temperatures of 250 C and 30 bar. Two case studies will be described: (i) the condensation and evaporation of cyclohexane in porous particles from which *in situ* determination of pore-size distribution can be made; and (ii) determination of the product distribution in an alkene oligomerisation reaction in a fixed-bed reactor during reactor operation.

PARALLEL SESSION LECTURES

PS 201

SOLID STATE MR SPECTROSCOPY AND IMAGING OF BONE MINERAL AND MATRIX

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Bone is a dynamic tissue whose changes in composition and geometry have enormous physiological impact. The rigid constituents of bone (inorganic calcium phosphate mineral nanocrystals and proteinaceous organic matrix) are solids with very short transverse relaxation times, and therefore yield weak or no signals with conventional MR imaging or spectroscopic methods. The focus of this presentation is on the composition of these solid bone constituents, and on the methodology necessary to carry out compositional measurements in both high field spectrometers and clinical scanners. The chemistry of calcium phosphate salts, in particular the apatite minerals, is highly complex, and indeed, the name "apatite" derives from the Greek *απατώ*, meaning "to deceive". Bone mineral, although similar to the well known synthetic compound calcium hydroxyapatite, $Ca_{10}(OH)_2(PO_4)_6$, is nanocrystalline, structurally disordered, and compositionally nonstoichiometric. It exhibits a high specific surface area containing labile chemical groups, and possesses a remarkable ability to support ionic vacancies and substitutions. Many of the biological and mechanical functions of bone are influenced by the mineral composition and crystal structure, especially by the subtleties of its nanocrystal surface chemistry. High resolution solid state NMR spectroscopy provides important information on bone mineral crystal chemistry and structure. We will show examples from developmental biology where solid state NMR spectroscopy has advanced the understanding of bone biology. Extending traditional solid state methods such as CP/MAS to clinical imaging of human subjects is obviously unworkable since we can neither spin patients nor expose them to 50 kHz RF decoupling fields. Clinical imaging of bone mineral and matrix may be accomplished with pulse sequences that avoid slice selection and spin or gradient echoes (short 180° pulses and gradient ramps are neither possible nor safe in clinical scanning). Image reconstruction methods must be employed to recover central k-space data lost in the receiver dead time. Electronic modifications to the scanner are required. We will show how high quality 3D bone mineral (^{31}P) and matrix (^{1}H) images with quantitatively accurate signal intensities may be acquired in humans using clinical MRI scanners.

PS 202

IN SITU MRI OF BATTERIES AND SUPERCAPACITORS

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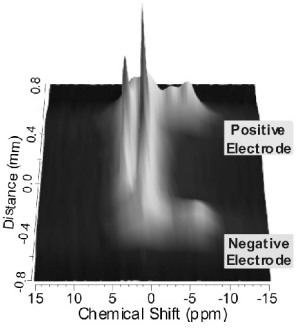
In situ NMR/ MRI has proven to be a useful tool to probe the structure of Li-ion batteries (LIBs) during real-time charge and discharge. *Ex situ* studies of batteries are limited by self-relaxation of the electrode materials before a measurement can be obtained. The application of advanced magnetic resonance techniques, such as MRI and complex NMR experiments, *in situ* has the potential to monitor dynamics and visually monitor changes in functioning electrochemical systems in real time. Although most *in situ* studies have studied structural changes, recently the monitoring of the electrolyte in a symmetric Li metal cell *in situ* was reported. The functionality of some energy storage devices where only the electrolyte is involved in the electrochemical process (such as supercapacitors) can only be studied *in situ*, as the electrolyte concentration gradients will relax as a potential is removed from the cell. Here we present techniques for *in situ* MRI of batteries and supercapacitors and discuss the challenges involved in this field.



^{1}H CSI image of a symmetric Yp17 carbon supercapacitor.

PARALLEL SESSION LECTURES

PS 203

IN SITU HP ^{129}XE MRI OF COMBUSTION

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Direct spin exchange optical pumping (SEOP) of ^{129}Xe-fuel mixtures enables MRI of combustion [1] using a fairly straightforward experimental procedure and a commercial 9.4 Tesla NMR microimaging system. A stream of combustible fuel that contains hp ^{129}Xe as an MRI contrast agent is produced through SEOP of a 85% CH_4 , 5% Xe , and 10% N_2 gas mixture. The hp fuel mixture is fed continuously from the SEOP cell into a combustor located within the superconducting magnet. MRI scans covering the pre-combustion zone, the flame region, and the exhaust area were taken with and without ignition. The MR images demonstrate that ^{129}Xe sustains its hyperpolarization to a high degree throughout the actual flame and exhaust regions.

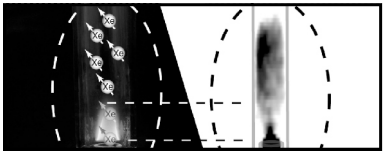

Figure 1.: Sketch showing a photograph of the actual combustion zone (left) and hp ^{129}Xe MRI (right) of the same region. Dark regions in the MRI indicate high ^{129}Xe signal intensity.

The combustion resistance of the ^{129}Xe hyperpolarized nuclear spin state allows for MRI studies of the fluid dynamics with combustion processes and a velocity profiles ($V_z(z)$) of the gas flow within the combustor is demonstrated. In addition, SEOP of hydrogen – xenon mixtures is explored. Hp ^{129}Xe MRI may be of potential usage for the study of flow fields within catalytic combustors and provide a better understanding of gas exchange with their porous surfaces. Practical aspects of MRI of combustion with hyperpolarized (hp) ^{129}Xe are presented.[1] Karl F. Stupic et al., Phys. Chem. Chem. Phys., **15**, (2013), 94-97.

PS 204

NMR AND MRI ANALYSIS OF ROCK CORE SAMPLES FROM OIL WELLS

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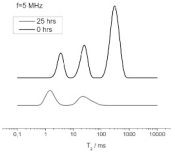
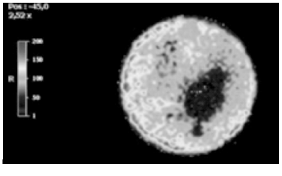
Oil and gas production is highly dependent on understanding key petro-physical parameters of reservoir rocks, such as porosity, permeability and wettability. Reservoir rock properties determine ultimately production volume as they define how and to what extent hydrocarbons can be recovered from rock deposits. These properties and the way they behave due to field factors, such as temperature, pressure and depth, remain of primary concern to the oil/gas exploration and production industry. Nowadays low field Nuclear Magnetic Resonance (NMR) core analysis is extensively used for oil exploration as a popular method to delineate relationships between core pore radii and permeability and to evaluate irreducible water saturation and residual oil saturation. The technique uses the radio-frequency resonance of protons in a magnetic field to determine characteristic relaxation mechanisms. These NMR mechanisms are attributed to the hydrogen relaxation signal originating from fluids within different regimes of the pore network. These regimes are reflected in the surface to volume ratio of the sample. Because there is a surface to volume ratio distribution, there are also relaxation distributions, which can be interpreted to provide information on pore size distribution, permeability, porosity, free fluid index and bound water volumes.
In addition, using Magnetic Resonance Imaging techniques (MRI) relaxation time values can be resolved spatially and provide noninvasive digital two- or three-dimensional images depicting hydrocarbon/water location and pore size distribution. MRI can be coupled with X-ray Computed Tomography (CT) and provide additional information about cracking, fracture orientation and density, pore volume connectivity and porosity. The technique is a prerequisite for sample selection and is frequently used as a saturation-monitoring tool.
In this work, the authors will present the results of an extensive NMR and MRI/CT study performed on three types of core specimens originating from different oil fields. Core plugs were saturated with water and oil separately and several key petro-physical properties were defined. The methods used to optimize experimental set-up and data manipulation will be presented, along with guidelines on how the above can be applied to improve oil recovery.

^{1}H NMR distribution of T_2 in a rock core sample saturated with water reveal a trimodal pore size distribution (black line). After a day water appears to have evaporated from larger pores and the distribution changes to bimodal (red line). The image on the left shows the spatial distribution of T_2 values in a slice vertical to the z-axis of the rock core sample immediately after water saturation. The colors represent T_2 values in the range 1-200 ms.





PS 205

HIGH SENSITIVITY/HIGH RESOLUTION PULSED ELECTRON SPIN RESONANCE IN SOLIDS – TECHNIQUE AND APPLICATIONS

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Electron spin resonance (ESR) is of significant importance in the field of solids in general and semiconductors in particular. For example, it enables to obtain detailed atomic information about paramagnetic defects, which affect the performance of semiconductor-based devices. However, conventional induction-detection ESR is often found not sensitive enough, especially in the case thin layered samples, or when the sample is heterogeneous and requires spatially-resolved analysis.
Recently, we have developed unique methodologies that significantly increase the sensitivity of ESR and also enable to obtain three dimensional images of solid samples of interest with sub-micron resolution. These capabilities enable now to pursue a whole new range of scientific and technological applications that were previously out of experimental reach. In the presentation we will describe the details of our new methodological approach and present some experimental results looking at important scientific applications. For example, measurement of diffusion of point defects in SiO_2, and quantifying the amount and the spectroscopic properties of defects in very thin amorphous silicon layers. We will also describe some potential future directions in the field, such as combining electrical detection with high resolution ESR micro-imaging, and increasing the static field of measurement.

PS 206

Ultra-high field DNP-ssNMR applied to cellular structural biology

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E.J. Koers, D. Mance, T. Sinnige, M. Weingarth and M. Baldus

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In our contribution we describe recent progress in using DNP-supported solid-state NMR (ssNMR) to elucidate the role of cellular membranes in the structural organization and function of complex biomolecules.
- We describe three-dimensional views of pH and ligand-activated bacterial potassium channels in different functional states and elucidate the potential influence of the cellular membrane for N-type channel inactivation [see, e.g., 1,2].
- We demonstrate how DNP-ssNMR can be used to obtain detailed structural information on lowly concentrated liposomal vaccine targeting Alzheimer's disease [3]. We find that DMPC/DMPG/cholesterol mainly stabilizes extended structures of the lipid-anchored peptide, while in DMTAP/cholesterol liposomes the peptide adopts a multitude of conformations including random-coil and extended structures.
- Finally, we report on recent progress in using cellular solid-state NMR spectroscopy [4,5] for the structural elucidation of cellular lipoproteins and for the study of protein translocation and insertion machines in bacteria.

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PARALLEL SESSION LECTURES

PS 207

RESONANCE ASSIGNMENT AND STRUCTURE INVESTIGATION BY HIGH RESOLUTION ^{1}H-DETECTED SOLID-STATE NMR UNDER ULTRA-FAST MAS: FROM MICROCRYSTALLINE PROTEINS TO LARGE PROTEIN ASSEMBLIES

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We present an overview of our recent advances using ultra-fast (60 kHz) magic-angle spinning (MAS) solid-state NMR spectroscopy and high magnetic fields. These include: (a) the use of deuterated/fully back-exchanged protein samples, which enable the acquisition of high resolution and sensitivity spectra with ^{1}H detection, (b) the design of a suite of scalar-based correlations for resonance assignment of backbone HN, N, C' and C_{α} and side-chains C_{β}, (c) the measurements of site-specific ^{1}H-^{1}H distance restraints using 3D NMR methods. These experiments enable the rapid assignment and the fast determination of the fold of medium-sized proteins, and open the way to the establishment of intermolecular contacts in larger protein assemblies. Examples are presented from **microcrystalline** and **non-crystalline** domains and assemblies from *E. Coli* DNA polymerase, as well as for **sedimented** viral capsids.

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PS 208

DYNAMIC NUCLEAR POLARIZATION FOR UNDERSTANDING BIOMINERALISATION IN DIATOMS

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Bioanalytical Chemistry, TU Dresden;
NMR Spectroscopy Research Group, Utrecht University

Solid-state NMR (ssNMR) spectroscopy is a well-established and powerful method for the characterization of various types of inorganic and hybrid materials as well as complex biomolecules. In principle, characterization of both bulk and surface species is possible with ssNMR. But with increasing interest in the surface functionalities of materials, the spectroscopic sensitivity becomes a crucial factor. The combination of ssNMR with DNP offers the possibility to selectively enhance the surface species in hybrid materials.
A hybrid material of biological origin is found in the biosilica of diatoms. The cell walls of these unicellular algae consist of amorphous biosilica with a species-specific micro- and nano-patterning. The isolation of the silica deposition vesicle (SDV) with its biomolecules which are supposed to be involved in the silica deposition and patterning process was not successful. A lot of work concentrated on the isolation and analysis of biosilica-associated or embedded biomolecules for this reason. Long-chain polyamines (LCPAs), polysaccharides, special peptide classes such as the silaffins, the silacidins, and cingulins have been identified.
In the present contribution we show, how dynamic nuclear polarization (DNP) can be used to investigate the low-surface-area biominerals for information about the involved biomineralisation processes. The aforementioned different biomolecules are located either deeply inside the silica or attached to the outer surface. DNP ssNMR offers the possibility to selectively enhance organic material which is located near the surface. This is a main advantage compared with conventional NMR or GC-MS analysis. The wetting with TOTAPOL as suggested by Emsley et al. [1] via incipient wetness impregnation resulted in enhancement factors between ε~20-50 for the accessible organic materials, mainly polysaccharides. This allowed multidimensional experiments in a reasonable time frame. The results will lead to new insights on diatom biomineralisation, especially the localization of the different organic constituents.

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PS 209

MAGIC ANGLE SPINNING NMR STUDIES OF INTACT BACTERIOPHAGE VIRUSES

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In recent years, we have shown that magic-angle spinning (MAS) NMR studies of intact filamentous viruses provide data of sufficiently high-resolution to study their capsid and DNA properties in great detail. We have managed to show that the capsids of phage particles are mostly helical and highly homogeneous, with the exception of very few individual residues. Those residues report on special structural features such as interactions with the DNA. NMR data could also be obtained for many resonances belonging to the single-stranded DNA reporting on structural properties such as sugar puckering and glycosidic torsion angles. DNA-protein interactions can be detected by various techniques and the use of sparsely labelled samples report on many inter-subunit interactions, which lay the basis for structure calculations.
Recently we have also begun looking into the larger T7 bacteriophage, which is structurally very different and possesses icosahedral symmetry. Very much like filamentous viruses mostly its structural features come from EM studies and lack real atomic resolution.
I will present the progress we have made towards modelling the quaternary structure of the filamentous phage M13 as well as our preliminary studies of T7, which is a 50 MDa complex macromolecular assembly composed mostly of its long double-stranded B-form DNA.

PS 210

CONFORMATIONAL EXCHANGE AND ION BINDING IN THE ION CHANNEL KCSA

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K^+ channels are inactivated subsequent to channel opening by a switch near their selectivity filter. Inactivation is central to their many crucial roles in cell signaling. We show that this dominant inactivation process (“selectivity filter collapse”) involves ion release, based on conformational exchange and ion binding studies of a prototypical prokaryotic ion channel, KcsA, carried out in membrane bilayers.
KcsA alters its conformation depending on the ambient potassium; at high potassium, it exists in a conductive but “closed” form, and at low potassium, it collapses into a nonconductive structure with reduced ion occupancy. We provide strong support for the hypothesis that the low K^+ state is the same as the inactivated state, using an inactivation-resistant mutant E71A.
Our data also show that E71 is protonated at pH 7.5 and must have an unusually perturbed pKa (>7.5) and undergoes a structural rearrangement (but not a protonation event) at low K^+. We elucidate transmembrane allosteric coupling between opening and ion release, using structural markers throughout the key functional regions of the protein.

PARALLEL SESSION LECTURES

PS 211

NEW DIFFUSION MRI METHODS FOR CHARACTERIZATION OF MICROHETEROGENEOUS MATERIALS

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Diffusion NMR and MRI are powerful methods for non-invasive studies of the pore space morphology of a wide range of materials – from rocks to brains. By following the self-diffusion of the pore fluid, parameters such as pore size, interpore distance, tortuosity, surface-to-volume ratio, and anisotropy can be estimated. This talk gives an overview of our recent methodological work of designing new experimental protocols specifically for determining the local self-diffusion inside cells [1], the rate of molecular exchange over cell membranes [2], the minimum length scale at which a material appears homogeneous [3], and the presence of microscopic pore anisotropy in a macroscopically isotropic material [4]. Examples are given from applications of the new methods to liquid crystals, emulsions, cell suspensions, and human volunteers.

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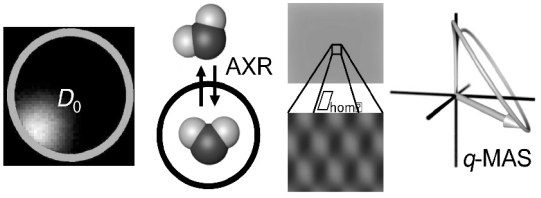

PS 212

CHARACTERIZATION OF (DOUBLE-)EMULSIONS BY NMR: DIFFUSOMETRY AND RELAXOMETRY

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Double emulsion are often opaque, and dilution influences the system's equilibrium states (confocal laser scanning microscopy image in Fig. 1), therefore NMR methods are exploited to give insight into geometrical structures as well as into composition and molecular dynamics.

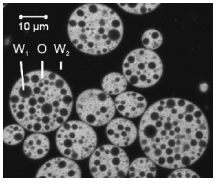

Fig. 1CLSM-image of a $W_1/O/W_2$ double emulsion.

Structure, i.e. droplet size distribution, can be addressed by Pulsed Field Gradient NMR (PFG-NMR). Signals of the different phases can be discriminated either by chemical shift, relaxation or diffusion contrasts[1-5]. Apart from the size distributions, which influence e.g. texture and mouth feel, the microbial, physical and shelf life stability, also the composition, i.e. the concentration of the dispersed phases, is of interest, mainly in the context of process and product development. In the case of simple emulsions (OW / WO), it can be determined by NMR spectroscopy. Additional differentiation is needed in case of multiple emulsions, which can be found in terms of diffusion or relaxation. Third, molecular dynamics is addressed by either PFG-STE NMR or spectroscopy in combination with paramagnetic relaxation enhancement. It is proven that, depending on the recipe of the emulsion, fast exchange between the phases occurs with a considerable impact on the understanding and impact of double emulsions in the context of controlled release.

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PS 213

^{1}H AND ^{19}F DIFFUSION AND RELAXATION IMAGING TO MONITOR DETAILED DRUG RELEASE PROFILES THROUGH EXTENDED RELEASE FILMS

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Capsules packed with pellets are commonly used as formulations for oral drug administration. The drug release from these pellets may be controlled for example by a thin extended release film based on locally phase separated hydroxypropylmethyl cellulose (HPC) / ethylcellulose (EC) mixtures. Upon contact with liquid, HPC will dissolve, leaving EC behind to create a network of various sized 'pores'. Detailed knowledge about the liquid transport through the film into the core of the pellet and the drug transport through the film into the aqueous phase is a prerequisite to tailor the film composition in order to “tune” the drug release profile.
A specifically designed release cell was used to follow both the water and a water-soluble Fluor-containing drug being transported through the film. Fast ^{1}H / ^{19}F diffusion and relaxation experiments, with a spatial resolution of 100 micrometres, were combined to monitor the water and drug dynamics focussing in particular on the initial dissolution of HPC and drug. This allowed also to quantitatively determining the water and the drug concentration in each voxel. The results showed that the drug transport through the film depends strongly on the fraction of water-soluble HPC. In addition, for low fractions of HPC, the film is less or almost impermeable for the drug. Here, the influx of water is resulting in a building up of pressure at the film interface towards the core. At high pressure, the drug was released followed by another pressure build up. This “pulsed” drug release profile was analysed quantitatively.

PS 214

TOWARDS A BETTER UNDESTANDING OF THE LOCAL STRUCTURE IN MOLTEN SALTS : COUPLING NMR CHEMICAL SHIFTS, SELF DIFFUSION COEFFICIENTS MEASUREMENTS AT HIGH TEMPERATURE AND MOLECULAR DYNAMICS SIMULATIONS

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Molten salts are ionic liquid mixtures at high temperature often involved in a wide range of industrial applications. Several kinds of molten salts can be distinguished, depending on the nature of the anions, O^{2-}, Cl^{-} or F^{-}… Inorganic molten salts can be strongly organized at unusually long distances due to the predominance of coulombic interactions and described by the formation of an intermediate range ordering. Because of particular experimental hindrances, the knowledge on molten fluoride salts chemistry has long remained limited to a few experimental approaches. These systems cumulate high temperatures (from 500 K to 1800 K), corrosive properties towards most of the materials and sensitivity to moisture and oxygen and make the NMR experiment a real challenge. Specific technical developments are required for the sample container, for the heating system adapted to the superconducting magnets and for the protection of the spectrometer and the probe. Thanks to a CO_2 laser heating and a container adapted to corrosive and reactive liquids, airtight and inert chemically towards molten fluorides, NMR measurements can be performed in molten fluorides up to 1500°C. The signal position, or the isotropic chemical shift, is the weighted averaged chemical shift of the different components in the melt. Knowing the chemical shift of the individual species, it becomes possible to extract their distributions depending on the composition. This approach of the liquid can be also combined with a more dynamical description with self-diffusion coefficients measurements in situ in the melts up to 1500K, thanks to the development of an NMR set up based on pulse field gradients associated with a CO_2 laser heating system. Large magnetic field gradients (up to 1200G/cm) allows measuring self-diffusion coefficients of different nuclei in a wide range of molten, glassy or even crystalline materials with high ionic conductivity.

PARALLEL SESSION LECTURES

PS 215

DYNAMICS OF POLYMERS AND LIQUIDS STUDIED BY THE MODULATED GRADIENT SPIN ECHO METHOD

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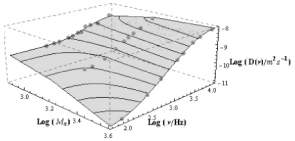
[2]*Institut Jožef Stefan, Jamova 39, 1000 Ljubljana, Slovenia,*
and[3]*University Ilmenau, Physics Institute, Unterpörlitzer Straße 38, 98693 Ilmenau, Germany*



Although, the polymers have a long-standing records of experimental and simulation investigations, the theory of entangled polymer dynamics still remains questionable. We present the investigation of molten polymers by the new technique of modulated gradient spin echo (MGSE)[1], which provides a new insight into details of polymer dynamic by unveiling the segmental velocity autocorrelation spectrum (VAS). With regard to other methods, the inquiry of molecular dynamics by the MGSE method is unique in the sense to provide not only information about the averaged displacements, but with the VAS also about the time-course of molecular motion in steps given by the frequency range of method. MGSE method applied to the NMR mouse set-up was used to measure the VAS of the mono-disperse poly(isoprene-14) with the molecular weights: M_n=*704, 1610* and *3920* within the frequency range from about *100* Hz to *10* kHz. As shown in the 3D contour plot, the frequency dependences of VASs fit very well to the spectrum for the model of Rouse chain motion, but with the differences in the scaling of its parameters: The centre-of-weight diffusion coefficient scaling goes from M_n^{-1} *to* $M_n^{-2.5}$ dependency at the transition from short to long polymers, the correlation time of the terminal mode, t_1, instead to increases with M_n^2, remains almost constant, while the mode amplitude $\langle X_1^2 \rangle$ scales as M_n. We explain it with the tube Rouse motion (not the chain Rouse motion)[2] that appears as a prevailing constraint release process in the frequency range of MGSE observation. MGSE method gives also a polymer like VAS for some liquids with the strong hydrogen-bondings (water, glycerol).

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PS 216

BRAIN-CP: BROADBAND ADIABATIC INVERSION-CROSS POLARIZATION – APPLICATIONS TO SOLID-STATE NMR OF SPIN-1/2 AND QUADRUPOLAR NUCLEI

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In this work, the BRAIN-CP/WURST-CPMG sequence, a broadband cross-polarization pulse sequence for the acquisition of solid-state NMR spectra of both spin-1/2 and quadrupolar nuclei, is discussed. Phase- and frequency-modulated WURST (Wideband Uniform-Rate Smooth Truncation) pulses are used as the elements of this new sequence to (i) provide broadband CP via the application of a broadband adiabatic inversion pulse on the X channel, while applying convention rectangular pulses on the H channel, (ii) to convert inverted spin polarization to observable signal over a broad frequency range, and (iii) to act as broadband refocusing pulses in a CPMG type sequence for signal enhancement. Spectra for a variety of nuclides from across the periodic table will be shown, practical advice on the set-up and utilisation of this sequence will be given, and a variety of interesting uses for BRAIN-CP/WURST-CPMG will be discussed, including signal enhancement, spectral editing for multi-site spectra and measurement of longitudinal relaxation times.

PS 217

PHOTOINDUCED CHARGE SEPARATION PROCESSES: FROM NATURAL PHOTOSYNTHESIS TO ORGANIC PHOTOVOLTAIC CELLS

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Photovoltaic (PV) cells are the most promising man-made devices for direct solar energy utilization. Detailed knowledge of the charge separation and charge transport in PV materials is crucial for improving the efficiency of the solar cells. Advanced EPR spectroscopy, especially light-induced multi-frequency EPR, has been essential for understanding the mechanisms of the light-induced generation, separation, and recombination of the charge carriers in natural photosynthesis. Here, we use light-induced EPR spectroscopy combined with DFT calculations to study mechanisms of charge separation and charge stabilization in active organic PV materials based on the composites of multiple polymers (P3HT, PCDTBT, and PTB7) and fullerene derivatives (C_{60}-PCBM and C_{70}-PCBM). Time-resolved EPR spectra show strong polarization pattern for all polymer-fullerene blends under study, which is caused by non-Boltzmann population of the electron spin energy levels in the radical pairs. Similar polarization patterns were first reported in molecular donor-acceptor systems, such as natural and artificial photosynthetic assemblies, and were understood within the models of spin-correlated radical pairs (SCRP) and sequential electron transfer. These models were used by us to explain the polarization pattern of the SCRP in polymer-fullerene blends and describe the charge separation process like electron jumps or tunnelling between neighbouring fullerene molecules. The first step of the charge separation process is exciton dissociation and electron transfer to the fullerene molecule neighbouring to the polymer. In order to outcompete the recombination process this state cannot live longer than a few picoseconds. Forward electron transfer forms an intermediate radical pair, with the separation distance within 15-20 Å. The third step is electron transfer to the secondary radical pair with a separation of 25-30 Å which is stable for tens-hundreds microseconds. The following electron transfer steps are on the slower time-scale and lead to further charge separation or charge recombination. The analysis presented here in combination with DFT calculations helps to improve our understanding of the mechanism of charge separation processes in the active organic photovoltaic materials.

PS 218

CHIRAL RECOGNITION IN METAL-ORGANIC FRAMEWORKS STUDIED BY SOLID-STATE NMR SPECTROSCOPY USING CHIRAL SOLVATING AGENTS

H.C. Hoffmann, S. Paasch, P. Müller, I. Senkovska, M. Padmanaban, F. Glorius, S. Kaskel, E. Brunner

TU Dresden and Westfälische Wilhems-Universität Münster

Metal-Organic Frameworks (MOFs) are hybrid materials formed by organic and inorganic building blocks. The interactions between organic and inorganic constituents are relatively strong. This results in three-dimensional network structures. Often, these networks exhibit micro- or mesopore systems of huge internal surface area. Applications of MOFs are envisioned in catalysis, gas storage, for gas and solvent purification, as nano reactors, highly selective molecular sieves, sensors, in microelectronics, and for optical purposes.
Moreover, chiral MOFs are currently gaining research interest for enantioselective separation and catalysis. We have described the synthesis of two chiral analogues, *i*Pr-ChirUMCM-1 and Bn-ChirUMCM-1, of the metal-organic framework UMCM-1 and their application in enantioselective separation.[1] Here, we demonstrate the solid-state NMR spectroscopic characterization of MOFs, especially with respect to their chiral properties. *Ad hoc*, NMR cannot discriminate different enantiomers of chiral compounds. However, liquid-state NMR offers approaches to detect chirality of dissolved molecules, e.g., *via* their interaction with so-called chiral shift agents. Here, we for the first time describe the use of chiral shift agents for chiral recognition in metal-organic frameworks by *solid-state NMR spectroscopy*. This has been shown for *i*Pr-ChirUMCM-1 and Bn-ChirUMCM-1 using enantiomerically pure (*R*)-/(*S*)-TFPE.[2] Pronounced chemical shift differences were observed exclusively for carbon atoms located in the modified, chiral BDC-linkers. Interactions between the chiral BDC-linkers and enantiomerically pure (*R*)- or (*S*)-TFPE result in the formation of NMR-distinguishable complexes with different chemical shifts which can be measured due to the excellent spectral resolution of their ^{13}C CP MAS NMR spectra.

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PS 219

SOLID STATE NMR STUDIES OF RECHARGEABLE BATTERY MATERIALS

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University of Cambridge, Cambridge, UK

Batteries are instrumental in efficient usage of clean and renewable energy sources. A comprehensive understanding of the battery operation mechanism is the key in the search for safer and cheaper battery electrode materials with high energy capacity. Due to its unique capability of detecting buried amorphous interfaces and identifying local structural/dynamical changes, solid-state NMR provides reliable experimental evidences, usually not accessible by other methods, for answering fundamental questions in the studies of rechargeable battery systems including Li, Na, and Mg ion batteries. High-resolution multinuclear solid-state NMR, complemented by X-ray and first principles calculations, has been employed in the investigation of the cause of additional capacities
(up to 40% excess to the theoretical capacity) found in metal oxides used as lithium ion battery electrodes. High-resolution ^{1}H, $^{6}Li/^{7}Li$, ^{17}O, and ^{19}F NMR spectra following the battery cycling reveal the reaction mechanism at each stage of the electrochemical process. Double- resonance correlation experiments help further to identify and confirm the formation of the amorphous solid-electrolyte-interface (SEI) and suggest that the true source of the additional capacity is the formation of LiOH and its subsequent conversion to LiOH and LiH. Isotope labeling (e.g. ^{17}O) helps to track the evolution of certain chemical phases through the electrochemical process. Sodium and Magnesium ion batteries are safer, cheaper, and more sustainable alternatives to Lithium ion batteries. The relationship of structure-electrochemical performance of promising Na/Mg ion battery electrode materials is studied by solid state NMR. For example, I of the high-performance polyanion Na electrode material, $Na_3V_2(PO_4)_2F_3$, as Na is extracted, the transition is observed from localized Fermi-contact interaction between Na ions and unpaired electrons to delocalized interactions between electrons in the conduction band and Na ions (source of Knight shift). The gradual increase of the electronic mobility directly affects the relaxation times (T_1 and T_2) of Na ions, observed also in lithium ion battery electrode materials. Analytical simulations are carried out to quantitatively analyze this phenomenon.

PS 220

NMR CRYSTALLOGRAPHY OF HIGH-PRESSURE SILICATE MINERALS AND RELATED INORGANIC MATERIALS

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Solid-state NMR has traditionally played a complementary role to diffraction techniques for minerals by providing detailed local structure information, such as cation (e.g. SI-Al-Mg, H) and anion order/disorder and hydrogen bonding. NMR spectroscopy can now be put to more efficient use in crystallography with the development of ab initio structural determination techniques for diffraction data that can easily incorporate local structural information from spectroscopy. The information content is further enhanced by first-principles calculations and double-resonance experiments utilizing dipolar couplings and J couplings. In this talk, we will present some of our recent work in applying these techniques to minerals synthesized under high pressure, focussing on the following two topics:

(1) ^{29}Si NMR of aluminosilicates: new insight from J-resolved spectroscopy: We have shown on the mineral K-cymrite ($KAlSi_3O_8 \cdot H_2O$) that the number of ^{29}Si MAS NMR peaks is determined not only by the number of crystallographically unique sites and the numbers of Si and Al in the neighboring T sites, but also by the number of nonequivalent Si-O-T angles around a given Si site.[1] J-resolved spectroscopy provided direct evidence for such a conclusion. 1D CPMG (and its variant) is particular useful for revealing J couplings of weak peaks.

(2) Crystal structure determination from combined NMR and powder synchrotron XRD: We have discovered and solved the crystal structures of several high-pressure silicate and $AlPO_4$ phases with this approach.[3,4,5] J-coupling based measurements and first-principles calculations provided complementary insights.

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Poster Presentation

Poster Presentation

300 MO

A SOLID STATE ^{31}P, ^{43}CA AND ^{29}SI MAS AND DOR NMR, AND GIPAW DFT STUDY OF A-TRICALCIUM PHOSPHATE AND SI-SUBSTITUTED A-TRICALCIUM PHOSPHATE BIOACTIVE MATERIALS

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Alpha-tricalcium phosphate (α-TCP, $Ca_3(PO_4)_2$) demonstrates both bioactive and resorbable characteristics. Substitution of SiO_4^{4-} for PO_4^{3-} in α-TCP (Si-α-TCP) is found to stabilize the structure at lower temperatures and improve mechanical (and possibly bioactive) properties. The mechanism of electroneutrality in the Si-α-TCP structure is not fully understood, though is thought to take place through the creation of O^{2-} vacancies or through excess Ca^{2+}. This study addresses some structural properties of *α*-TCP using ^{31}P MAS NMR at intermediate B_0 fields (11.7 T) and ^{43}Ca DOR NMR at multiple fields (20.0 T, 14.1 T, 11.7 T), and via correlation of the measured ^{31}P and ^{43}Ca isotropic chemical shifts (δ_{iso}) against calculated values obtained with GIPAW DFT methods using the CASTEP code. These results show that the structure has high short range order and clearly support the monoclinic $P2_1/a$ (12 P site/18 Ca site) model. In contrast, solid state ^{31}P MAS and ^{43}Ca DOR NMR studies of Si-*α*-TCP demonstrate that significant disorder broadening is characteristic of these data, however the corresponding ^{29}Si MAS NMR data affords reasonably resolved resonances at shifts in the δ_{iso} range of ~–70 - –75 ppm indicating a predominance of Q^0 Si speciation accompanied by a low intensity Q^2 resonance at δ_{iso} –84.5 ppm. ^{31}P-^{29}Si HETCOR data from these systems suggests that, despite the intrinsic disorder, explicit PO_4^{3-} framework species can be associated with the different Q^0 Si speciation, and while there is some dispersion of the silicon throughout the structure it is predominantly associated with a small number of P sites. The NMR data and accompanying DFT calculations for the Si-α-TCP system suggest that the more favourable mechanism for charge balance is a calcium excess, where a Ca^{2+} goes into an existing vacancy in the structure and two nearby PO_4^{3-} units are replaced by two SiO_4^{4-} moieties.

301 TU

MEMBRANE CURVATURE AND CHOLESTEROL EFFECTS ON LIPIDS PACKING AND SPIN LABELED LIPIDS CONFORMATIONAL DISTRIBUTIONS

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Nitroxide spin labeled lipid analogs are often used to study model membrane properties using EPR spectroscopy. Whereas in liquid phase membranes the spin label assumes, on average, its putative location, in gel phases and frozen membrane, depending on its position along the acyl chain, it may exhibit a different average location. Here we used ^{2}H three-pulse Electron Spin Echo Envelope Modulation (ESEEM) of phospholipid spin probes, combined with various deuteration schemes to detect the effect of the model membrane curvature and cholesterol on vertical migrations of the spin label. We compared large and small unilamellar 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) vesicles with and without cholesterol (10%). The vertical displacement of the spin label was manifested as an apparently flat trans-membrane profile of water concentration and of label proximity to the head group choline. The spin label propensity to migrate was found to increase with vesicle curvature and decrease in the presence of cholesterol. This in turn reflects the effect of packing and ordering of the membrane lipids. The results show that in curved vesicles lacking cholesterol, the label attached to carbon 16 may travel as far high along the membrane normal as the location of the label on carbon 5, due to the presence of U shaped lipid conformations. This phenomenon must be taken into account when using spin labeled lipids as membrane depth markers or to trace transmembrane profiles.

Poster Presentation

302 TH

STUDY OF THIN FILAMENT COMPLEXES BY SOLID STATE NMR SPECTROSCOPY

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Actin plays a key role in muscle contraction. Even if the knowledge of the molecular mechanism and regulation of events driven by actin or actin binding is in continuous development, obtaining the atomic structure of the actin filaments and their complexes is a substantial challenge. In fact, only low resolution data has been obtained by electron microscopy and X-ray diffraction because the filamentous state prevents the use of the techniques which can provide data at atomic resolution, such as solution NMR spectroscopy or X-ray crystallography. Indeed, the size of the actin filaments exceeds the limit of detection of NMR and moreover they cannot be crystallized. Even if the low resolution data are enough to obtain general models of actin filaments, they have been unable to produce details for the characterization of the interactions which allow the development of the filaments. However, nowadays solid state NMR spectroscopy has reached a stage where atomic resolution data can be collected for protein complexes. In this study, initially we want to evaluate the potential of this technique to the structural study of F-actin and its complexes.
As a test system we study the complex of the Villin Head Domain (VHD), the 8kDa domain of ABLIM2 involved in actin interaction, and actin filaments. VHD domains have been are extensively characterized by solution NMR spectroscopy and due to its stability, expression yield and size has been chosen as a model protein for F-actin complexes.
We will show first results of using the VHD/F-actin interaction as a model system for the exploration of this newly emerging methodology.

303 MO

HEAVY MICE AND LIGHTER THINGS: USING NMR TO ELUCIDATE MOLECULAR STRUCTURES IN TISSUES

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The successful development of a ^{13}C, ^{15}N-labelled "heavy" mouse allows two and three-dimensional correlation NMR spectra to be recorded on intact tissues, giving, for the very first time, access to detailed molecular structural information of proteins in native tissues, in the environment in which they normally operate.
Tissues such as muscle, tendon, ligament, bone, blood vessels and skin are primarily comprised of an extracellular matrix (ECM) which is composed of fibril-forming collagen proteins interspersed with an aqueous matrix of proteoglycans and other molecules. Collagens are triple helical proteins with of order 1000 residues per chain and form microfibrils in which the collagen triple helices are glycosylated and interact with a wide range of other molecules, including those in the aqueous matrix. The structure of collagens in their native environments is not currently known, yet it is this structure that confers the material and biological properties of the tissue and that which cells of the tissue interact with. We show that the increase in resolution afforded by two-dimensional NMR spectra has allowed assignment of the vast majority of amino acid species in collagen type I.
More importantly, a library of multidimensional NMR spectra of a tissue act as "fingerprints" of the underlying molecular and intermolecular structures within a ~0.2 – 1 nm lengthscale range and so can be used as reference data in order to develop and validate ^{13}C, ^{15}N-enriched *in vitro* or engineered models of mammalian tissues. We have used this principle to refine *in vitro* models of bone and vascular tissue. An *in vitro* model of a tissue allows specific isotope labelling regimes to be used to assist spectral assignment. We have used this feature to examine the collagen helix conformation and the possible role of collagen glycosylation.
These developments allow structural study at a molecular level within truly native-like collagenous systems, we are currently working towards a quantitative structural model for the fibronectin site of collagen type I.

Poster Presentation

304 TU

CELLULAR STRUCTURAL BIOLOGY: SOLID-STATE NMR STUDY ON THE CELL-EMBEDDED TYPE IV SECRETION SYSTEM

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Structural Biology relates to the elucidation of biomolecular structure under in-vitro conditions and its interpretation in terms of molecular function and interactions in cells. Recently, we introduced an experimental approach termed cellular solid-state NMR[1,2] aiming at bridging the gap between cellular and structural biology. Here, we have extended this concept to study the bacterial type IV secretion system (T4SS) in its physiological environment. T4SS is a macromolecular machine used by gram-negative bacteria to transport diverse molecules including proteins and DNA. In spite of its biological relevance,ranging from multidrug resistance to in-vivo gene delivery, structural information on the T4SS has so far only been obtained using a combination of EM on X-ray crystallography on purified complexes[3]. Moreover, structural insight at atomic scale is currently only available for about half of the amino acids of the core complex of this molecular machinery.
For cellular ssNMR, we coexpressed the entire core complex consisting of three core proteins VirB7, BirB9 and VirB10 using [15N ,13C] media in WT as well as OmpA/F deficient *E. coli* strains. We subsequently conducted a suite of multidimensional ssNMR experiments on isolated cell envelopes and compared our data to negative controls. We could readily distinguish ssNMR resonances from T4SS from the cellular background. Moreover, ssNMR spectra of T4SS in cellular environment reveal good agreement with spectroscopic predictions resulting from structural modelling of the core complex in FANDAS. [4]. In our contribution, we will report on our latest findings to probe the supramolecular structure of T4SS, including DNP studies at 800 MHz.

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305 TH

STRUCTURAL STUDIES OF A FILAMENTOUS BACTERIOPHAGE BY MAGIC-ANGLE SPINNING SOLID-STATE NMR

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Filamentous bacteriophages (phages) are viruses that infect bacteria. These large biological assemblies (~20-35 MDa) are composed mainly of a single-stranded DNA (ssDNA) encapsulated in a capsid that contains several thousands of copies of the structurally identical major coat protein subunits. Some phages are related to pathogens and others are utilized in molecular biology, biotechnology and nano-technology; they can be used as anti-bacterial agents, for phage-display techniques and are widely used for developing and improving pharmaceutical drugs.
The structure of the phage is governed by capsid symmetry, subunit conformation, the interaction between subunits, the interactions (or interface) between the subunits and the DNA and the structure of the DNA itself. We have demonstrated that magic-angle spinning (MAS) solid-state NMR experiments applied to M13 phage provide excellent resolution reporting on the subunit conformation and dynamics1,2 . We present here a new approach for studying the ssDNA-capsid interface by MAS NMR using proton-mediated ^{31}P-^{13}C polarization transfer experiments. These PHHC experiments allow us to map the interface residues in the C-terminal part of the major coat protein and to show that the ssDNA-capsid interactions are sustained mainly by electrostatic interactions between the positively charged lysine sidechains and the negatively charged phosphate backbone.
In order to characterize the interactions between subunits, we prepared sparsely labeled samples, and applied homonuclear correlation experiments which revealed inter-subunit contacts that are crucial for structure calculation of the entire capsid assembly. Indeed, preliminary structure calculation of the M13 capsid using ROSETTA modeling and a pre-determined C_5S_2 symmetry (from fiber diffraction) provided excellent convergence of the lowest energy structures.

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306 MO

NMR STUDIES OF THERMO-RESPONSIVE POLY(ASPARAGINE) DERIVATIVES

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Poly(asparagine) based derivatives (PAD) are unique biocompatible polymers that hold the potential for functioning as alternatives to non-biodegradable poly(acrylic acid), for use as hair setting or super-absorbent materials, and more generally as thermo-responsive polymers; a complex aggregation and dissociation process resulting in a liquid-gel transition in the aqueous solution that occurs in the temperature range of 40 to 70C. In this poster we highlight experimental findings aimed at probing the details of the molecular mechanism that gives rise to this phase transition.
The dynamics and distribution of waters of hydration was also investigated through the phase transition. The 13C solution NMR of the PAD in DMSO-d6 was used to investigate the isolated polymer including the peak assignment. By systematic addition of D2 O we have tracked structural changes due to aggregation and found hydrophilic side chains were contracted. The 13C CP/MAS NMR was also implemented to investigate the aggregates during the phase transition.
Deuterium T1-T2 and T2-T2 two-dimensional relaxation spectroscopies, using an Inverse Laplace Transform, were used to monitor the water-PAD interaction during the phase transition.

307 TU

DETERMINATION OF THE LITHIUM BINDING SITE IN INOSITOL MONOPHOSPHATASE, THE PUTATIVE TARGET FOR LITHIUM THERAPY, BY MAGIC-ANGLE SPINNING SOLID STATE NMR.

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Inositol monophosphatase (IMPase) catalyzes the hydrolysis of Inositol monophosphate to inorganic phosphate & inositol. For this catalytic process to occur, IMPase is activated by Mg^{2+} cations. In patients suffering from bipolar disorder, IMPase activity is assumed to be higher than normal thereby increasing inositol levels.
Treatment with Lithium salts reduces the activity of IMPase by means of enzyme inhibition, but the mechanism by which Lithium exerts its therapeutic effects is still in conjecture. Structural analysis by means of X-ray crystallography was unable to detect Li^{+} cations in IMPase active site. Solution ^{7}Li NMR showed no separation between free and bound Lithium.
In this study, we determine and characterize the lithium binding site in *E. Coli* SuhB IMPase using magic angle solid-state NMR spectroscopy by the use of lithium detection and by heteronuclear correlation experiments between the lithium atom and its surrounding spins.
These are the first experiments that demonstrate direct polarization transfer between a metal-ion and the surrounding spins in enzymes. Therefore, we also provide examples on model systems that mimic the enzyme binding site.

308 TH

PROBING INTERMOLECULAR STRUCTURES OF BIOMOLECULES IN SOLID STATE USING HIGH RESOLUTION ^{1}H SOLID STATE NMR UNDER ULTRA HIGH SPEED MAS

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Abstract: (Your abstract must use Normal style and must fit into the box)The well-resolved ^{1}H solid state NMR spectra of alanine tripeptide (Ala_3) with anti-parallel and parallel β sheet structures are assigned by using double-quantum magic-angle-spinning (DQMAS) measurements with ultra high field and ultra fast MAS NMR.
The observed ^{1}H chemical shifts are in excellent agreement with the ^{1}H chemical shifts calculated by gauge-including projector augmented wave (GIPAW) method for ^{1}H geometry optimized structures. This indicates the accurate ^{1}H positions of the structure. The chemical shifts of the amide protons are described by the inverse third power of inter-molecular NH…OC direct hydrogen bond distance (Yazawa et al., Chem. Comm.,2012).
We applied these intermolecular structural analyses using ^{1}H solid state NMR to the crystalline structural analysis of *Bombyx mori* silk after spinning, Silk II. Based on the results, we propose a new crystalline model of Silk II with two different packing manners.

309 MO

CROSSLINKING STUDIED BY MAS SOLID-STATE NMR

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The process of protein fixation, whereby proteins are chemically crosslinked by covalent bonding between reagents with reactive end groups and the functional groups of specific residues, is used in electron-microscopy (EM) for tissue fixation, to study protein-protein interactions, and more recently for deriving structural information on protein complexes using mass spectroscopy. In the simple idea of using a bridging reagent for conjugating proteins that are in close proximity, the unambiguous identification of crosslinked proteins, detection of the actual linkage sites and characterization of the type of bonding are sometimes entangled. Moreover, the integrity of protein complexes determined by artificial crosslinking, where the biological system is usually studied under nonphysiological conditions, is arguable.
Here we present atomic-resolution insights into glutaraldehyde-protein coupling, using magic-angle spinning (MAS) solid-state NMR. The biological system chosen for this purpose is the fd filamentous bacteriophage, a threadlike E. coli-specific virus. Each phage particle is a highly symmetrical array of ~3430 copies of a 50-residue long protein subunit, coating a circular single-stranded DNA molecule. We previously reported through chemical-shift analysis that the coat protein in fd is mostly a rigid helix. Foregoing characterization of the wild-type capsid, combined with our high-yield preparation protocol of [U-^{13}C, ^{15}N] labeled intact phage provide a convenient platform to study protein crosslinking. We show that through basic two-dimensional ^{13}C-^{13}C and ^{15}N-^{13}C correlation experiments we are able to study the effect of glutaraldehyde on specific residues in the protein shell, and hopefully could further implicate on the dynamic properties of proteins under these conditions. It is also shown how the infectivity of the phage is affected by addition of different glutaraldehyde concentrations.

Poster Presentation

310 TU

$P^{31}P$ NMR RELAXATION STUDIES OF STRONTIUM DOPED BIOLOGICAL HYDROXYAPATITE

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Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a major component and an essential ingredient of normal bone and teeth. It is one of few materials that are classed as bioactive, meaning that it will support bone ingrowth and osseointegration when used in orthopaedic, dental and maxillofacial applications.
The chemical nature of hydroxyapatite lends itself to substitution, meaning that it is not uncommon for non-stoichiometric hydroxyapatites to exist. The most common substitutions involve carbonate, fluoride and chloride substitutions for hydroxyl groups, while defects can also exist resulting in deficient hydroxyapatites.
Among the many cations that can substitute for calcium in the structure of hydroxyapatite, strontium provokes an increasing interest because of its beneficial effect on bone formation, and prevention of bone resorption. Strontium (Sr) is one of the essential trace elements in human body, which can enhance the strength of bone and prevent caries.
In this work, the effect of strontium substituted biological hydroxyapatite obtained from cuttlefish has been investigated by ^{31}P solid state NMR T_1 and T_2 relaxation. In particular, the incorporation of Sr into the crystal lattice has been examined through the analysis of the homonuclear ^{31}P-^{31}P spin-spin relaxation which is the main T_2 relaxation mechanism in hydroxyapatite. The Van Vleck second moment, which is highly sensitive to the internuclear distance over a relative short range, was found to be Sr dependent, suggesting the successful incorporation of Sr into the crystal lattice. The variation of the T_1 relaxation with Sr content was also investigated. The NMR results are correlated with those obtained from additional XRD and infrared experiments

311 TH

PREDICTING NMR SPECTRA OF NATURAL SUBSTANCES BY DFT CALCULATIONS AS A TOOL FOR STRUCTURE DETERMINATION

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Natural sources supply a fascinating variety of molecules having intricate structures and unusual functionalities. NMR spectroscopy has become a staple methodology for the determination of their structures; however, despite steady technical advancements, this information may not lead to an unambiguous molecular structure. As a result, it is not unusual to see structure revisions where a proposed molecular structure is challenged in view of more compelling arguments (often, total synthesis). The conclusive proof of structure is commonly accepted to be the match between the NMR spectrum of the unknown species and of a species deriving from total synthesis (quite often, not a trivial task). On the other hand, if the NMR spectrum of a molecule whose structure is certain were available, one could work on the problem, which requires *a priori* knowledge of the chemical shifts and coupling constants for all spins of interest. Density-functional theory (DFT) methods have enabled such computations with great accuracy. The scope and application of DFT calculations will be presented in case histories concerning natural substances including arsenicin A,[1] hexacyclinol [2] and vannusal B.[3]

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Poster Presentation

312 MO

TOWARDS A FRAGMENT BASED DRUG DESIGN STRATEGY TO DESIGN SMALL MOLECULES AS INHIBITORS OF ABETA AGGREGATION

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Intrinsic disorder is observed among proteins implicated in neurodegenerative disorders like Alzheimer's (AD) and Parkinson's diseases. In this study, a strategy for the therapeutic treatment of neurodegenerative diseases is investigated by developing a computational method to enable the screening of drug-like compounds for intrinsically disordered proteins (IDPs). Application is focused on Abeta whose abnormal self-assembly into neurotoxic oligomers and fibrils is believed to be the cause of Azheimer's Disease. Disrupting the self-assembly of Abeta will target the underlying cause of the disease. To this end, we have assessed the druggability of Abeta through identification of hotspots by performing fragment based mapping calculations on Abeta structures obtained from Molecular Dynamics Simulations and Metadynamics. These fragments are used to build a library of small molecules to computationally probe interactions with Abeta and further, understand the mechanisms of binding. Such a fragment screen is expected to successfully identify new molecular scaffolds in Alzheimer's drug discovery and provide insights into small-molecule binding to IDPs.

313 TU

GRID-FREE POWDER AVERAGING IN MAS-NMR SIMULATIONS

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The stochastic Liouville equation formalism allows for the inclusion of explicit rotational diffusion into simulations of spin dynamics in a very straightforward manner [1]. In a similar way, one can include coherent rotations such as those required in simulations of MAS-NMR spectra. The formalism that arises from doing this is similar to Floquet theory [2] in that the periodic time dependence of the orientations may be taken into account using a time independent 'Hamiltonian'. However, unlike Floquet theory, this formalism allows expansion in a general class of functions; importantly, one may use as basis functions the elements of the Wigner rotation operator [3] allowing an analytical average to be taken over all orientations to give a powder-averaged spectrum without the need for approximation with an explicit grid of angles.

The basis of functions is formally infinite; truncation to a reasonable basis dimension is however found to be possible if one includes spinning about the magic angle at rates similar to those used experimentally. Several simulated MAS-NMR spectra and their respective truncated basis dimensions are presented. We also demonstrate numerically the validity of state space restriction in these simulations, confirming what was shown previously by Dumez, et al. [4], and providing further evidence that polynomially scaling simulations [5] are possible for general simulations of MAS-NMR spectra.

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Poster Presentation

314 TH

THE PROGRAM CASPER - A USEFUL TOOL IN THE ANALYSIS OF LIQUID STATE NMR DATA OF GLYCANS

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Carbohydrates, the most abundant biomolecules found in nature, are present in all forms of life, playing essential roles in a wide range of biological processes. In order to understand the role of these compounds in biological systems it is of key importance to have knowledge of their structures, and NMR spectroscopy is by far the most powerful tool to address this problem. However, the limited spectral dispersion of both ^{1}H and ^{13}C nuclei in these types of molecules can make the process of assignments of certain resonances tedious and time consuming. The program CASPER (http://www.casper.organ.su.se/casper/), on the other hand, is a promising tool to help to overcome this problem, as the whole analysis of the NMR data can be carried out in an automatized or semi-automatized manner. This software uses liquid state NMR data to elucidate the structure of glycans based on their ^{1}H and ^{13}C chemical shifts as well as ^{1}H-^{1}H or ^{1}H-^{13}C correlations from 2D experiments such as ^{1}H,^{1}H-TOCSY , ^{1}H,^{13}C-HSQC (or ^{13}C,^{1}H-HETCOR), ^{1}H,^{13}C-H2BC and ^{1}H,^{13}C-HMBC experiments. In addition, a module for component and absolute configuration analysis has been implemented, allowing the fully automatized analysis of glycans using solely *unassigned* NMR data as input information.
Herein, we demonstrate that this program can successfully and rapidly determine the structure of the repeating unit of polysaccharides (including the absolute configuration of their component monosaccharides), or be used to predict ^{1}H and ^{13}C chemical shifts to assist in the manual interpretation of NMR data of carbohydrates in general (such in the case of preparations comprising a mixture of compounds or when the identification of a minor constituent is required). Consequently, what is considered the most tedious and time-consuming part of the structural elucidation process of carbohydrates, can be considerably reduced from several hours of manual interpretation (or even days, depending of the complexity of the system and the experience of the interpreter) to a few minutes of automatized or semi-automatized analysis.
The different features supported by CASPER till date will be exemplified and the next challenges in the development of software will be discussed.

315 MO

DETECTING FUNCTIONALLY IMPORTANT AMINOACIDS FROM 3D PROTEIN STRUCTURES

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Proteins and their intra and inter molecular interactions are dependent on the amino acids involved in the particular interaction. Interactions that are energetically favorable, have been evolved and optimized energetically over time. On the other hand, most energetically unfavorable interactions have been dismissed in nature. Thus, the maintenance of an unfavorable interaction in nature could be interpreted as functionally significant. Assuming that statistically frequent interactions can be interpreted as energetically favorable, we developed an algorithm to detect energetically unfavourable interactions.
Based on the study of Jha et al. (2011) we score each aminoacid of a Protein DataBank file. Jha et al. (2011) described how to summarize interactions of aminoacids in a matrix form. They demonstrated their approach on membrane proteins. Our algorithm assigns high scores to aminoacids with several statistically unfavorable interactions. The aminoacids with the highest score are considered statisistically unfavorable and thus they may have a significant functional role. We tested our algorithm on Sensory Rhodopsin II (phoborhodopsin) and detected several aminoacids that have been reported in the literature as having a significant role on the protein function. For example, Asp193 (Kitade et al. 2009; Ikeura et al. 2004), and Arg72 (Kitade et al. 2009) which are among the highest scored aminoacids have a role in the photochemistry of Sensory Rhodopsin II.

Poster Presentation

316 TU

QUANTUM MECHANICAL NMR SIMULATIONS OF WHOLE PROTEINS

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The reduced state space approximation in Magnetic Resonance is the observation that many spin states either remain unpopulated altogether or do not contribute to the observable magnetization dynamics in any way [1]. Such states may be excluded from consideration – the result is a family of very efficient simulation algorithms presently implemented in our *Spinach* library [2]. Importantly, the reduced matrices are a different representation of the same Lie algebra, meaning that all existing Magnetic Resonance simulation machinery (relaxation theories, exponential propagators, diagonalization methods, average Hamiltonian theories, *etc.*) remains unchanged – the only difference is smaller matrices.
We recently started to implement standard NMR, EPR, DNP and Spin Chemistry experiments in the *Spinach* library [2] – this communication reports the use of the reduced state space approximation to run quantum mechanical simulations of NOESY, HSQC, HNCO, HNCOCA, TROSY, TOCSY and other liquid-state 2D/3D NMR experiments on small- to medium-sized proteins, such as α-lactalbumin and ubiquitin. The simulations are carried out for the whole protein in one go, using a carefully pruned Liouville space that excludes unimportant and unpopulated spin states. Redfield relaxation superoperators (with a single global rotational diffusion tensor), including all cross-correlations are used in all calculations. In practice the simulations require shielding tensors, J-couplings, quadrupolar tensors and Cartesian coordinates for each participating spin. These may be supplied directly or imported from a quantum chemistry package. On modern workstation hardware, the above listed NMR simulations for ubiquitin take 24-48 hours of CPU time.
Spinach is a well-commented and well-documented open-source *Matlab* library [2] available for download at http://spindynamics.org. It is capable of simulating almost any type of spin dynamics in NMR, EPR, DNP or Spin Chemistry.

Literature

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317 TH

NEW FEATURES OF THE MMM MODELLING SOFTWARE PACKAGE

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The open-source Matlab-based software package MMM provides fast and reasonably accurate simulation of the conformational distribution of common nitroxide spin labels and of label-to-label distance distributions, including multi-spin effects for up to five labels. The graphical user interface allows for visualization of protein and nucleic acid structures symmetry or lipid-bilayer related transformation of protein coordinates. Combined with the possibilities to read PDB structures and primary DEER/PELDOR experimental data or distance distributions computed with the DeerAnalysis package, MMM can be used for testing whether structures or structure models are consistent with experimental label-to-label distance measurements.
Versions 2011.2, 2013 and 2013.2 of MMM include new features for modelling structures from a limited number of constraints and a structural template and for localizing label positions with respect to each other or in only partially resolved structures where the label position is in an unresolved domain. Modelling from a template and sparse constraints is based on anisotropic elastic network models and works best for proteins undergoing hinge motions. Alternatively, the Modeller interface of MMM provides convenient access to homology modelling with additional label-to-label distance constraints.
Positions of a label at an unresolved site with respect to a known part of a structure can be computed with two-fold ambiguity if three distances to known sites were measured and unambiguously if at least four distances were measured. Depending on the width of the label-to-label distance distributions and consistency of the experimental data, such trilateration or multilateration leads to position uncertainty, which is accounted for in MMM by computation of probability density surfaces. Relative positions in a network of labels can be computed without resorting to a structural template by a distance matrix geometry approach. Such networks can also be fitted to a structural model if at least six labelled sites are resolved in the model.
Finally, a full grid search protein-protein docking approach that we earlier introduced on the NhaA homodimer is generalized to symmetric homooligomers consisting of more than two protomers and to heterodimers and is made available through MMM.

318 MO

A NOVEL APPROACH TO COMPARE NMR PREDICTIONS BY MEASURING SIMILARITY USING BINARY TREES

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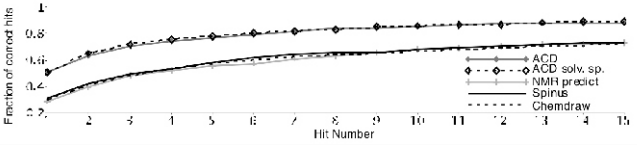
A novel approach to compare NMR prediction algorithms is proposed that doesn't require the manual assignment of data, but relies on a spectral similarity algorithm developed by the authors based on trees. This makes benchmarking of NMR predictors less tedious.
The ranking of predictors reflects their ability to predict a spectrum that is more similar to its experimental counterpart than to the other spectra predicted for molecules of the dataset. A goodness factor is established for each experimental spectrum and since the correct match is known, a Mean Reciprocal Rank (MRR) analysis can be used to summary the performance of each prediction algorithm. Here, a comparison of four popular NMR predictors (ACD/Labs, Modgraph, Chemdraw, Spinus) has been performed using 1000 molecules and their experimental spectra.

It was found (Figure 1) that ACD/Labs performs better by far (0.6 MRR for ACD, ~0.4 MRR for the other predictors). It is worth noting that the freely available Spinus platform performs as good as others commercial alternatives. In order to validate our approach, these results were compared and found consistent with those obtained using the traditional method: comparing the deviations between predicted and experimental shifts, by manually assigning our dataset.

This project was funded by Colciencias – Renata (RC-561-2009)



319 TU

THE ROLE OF SYMMETRY IN NMR ASSIGNMENT

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One of the main challenges of automatic NMR assignment consists in dealing with the huge size of the associated search space. This makes full-search strategies unsuitable even for small molecules. Branch-and-bound is an interesting approach that may allow the exploration of the space and the convergence to acceptable solutions using an acceptable number of operations. It allows to avoid the construction of non-viable branches while building the tree, when their estimated score is lower that a certain value (bound).
The success of this approach, however, relies on the design of efficient branching and bounding procedures. Here we present three recommendations that make them efficient for the assignment of small molecules: 1. The assignment process can be carried on the quotient graph of the structure formula under its group of symmetry, which discards a vast number of *a priori non-viable* solutions; 2. Symmetries of the molecule and of the spectrum preserve assignment scores, which allows for a more efficient branching; and 3. Evaluation of restrictions associated with highly reproducible and reliably predictable properties, such as intensity, increases the gap between branch bounds, which accelerates pruning of non-viable branches if performed early during the search. We were able to apply manually this strategy to perform the assignment of 5 molecules and to illustrate the efficient reduction of the solution space, step by step.

Poster Presentation

320 TH

EQUIVALENCE BETWEEN EULER ANGLE CONVENTIONS FOR THE DESCRIPTION OF TENSORIAL INTERACTIONS IN LIQUID NMR: APPLICATION TO DIFFERENT SOFTWARES

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Long-range orientational restraints derived from alignment or rotational diffusion tensors have expanded the application field of biomolecular NMR. The orientation of the principal axes system of these tensors is usually described by the so-called Euler angles.
However, no clear consensus has emerged concerning the convention of the orthogonal rotations associated with.
As a result, the different softwares that derive or predict them have adopted different rules, which make the comparison between their results difficult. Moreover, the rotation schemes are seldom completely described. Here, we resume the different conventions, determine which ones are adopted by commonly used softwares and establish the formal equivalences between the different Euler angles they calculate.

321 MO

STRUCTURAL IDENTIFICATION OF THE REACTION INTERMEDIATES IN [NiFe] HYDROGENASES BY THEORETICAL SPECTROSCOPY

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Max Planck Institute for Chemical Energy Conversion

[NiFe] hydrogenases catalyze the reversible oxidation of dihydrogen. The corresponding catalytic cycle involves a formidable number of redox states of the Ni-Fe active site, which can be distinguished experimentally by their specific IR stretching frequencies of the CN and CO ligands coordinated to iron. These spectroscopic fingerprints serve as sensitive probes for the intrinsic electronic structure of the metal core and indirectly, for the structural composition of the active site.
Density functional theory (DFT) was utilized to calculate vibrational frequencies and EPR parameters.
By carefully comparing theory and experiment, we have identified candidates for the Ni-SI_r, Ni-SI_a and Ni-R states by matching the predicted relative frequency shifts with experimental results. The Ni-SI_r and Ni-SI_a states feature a water molecule loosely bound to nickel and a formally vacant bridge. Both states are connected to each other through protonation equilibria, i.e. in the Ni-SI_a state one of the terminal thiolates is protonated, while in Ni-SI_r this thiolate is unprotonated.
For the reduced Ni-R state two models emerge as feasible options, one in which H_2 coordinates side-on to nickel, and a second that features a hydride bridge and a protonated thiolate.
For the light-induced Ni-L state, the formation of a functionally relevant nickel-iron bond upon dissociation of the hydride is unequivocally observed and is in full agreement with the observed g values, ligand hyperfine coupling constants and FTIR stretching frequencies.
The Ni-SU state remains elusive since no unequivocal correspondence between the experimental and calculated IR frequencies of the employed models has been found, indicating that a large structural arrangement may occur upon reduction from Ni-A to Ni-SU in which the bridging ligand may dissociate.

Poster Presentation

322 TU

EPR AND CALCULATIONS ON ELECTRONIC STRUCTURE OF PHENALENYL AND PHENALENYL DERIVATIVES RADICALS

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Organic free radicals are present in the asphaltene fraction of oils, but little is known about their molecular nature due to the multiplicity of molecular structures that causes the appearance of an unresolved single line with a width between 4 and 6 Gauss. When hyperfine split was observed in the EPR spectrum of marine diesel (bunker), it was possible investigate the free radical molecular structure. The results obtained for hyperfine interaction (*hf*) indicated that perinaphthenyl radicals (Figure 1a) are probably responsible for the septet-quartet EPR spectrum of this oil by-product. Further study, with marine diesel temperature variation, pointed to perinaphthenyl radical plus two phenalenyl derivatives radicals (Figure 1b and 1c) compose the EPR spectrum after heating. According to the results obtained for *hf*, hydroxyperinaphthenyl and 4,6-dimethylperinaphthenyl radicals are the most likely phenalenyl derivatives yielded upon heating the marine diesel.

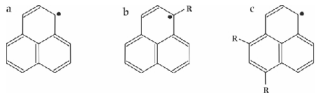

Figure 1: Structures of the phenalenyl radical (**a**) and phenalenyl derivatives (**b** and **c**).

The proposed model to explain the marine diesel spectrum with three overlapping paramagnetic species generates with great accuracy all lines of the EPR spectrum. However, with three overlapping spectra it is impossible to observe hyperfine splits caused by any functional group which replace the hydrogen in the perinaphthenyl structure. Faced with this difficulty, we resort to calculations on electronic structure of molecules responsible for the composition of the EPR spectrum. The hyperfine coupling constants were the theoretical parameters calculated and used for comparison with experimental data. The energy-minimized structures were obtained with Theory of Density Functional (DFT). The difference between experimental and theoretical values was below 7% to hyperfine coupling constants in first order (*A*) and 20% in second order (*A´*), to all structures analyzed, thereby confirming these molecular structures in marine diesel EPR spectrum.

323 TH

ADVANCED METHODS IN ORIENTATION PELDOR: A METHODICAL APPROACH TO SOLVING STRUCTURE

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Pulsed electron-electron double resonance (PELDOR) spectra become strongly dependent on spin label orientation at high fields, and it becomes possible to measure the relative orientations of rigid spin labels by performing several optimised PELDOR experiments at W-band[1], allowing for symmetries imposed by the experiment. Using the accurate orientation information from these developed methods it is possible to elucidate structures with less ambiguity and fewer measurements.

This paper will review the novel methods which have been developed to quickly and accurately analyse orientation PELDOR measurements and illustrate that:

*By applying orientation PELDOR methods with recently developed RX rigid spin labels we can accurately measure structure and flexibility of biological molecules [2].

*The experiment can be tailored to give a clear measurement pathway to solving specific structural questions – minimising experiment run time and cost.

*The accuracy of these measurements appears to be better than 5degrees with representative signal to noise.

To illustrate the technique we will show experimental results of biological systems – comparing site attachments with subtle changes in spin label conformation and showing that these changes can be resolved. We also show that by applying these techniques in specific cases, the orientation and distance of multiple spin labels can be resolved.

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Poster Presentation

324 MO

CONTINUOUS WAVE-EPR AND PULSED-EPR STUDIES OF OXYGEN-INDUCED RADICALS IN NEURONAL NITRIC OXIDE SYNTHASE

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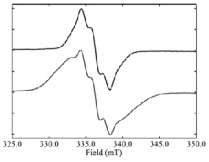
Nitric Oxide Synthase (NOS) is an enzyme that exists in three isoforms in the human body and uses L-arginine to produce nitric oxide and citrulline by a pair of monooxygenase reactions. Nitric oxide plays a major role in nerve transmission signalling (neuronal NOS), physiological processes (endothelial NOS), and pathophysiological processes (inducible NOS). We focused on radical production by the neuronal NOS (nNOS) cycle during abnormal turnover using continuous wave-EPR and pulsed-EPR. Freeze-quenched samples of nNOS with and without substrate were studied for radical production during turnover in the nNOS cycle. L-arginine and the cofactor tetrahydrobiopterin (BH_4) are two components of the nNOS cycle that are the focus for the following study. BH_4 functions as an electron donor and forms a radical cation during turnover. Samples, with and without substrate, were made in D_2O and H_2O. CW EPR spectra were simulated and fit using easyspin. The simulations of spectra with substrate (Black) are very similar to previous reports on eNOS. Without substrate (Blue), nNOS produces two radicals: one radical being the same as nNOS with substrate (BH_4^+), and the other radical is a broad species. The second radical has the same couplings as the first radical but with much larger proton couplings. Pulsed EPR showed that the two radicals had noticeably different relaxation properties. Relaxation studies find the location of the radicals with respect to the heme site: the first radical interacts with high spin heme and the second radical interacts with a low spin heme. This work is supported by National Institutes of Health HL095820.



325 TU

ORGANIC AND CONVENTIONAL WINES DIFFERENTIATION ON THE BASIS OF THEIR PHYSICO-CHEMICAL PROPERTIES ASSESSED BY EPR, UV-VIS, HPLC AND AAS

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Continuous development of organic agricultural practices and the increasing number of products labelled as “bio” or “organic”, including wines increases also the demands of both, consumers and respective food control authorities on reliable methods and practices of food authenticity verification and confirmation.
In our complex study, basic physico-chemical characteristics of certified Slovak, Czech, Austrian, Hungarian and Italian organic and conventional wines, altogether 73 samples, were evaluated. Besides the radical-scavenging and antioxidant properties and other characteristics evaluated by EPR and UV-VIS, also the capillary isotachophoretic separations was performed for organic acid determination. In addition, the content of some minerals and trace elements in wines was monitored by AAS and HPLC was employed for quantification of selected wine phenolic constituents, including (+)-catechin, (–)-epicatechin, hesperidin, rutin, quercetin and trans-resveratrol. Last but not least, objective colour determination of all the samples was performed using the UV-VIS-NIR spectrophotometer. In view of the complex data of different assays and number of samples, ANOVA and multivariate statistics were used to compare, explore, discriminate and model the data.
The results obtained demonstrate high potential of chemometrics applied on multi-compositional data for the purposes of wines differentiation and classification according to their affiliation to either organic or conventional production system. It is also very nicely applicable for the purposes of wines' geographical origin authentication. Very high classification scores in both recognition and prediction ability evaluation indicate some significant differences between the organically and conventionally produced wines, independently on the wine variety. Further studies focused on evaluation of data of a larger group of European organic and conventional wine samples are desirable.

This contribution is the result of the project implementation “Centre of Excellence for Contaminants and Microorganisms in Food – ITMS 26240120024” supported by the Research & Development Operational Programme funded by the ERDF.

Poster Presentation

326 TH

TRACING THE TRANSIENT CONFORMATIONAL SIGNAL IN BACTERIAL PHOTOTAXIS USING SDSL-EPR SPECTROSCOPY

Daniel Klose*, Julia Holterhues*, Meike Müller-Trimbusch*, Enrica Bordignon*[‡], Ines Heinrich[+], Lin Li[+], Martin Engelhard[+], Johann P. Klare* and Heinz-Jürgen Steinhoff *

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In microbial photo- and chemotaxis a two-component signaling cascade mediates a regulated response of the flagellar motor to environmental conditions. Upon activation, photo- and chemoreceptors transfer a signal across the plasma membrane to activate the histidine kinase CheA. Successive regulation of the CheY-phosphorylation level controls the flagellar motor.

In Natronomonas pharaonis a sensory rhodopsin II – transducer complex (SRII/HtrII) mediates negative phototaxis.[1] As the initial signal, a light-induced outward movement of receptor helix F leads to a conformational change of transducer helix TM2, which in turn propagates the signal to the adjacent HAMP domain.[1,2]

For the HAMP domain, a widely abundant signaling module, several mechanisms were suggested[3], all comprising two distinct conformational states. These can be observed by two-component cw-EPR spectra at ambient temperatures existing in a thermodynamic equilibrium which can be driven by salt-, temperature- and pH-changes.[4]

To trace the conformational signal and it's propagation throughout the elongated transducer, we applied cw- and pulse-EPR spectroscopy in conjunction with nitroxide spin labeling. We follow transient changes by time-resolved cw-EPR spectroscopy and compare the resulting spectral changes to difference spectra corresponding to the above shifts in the thermodynamic equilibrium. The light-driven conformational changes are in agreement with a shift towards a more compact state of the HAMP domain.

Following this signal beyond the HAMP domain requires a mechanism compatible with the formation of trimers of SRII/HtrII dimers which activate CheA. An activation scheme within the framework of hexagonal arrays formed by the trimers of SRII/HtrII will be the key step to understanding the enormous cooperativity leading to signal amplification in networks formed by clusters of interacting receptors.

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327 MO

NEW 2,5-BIS(SPIROCYCLOHEXANE)-SUBSTITUTED NITROXIDES OF PYRROLINE AND PYRROLIDINE SERIES AS SPIN LABELS: ADVANCED PROPERTIES AND APPLICATIONS TO DISTANCE MEASUREMENTS IN RNA

O. A. Krumkacheva[1], I. A. Kirilyuk[2], Y. F. Polienko[2], I. A. Grigor'ev[2], R.K. Strizhakov[3], E. S. Babailova[3], A. V. Ivanov[3], M.A. Vorobjeva3, A.G. Venyaminova[3], A. A. Malygin[3], G.G. Karpova[3], M. V. Fedin[1] and E. G. Bagryanskaya[1,3]

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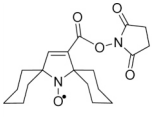
Over the past few years the development of site-directed spin labeling methods allowed measurements of distances and distance distribution functions in double labeled oligonucleotides using pulsed electron–electron double-resonance (PELDOR) spectroscopy. It has been found recently that piperidine nitroxides with spirocyclic moieties at α-carbons of nitroxide group may have advantages over 2,2,6,6-tetramethyl analogues in structural studies using PELDOR [1].

In this work [2] we investigated chemical and spectral properties of new 2,5-bis(spirocyclohexane)-substituted nitroxides of pyrroline and pyrrolidine. New spin labels demonstrate clear advantages over 2,2,5,5-tetramethylpyrroline nitroxides with respect to electron spin relaxation rates, which should favor PELDOR distance measurements at liquid nitrogen temperature range. Moreover, new nitroxides demonstrate much higher stability toward reduction by ascorbate than spirocyclohexane-substituted nitroxides of piperidine series and showed 1.3−3.1 times lower reduction rates compared to corresponding 2,2,5,5-tetramethyl nitroxides.



For the first time we used new spirocyclohexane -substituted spin label with advanced relaxation properties and high stability toward reduction by ascorbate to measure distances for oligonucleotides using Q-band PELDOR. To test this spin label, we applied it to model RNA duplex with known distances between corresponding residues and compared results with standard 2,2,5,5-tetramethyl-substituted nitroxides. The obtained distance distributions correspond well to the expected ones. It implies that novel spirocyclohexane-substituted nitroxides are promising spin labels for distance measurements by PELDOR, including structural investigation of RNA and DNA in cell.

This work was supported by the Russian Foundation for Basic Research (12-03-33010, 12-04-01435).

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Poster Presentation

328 TU

IMPROVING THE SENSITIVITY OF THE FREQUENCY DOMAIN MAGNETIC RESONANCE

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The high-frequency electron paramagnetic resonance (HFEPR) and frequency domain magnetic resonance (FDMR) spectrometer recently installed at University of Stuttgart will be presented together with first obtained results. Our aim is to improve sensitivity in high-field EPR in order to enlarge the scope of this powerful method. Especially, we are interested in the possibility to perform measurements in the frequency domain, in zero applied field, which excludes the possibility of the large external magnetic field influencing the sample properties. The spectrometer operation frequency is from 85 GHz to 1100 GHz with a maximal field of 17 T.
For the low loss propagation of microwave quasi-optics in combination with a corrugated waveguide is used. The sample is placed either in a non-resonant cavity or in a Fabry-Pérot (FP) resonator, located in a variable temperature cryostat. The cryostat allows measuring in the temperature range from 1.8 K to 300 K. The measurement is controlled with software written in LabView.

329 TH

HIGH SPIN EFFECTS IN DEER DISTANCE MEASUREMENTS OF GD^{3+}-GD^{3+} MODEL SYSTEMS

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3. *Department of Chemistry, Bielefeld University, Bielefeld, Germany.*

Gd^{3+} (S=7/2) based spin labels have been shown in the past few years to be a viable alternative to the standard nitroxide tags, for DEER experiments at high fields[1,2]. The analysis of the DEER trace data has been typically performed as though it was a system of two S=1/2 spins, rather than two S=7/2 spins. This has been justified by estimations based on second order perturbation theory which show that the correction to the quantization axis due to the crystal field interaction (cfi) does not result in a change in the frequency of the dipolar modulations observed in the DEER traces as long as $D/v_0<<1$[1], where D is the cfi parameter and v_0 is the spectrometer frequency. These calculations were based on two assumptions:(i) The magnitude of the dipolar interaction is much smaller than the frequency difference of the 2 spins (weak coupling) and (ii)multiple transitions per spin packet are avoided[3]. Indeed, distance measurements on several biological systems have produced distances similar to the distances predicted by other methods[2,4,5]. In this work we will show high field DEER measurements performed on two different bis-Gd model systems which are highly rigid. Analysis of the DEER traces show that the observed modulations decay much faster than expected, resulting in a FT which is not an ideal Pake pattern. Structural calculations of the models indicate that this modulation damping cannot be attributed to the expected distance distribution. To obtain a better understanding of this phenomenon we will show full quantum mechanical density matrix simulations of the DEER experiment on a spin pair with S>1/2.

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Poster Presentation

330 MO

PULSE EPR, ENDOR AND ELDOR-DETECTED NMR SPECTROSCOPY ON THE WATER-OXIDIZING MANGANESE CLUSTER IN PHOTOSYSTEM II

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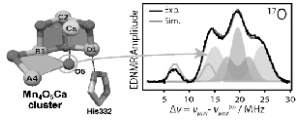

The mechanism of light-driven oxidation of water to O_2 by the protein complex photosystem II (PSII) is a major unre-solved question in bioinorganic chemistry. The water-oxidizing complex (WOC), a Mn_4O_5Ca cofactor, passes through five distinct redox states ($S_0 – S_4$) in the course of its catalytic cycle. Of those states that can be isolated and trapped, S_2 and S_0 possess total spin S_t = ½ ground states and thus are readily accessible to pulse EPR techniques. By a combined multi-frequency EPR/ENDOR/ESEEM/DFT approach, we have developed a detailed picture of the geometric and electronic structures of these S states. Specifically, we can assign the local valence states of all Mn ions in the cluster, with the total valence state configurations for the S_2[1,2] and S_0 states being $Mn^{III}(Mn^{IV})_3$ and $(Mn^{III})_3Mn^{IV}$, respectively. This is in part achieved by using intrinsic local spin probes such as the $^{14}N/^{15}N$ ligand of the histidine coordinated to the Mn_{D1} ion, as well as introduced spin probes including small ligating molecules (e.g. NH_3). The model we have developed for the Mn_4O_5Ca complex allows us to identify the sites of substrate water binding by means of isotopic labelling using $H_2{}^{17}O$ and W-band (94 GHz) ELDOR-detected NMR (EDNMR). These experiments identify an exchangeable μ-oxo bridge in the S_2 state as a likely substrate water[3], namely O5. This assignment is supported by site-directed perturbations of the bridge via substitution of the Ca^{2+} site with Sr^{2+} and ligation of the NH_3, displacing the Mn_{A4}-bound water *trans* to the O5 bridge. We also demonstrate that O5 is bound already in the S_0 state, supposedly being protonated, i.e. a μ-hydroxo bridge. These results provide a comprehensive picture of the early S states and reaction steps and allow us to limit possible catalytic mechanisms to those that include O5 as a substrate.

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331 TU

YARDSTICKS AND SLIDE-RULES TO IMPROVE PULSED EPR DISTANCE MEASUREMENTS

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PELDOR (Pulsed Electron-Electron Double Resonance) is an emerging technique for nanometre distance measurements between specific sites of biomolecules for structural modelling[1]. Most commonly nitroxide radicals are used as probes for EPR distance measurements because they are straightforward to introduce in biological systems such as soluble and membrane proteins and nucleic acids[1][2].

PELDOR distance measurements currently rely on Tikhonov regularisation method to process frequency modulation to distance distributions. This method has been proven to be reliable and robust when considering two spins, although when more than two spins are present in the system analysed artefacts in distance distributions can be generated leading to uncertainties in data interpretation[3]. Tikhonov regularisation in all current implementations uses a kernel function which considers only spin pairs, omitting multi-spin effects[4].

Experimentally benchmarking the problem and developing and implementing solution strategies requires acquisition and processing of high quality data from multiply spin-labelled systems. Thus, we chose to synthesise chemical model compounds containing two, three or four nitroxide moieties and short or long distances between the radicals.

Synthesis of model compounds, first results on distance measurements and data processing will be presented.

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Poster Presentation

332 TH

PELDOR IN ROTATIONALLY SYMMETRIC HOMO-OLIGOMERS

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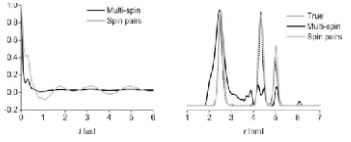
PELDOR (pulsed electron-electron double resonance) has been proven reliable in extracting nanometre distances for structural biology. The theory behind the experiment is well-defined for two spin systems, however recently research has been extended to homo-oligomers, such as membrane transporters, which lie beyond the scope of common analysis.

In this work, we have numerically explored multi-spin systems (up to octamers) assuming spin labels to be arranged in regular convex polygon symmetry. The results demonstrate that the dipolar evolution and consequentially the distances obtained will be significantly distorted, due to the appearance of both spin pair and multi-spin frequencies.



Time (left) and distance domain (right) data of a hexagon. Black traces treat all coupled spins, whereas grey traces explicitly assume spin pairs.

In order to improve data analysis approaches in homo-oligomers, we have implemented a minimisation approach which treats the above mentioned frequencies and we demonstrate its feasibility on a seven-fold labelled heptameric Mechanosensitive Channel of Small Conductance of *E. coli*.

A. Giannoulis[1,2,3], R. Ward[2,3], E. Branigan[2], J. H Naismith[2], B. E. Bode[1,2,3,], Mol. Phys., in press*

333 MO

DISTANCES AND ORIENTATIONS WITH DEER/PELDOR AT HIGH EPR FREQUENCIES

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PELDOR spectroscopy is a well-known technique to measure inter-spin distances in a nanometer range. Besides this, if applied at high fields/frequencies (95 and 263 GHz), the technique exhibits an enhanced orientation selectivity. Thus, it can be used to provide information on the relative orientation of spin-labels in a pair, assuming those are rigidly oriented in a studied bio-macromolecule. Nevertheless, general applicability of the method is hampered by difficulties related to the performance of a two frequency experiment at such frequencies. Furthermore, the inherent symmetry of the involved interactions complicates the analysis of the orientation selective data.

We attempt to refine the technique, and in general, to extend the applicability of high field DEER/PELDOR by implementing a dual-mode resonator[1-3] and by increasing the frequency of the measurements. Our recent results on two representative biological systems, i.e. an RNA and an α-helical peptide[2], permit to explore the feasibility of the approach. We show how the performance of the method can be improved by enhancing resolution toward orientation selectivity and by setting proper constraints for the orientation analysis. Finally, initial results[3] and further prospects of using spin-pairs with different spectral properties for selective distance measurements at high fields are discussed.

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Poster Presentation

334 TU

STRUCTURAL ARRANGEMENT OF A METHIONINE MOTIF IN THE PRESENCE OF THE C-TERMINAL DOMAIN OF THE HUMAN COPPER TRANSPORTER, CTR1, CU(I) AND AG(I) IONS, WAS REVEALED BY EPR SPECTROSCOPY

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The chemistry department, faculty of exact science, Bar Ilan University, Ramat-Gan, Israel, 52900

Copper is an essential element whose localization within cells must be controlled to avoid toxicity in human, yeast, and bacteria cells, requires the presence of specific mechanisms for acquisition that are intimately linked to controlled distribution, which are yet to be fully understood.
Herein, we utilize continuous wave (CW) and pulsed electron paramagnetic resonance (EPR) spectroscopy together with site-directed spin label to explore structural changes that occur in the methionine motif upon binding a specific metal ion and controlled copper transportation mechanism by probing structural changes that occur in the c-terminal domain of the human copper transporter, CTR1, upon interacting with a methionine segment. The copper transporter CTR1 transports Cu(I) and Ag(I) ions to various intracellular pathways. Methionine motifs are methionine-rich metal binding segments found in many proteins involved in the transportation of copper ions to other cellular pathways, and are found to bind Ag(I) with an affinity comparable to Cu(I).
This study indicates that the methionine motif experiences conformational changes while coordinating to metal ions and CTR1 c-terminal domain. In addition, the data of this study emphasizes the importance of the cysteine residue of the CTR1 c-terminal domain to a correct conformational state of the target metal binding site.

335 TH

LOW-TEMPERATURE EPR STUDY OF GLASSY MODES IN DISORDERED SOLIDS

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Electron spin-lattice relaxation time measurements (T_1) of two different types of paramagnetic centers incorporated in solid host matrices experiencing glassy and crystalline type of molecular packing are presented. The systems under investigation consist of nitroxyl radical labeled solid ethanol and irradiated anhydrous solid trehalose. The largest difference in T_1 is detected below ca. 80 K pointing to the universal properties of amorphous/disordered solids, which can be studied by electron paramagnetic resonance spectroscopy (EPR). In specific, the involvement of glassy/soft/boson peak modes, which enhance the energy exchange between the spin system and the lattice in the glassy as compared to the crystalline state, is discussed [1,2].

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Poster Presentation

336 MO

CROSS-POLARISATION EDITED ENDOR

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Electron nuclear double resonance is a fundamental technique in EPR spectroscopy that directly detects hyperfine transitions of nuclei coupled to a paramagnetic center. Despite its wide use, spin-sensitivity and restricted spectral resolution in powder samples pose limitations of this technique in modern application fields of EPR. In this contribution we examine the performance of an ENDOR pulse sequence that utilizes a preparation scheme different from conventional Davies ENDOR. The scheme is based on electron-nuclear cross polarization (eNCP), which requires concomitant microwave (MW) and radio-frequency (RF) irradiation satisfying specific matching conditions between the MW and RF offsets and the hyperfine coupling. Changes in nuclear polarization generated during eNCP can be detected via a conventional ENDOR read-out sequence consisting of a RF -pulse followed by EPR-spin echo detection. Using ^{1}H BDPA as a standard sample, we first examine the CP matching conditions by monitoring the depolarization of the electron spin magnetization. Subsequently, so-called CP-edited ENDOR spectra for different matching conditions are reported and analyzed based on the provided theoretical description of the time evolution of the spin density matrix during the experiment. The results demonstrate that CP-edited ENDOR provides additional information with respect to the sign of the hyperfine couplings. Furthermore, the sequence is less sensitive to nuclear saturation effects encountered in conventional ENDOR.

337 TU

USING BOUND FATTY ACIDS TO DISCLOSE THE FUNCTIONAL STRUCTURE OF SERUM ALBUMIN

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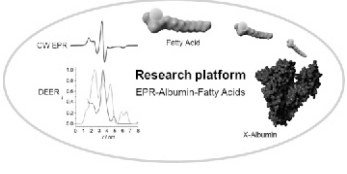
Serum albumin is a major transport protein in mammals and is known to bind to a wide variety of small molecules such as for long-chain fatty acids (FAs). Using a new electron paramagnetic resonance (EPR) spectroscopic approach, we aim to gain information on the functional structure of serum albumin in solution as a "coarse-grained" picture from the ligands' point of view. This approach is based on using spin labeled (paramagnetic) stearic acids self-assembled with albumin and subsequent nanoscale distance measurements between FAs using double electron–electron resonance spectroscopy (DEER). Simple continuous wave (CW) EPR spectroscopy complements our studies, e.g. by quantification of bound ligands.

Based on DEER distance measurements, nanoscale differences in the functional solution structures between different albumins can be revealed with the aid of crystal structure evaluation, comprising symmetry of ligand alignment and flexibility of the protein interior and surface, respectively.

Going beyond fundamental structural studies, we are able to establish a research platform utilizing the albumin model system for general dynamical and functional studies of proteins or albumin-based hybrid materials and their corresponding ligand interactions.



338 TH

EPR CHARACTERIZATION OF POLARON STATES IN ORGANIC CONDUCTING MATERIALS

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University of Antwerp, Belgium
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Organic semiconductors are becoming more and more important in daily life as modern functional materials, with wide potential applications in LEDs, sensors and photovoltaics.
In this work, we focus at understanding the structural and electronic properties of the polaron states of series of organic oligomers and small molecules. Polaron states can be induced electrochemically or chemically, or by charge transfer of the molecules blended in suitable polymers (photovoltaic effect). Two families of materials are studied: poly-phenylvinylene (PPV) –like oligomers and 5-aryl-2,5-dithienylthiazolo[5,4-d]thiazole derivates. While most molecules turn out to be good electron donors, some show electron-accepting capacity in blends with polymers. Advanced EPR techniques and DFT are combined to study the positive and negative polarons, revealing information about the electronic structure and stacking properties of the small molecules.

339 MO

SPECIES-SPECIFIC DIFFERENCES IN ABC EXPORTERS: ASYMMETRY AND CONFORMATIONAL TRANSITIONS

Simon Böhm[1], Michael Hohl[2], Markus Seeger[2] and Enrica Bordignon[1]

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The common understanding of the mode of action of ABC exporters is that the nucleotide binding domains (NBDs) close in presence of ATP/Mg leading to an outward-facing conformation of the transporter followed by hydrolyzation to ADP and reopening to the inward-facing state.
The crystal structure (3QF4) of the heterodimeric, hyperthermophilic ABC exporter TM287/288 shows an asymmetric inward-facing conformation with only one molecule of the non-hydrolyzable ATP analogue AMP-PNP bound to the degenerate site. The NBDs show only partial interaction and the overall conformation of the transporter is an inward-facing state.
The aim of this work is to compare the nucleotide- and substrate-induced conformational transitions of different heterodimeric ABC exporters to address species-specific differences. The systems chosen are TM287/288 from a hyperthermophilic and EF789/790 as well as LmrCD from mesophilic bacteria. Homology models were created where no structure is available yet as a starting point. Positions for spin-labeling were screened to be in similar regions of the different transporters.
The results obtained on pairs of all three spin-labeled exporters are presented. Distance distributions on several double mutants of TM287/288, of EF789(189R1)/790(198R1) and of LmrC(186R1)/D(313R1) in the apo and AMP-PNP/Mg states are presented. Interestingly, and in contrast with observations in other ABC transporters such as MsbA, e.g., in the AMP-PNP/Mg state no significant distance changes are observed with respect to the inward-facing state. How this is related to species-specific differences or to the heterodimeric nature of the ABC exporters will be subject of further analysis.

POSTER PRESENTATION

340 TU

POLARITY AND HYDROGEN BONDING WITHIN THE RADICAL TRANSFER OF RIBONUCLEOTIDE REDUCTASE IA *E. COLI* STUDIED WITH PULSED 263 GHZ EPR AND W-BAND ^{2}H ENDOR SPECTROSCOPY

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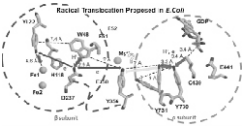
The *de novo* synthesis of DNA precursors in all living organisms, essential for DNA synthesis and repair, depends on Ribonucleotide Reductases (RNRs). The catalyzed reduction from ribonucleotides to deoxyribonucleotides is highly regulated. Nearly all eukaryotes use RNR subtype Ia. In the active form, *E. Coli* RNR Ia is composed of two homodimeric subunits: α^2 and β^2.



A radical transfer from the diiron center to the nucleotide reducing thiyl radical (C_{439}•) is proposed to occur as a Proton Coupled Electron Transfer (PCET) from a stable tyrosine radical (Y_{122}•) via W_{48}[?] to Y_{356} in the β-subunit and via Y_{731} and Y_{730} to C_{439} in the α-subunit. By introducing a new rate limiting step in the reaction, it was possible to observe on-pathway radicals. We employed the successive incorporation of 3-aminotyrosines (NH_2Y) - with the nonsense suppression methodology - on the positions (β-Y_{356}, α-Y_{731}, α-Y_{730}). The radical can be trapped at a single amino acid position, due to the altered redox potential (ΔE = 190 mV) of the amino acid mutant. Here we report the first pulsed EPR spectra of the three transient NH_2Y radicals at 263 GHz that allow a detailed analysis of the g-values. A trend in polarity of the three mutants is derived. This trend is consistent with ^{2}H hyperfine spectra of $N^{2}H_2Y_{730}$• and $N^{2}H_2Y_{731}$•, probing the local exchangeable hydrogen nuclei in their surrounding (5 Å). H bonding interactions can be assigned. In contrast, NH_2Y_{356}• shows a polar environment, but has no exchangeable ^{2}H nuclei within the hydrogen bond range. The possibility of Mg^{2+} in the surrounding of Y_{356}• is discussed.

341 TH

RELAXATION ENHANCEMENT BASED DISTANCE MEASUREMENTS ON ORTHOGONALLY LABELLED PROTEINS

Sahand Razzaghi[1], Enrica Bordignon[1], Luca Garbuio[1], Petra Lueders[1], Evan Brooks[2], Wayne L. Hubbell[2], Maxim Yulikov[1] and Gunnar Jeschke[1]

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[2] *Jules Stein Eye Institute, UCLA, Los Angeles USA*

The relaxation enhancement of a nitroxide spin-probe, induced by a fast relaxing paramagnetic lanthanide center can be used as an alternative to DEER/PELDOR experiments to measure distances in macromolecules [1].

In the presented work we apply the longitudinal relaxation enhancement approach to determine distances in orthogonally spin labelled T4-Lysozyme [2] and on WALP23 polypeptides [3].
We compare extracted distances to Gd(III)-nitroxide DEER data, discuss the shape of the relaxation enhancement curve, maximum relaxation enhancement temperature, and the overall performance. Key steps of data processing, types of obtained distance information are discussed and single temperature measurements are shown to be sufficient for reliable distance determination. Studies of conformational changes and of biomacromolecule association-dissociation are proposed as possible application area of the RE-based distance measurements.

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Poster Presentation

342 MO

EXTENDING THE ELECTRON SPIN COHERENCE TIME OF ATOMIC HYDROGEN BY DYNAMICAL DECOUPLING

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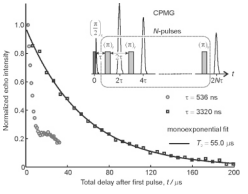

Hybrid electron-nuclear spin systems and in particular paramagnetic atoms trapped in molecular cages like endohedral fullerenes (e.g. $N@C_{60}$ or $P@C_{60}$) are promising components of spin-based quantum computing because they have long spin relaxation times and can be precisely placed into large arrays by chemical engineering. Atomic hydrogen encapsulated in polyhedral octa-silsesquioxanes (POSS) can be even more attractive due to its simpler electronic 1s state and the exceptionally large hyperfine coupling of 1420.406 MHz. Crucial properties for quantum computing like the spin-lattice T_1 and spin-spin T_2 relaxation times depend strongly on the type of the peripheral organic substituents. Recently,[1] we showed that the room-temperature phase memory time T_M=13.9 µs for the species with $R=OSiMe_2H$ is the longest observed so far for this kind of cages. Here we study the electron spin decoherence of $H@Q_8M_8$ ($R=OSiMe_3$) induced by the ^1H and ^{29}Si nuclear spin bath. By applying the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence we significantly suppress the low-frequency noise due to nuclear spin flip-flops up to the point where a maximum T_2 = 56 µs is observed. Moreover, dynamical decoupling with the CPMG sequence reveals the existence of two sources of high-frequency noise: first, a fluctuating magnetic field with the proton Larmor frequency, equivalent to classical magnetic field noise imposed by the ^1H nuclear spins of the cage organic substituents, and second, decoherence due to entanglement between the electron and the inner ^{29}Si nuclear spin of the cage.

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343 TU

APPLICATION OF COARSE-GRAINED MODELLING CONSTRAINED BY EPR DISTANCE DATA TO INVESTIGATE THE FOLD OF MEMBRANE PROTEINS

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Lab. Phys. Chem., ETH Zurich, Switzerland; Biology Department, LMU Munich, Germany

Classically used methods of structural biology like X-ray diffraction or NMR may have serious limitations when applied to membrane proteins. Among the factors responsible for that are big protein size, an often large conformational flexibility, and the necessity to use detergent for crystallization. Spin labeling combined with pulse EPR measurements and computational methodology offer an independent, complementary approach to study structure and function of such systems.

Here we present a coarse-grained procedure based on the matrix geometry approach and long-range distance constraints from the Double Electron-Electron Resonance (DEER) experiment to model the architecture of membrane proteins. Within the procedure helices are taken as straight cylinders (ideal helices), spin labels are treated explicitly, while the experimental distances provide lower and upper bounds for the modeling.

We tested our approach on proteins with a known structure, which were different either in the size or in the degree of deviation of the helical shape from ideal. On the example of a bovine rhodopsin with seven relatively straight transmembrane helices, our method was shown to predict the fold successfully when distances between backbone atoms at all helix ends are known. A proper fold is still obtained when the distances are between spin labels. In the case of the sodium galactose transporter vSGLT direct application of the approach is hindered by a strong non-ideality of the helices. Interestingly, the presence of spin labels helps to overcome this limitation at the cost of precision.

We applied our procedure also to the sodium proline secondary symporter PutP, whose structure is unknown. As the number of available distance constraints (about 80) is not sufficient to uniquely position 13 transmembrane helices in space, similarity to other proteins that are presumably sharing the same fold was included by using a template for a ten-helix core during calculations. Although the template information dominates the obtained fold of the core, our approach does allow to place non-core helices of PutP with respect to the protein core, based solely on the DEER distance data.

Poster Presentation

344 TH

NUMERICAL SIMULATION OF OFF-RESONANCE SATURATION, AN MRI SEQUENCE FOR POSITIVE CONTRAST WITH SUPERPARAMAGNETIC PARTICLES

Delangre Sébastien, Gossuin Yves

UMONS

Magnetic Resonance Imaging (MRI) is a powerful non invasive medical imaging technique that provides images with excellent resolution and intrinsic contrast. MRI sometimes requires contrast agents to increase the visibility of the area of interest such as tumors.
Two types of contrast agents are currently used in MRI: paramagnetic ion complexes such as gadolinium complexes and superparamagnetic iron oxide nanoparticles (SPIO). Ion complexes produce positive contrasts in opposition to SPIO, which produce only a negative contrast with conventional imaging sequences. SPIO presents some advantages over ion complexes: they are biocompatible and one unit carries many active ions, which leads to a lower detection threshold than ion complexes. Unfortunately, negative contrast can be difficult to interpret because it can also be produced by a lot of sources independent of SPIO as air bubbles or tissues interfaces. Some new imaging sequences which allow obtaining positive contrast with SPIO to avoid this problem were developed these last years. One of them is the Off-Resonance Saturation (ORS) technique. The idea behind this sequence is to saturate the signal near the SPIO and subtract the resulting image to an image obtained without saturation of the protons near the SPIO. The resulting image is an image with a positive contrast near the SPIO. ORS works well experimentally both in vitro and in vivo but there have so far been no systematic studies to ascertain the influence of the particles and sequence characteristics on the resulting contrast. In the present work, we detail the results of such a study carried out using a numerical simulation approach. Our numerical simulations reveal that good contrast can be obtained by SPIO with very large and very small radius, while poor results are obtained at intermediate particles sizes. The radius limits depend on the sequence and SPIO characteristics. The SPIO concentration also affects the contrast obtained by ORS. The contrast increases with concentration until a limit value is reached. In conclusion, numerical simulations allow determining a set of optimum parameters leading to a maximum contrast with ORS.

345 MO

SELECTIVE LABELING OF BIOMOLECULES AND APPLICATION TO CELL IMAGING

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Selective labeling of biomolecules has judicious implications and applications, especially on localization of biologics and drugs in discrete subcellular compartments within living cells. Tat^{48-60} is a dodecapeptide which is derived from the Tat protein excreted by virus-infected HIV cells and has been established to present very high cellular permeability[1,2]. The importance of such cell penetrating peptides (CPPs) is that can be used to carry into the cell various biological cargoes (i.e. proteins, nucleic acids, drugs) without activating any cellular pathway or presenting toxicity. This is especially important since numerous drugs fail to harness their full therapeutic potential due to their inadequate cellular delivery properties. The covalent attachment of a chromophore group on the Tat may allow the detection region within the cell. In an effort to develop a more photostable chromophore, with respect to the classical used fluorescein, we conjugated on Tat peptide, through amide bond, two distinct chromophore groups that present strong luminescence in the visible spectrum: (a) (green) carboxyfluorescein (FC) and (b) (red) polypyridine ruthenium complex ($[Ru(bipy)_3]^{2+}$). By fluorescence microscopy experiments on HELA tumor cell line, we observed that in both cases the labeled transmembrane carrier was able to enter the cell with an unknown, but non-specific mechanism. In the case of $[Ru(bipy)_3]^{2+}$ greater photostability occurs after continuous irradiation of molecules and the extra resolution may be increased significantly due to the long life time of luminescence introduced (about 500 ns).

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Poster Presentation

346 TU

COMBUSTION RESISTANCE OF THE ^{129}XE HYPERPOLARIZED NUCLEAR SPIN STATE

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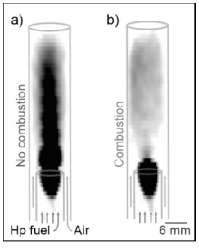
This work tackles the technical difficulties associated with *in situ* MRI of combustion processes. Previously, Glover *et al.* have presented *in situ* combustion studies that allowed for MR images of fuel entering the reaction zone however the combustion zone itself remained elusive [1]. Using the MR signal of combusting molecules is difficult due to the very short relaxation times associated with the radical reaction mechanisms and the low spin density in the combustion zone. Anala *et al.* demonstrated that a hyperpolarized ^{129}Xe mixture could be added to a fuel for two-dimensional nuclear magnetic resonance spectroscopy of combustion [2] and ongoing research with hp^{129}Xe by Pines and co-workers is attempting flow field measurements in micro-combustors [3]. However, the extent to which the hyperpolarized state of the noble gas survived the combustion process remains unknown and the experimental procedures are cumbersome.



Using a methane-xenon mixture directly for spin exchange optical pumping (SEOP), instead of mixing the gases after SEOP, allows for fairly straightforward MRI protocols with a continuous flow of fuel and hp^{129}Xe as contrast agent. The ^{129}Xe hyperpolarized spin state was found to survive the complete passage through the harsh environment of the reaction zone as shown by the MR images in the adjacent figure. A velocity profile demonstrates the feasibility of MRI velocimetry of transport processes in combustors.

References:
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347 TH

MAGNETIC RESONANCE MICROSCOPE USING A BULK SUPERCONDUCTING MAGNET

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We have developed the magnetic resonance (MR) microscope using a bulk superconducting magnet.
The bulk magnet comprises six annular bulk superconductors (60 mm outer diameter, 28 mm inner diameter, 20 mm high) made of c-axis oriented single-domain $EuBa_2Cu_3O_y$ crystals. These bulk superconductor cool down below Tc by pulse-tube GM cryo-cooler. Therefore, the magnet system is cryogen free.
The magnet was energized using a superconducting 300 MHz Wide Bore NMR magnet operating at 4.7 T by using Field Cooling (FC) Method. Three-dimensional MR images of an apple seed, acquired with voxels of 50 μm^3 demonstrated the potential of our system.

Poster Presentation

348 MO

EFFECTS OF STENT ON INTRA-ANEURYSMAL BLOOD FLOW ASSESSED BY MEANS OF 3D VELOCITY MAPS USING LOW FIELD NMR TOMOGRAPHY

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Aneurysms are originated by a weakness of the vessel walls, locally increasing the normal size of the artery or vein. The two events that dominate the evolution of an intracranial aneurysm are growth and rupture, being both dependent on intra-aneurysmal flow. Decrease of intra-aneurysmal flow is considered an alternative for treating intracranial aneurysms. Such modification can be achieved by inserting stents or flow diverters. Before the insertion of the stent a study can be done to determine if the flow alteration will lead to the formation of a thrombus in the aneurysm and thus an aneurismal occlusion. Such study helps at choosing the flow diverter with correct construction parameters for the treatment. The measurements of the flow alteration are done using silicon models that copy the patient anatomy. Dynamic NMR imaging is the only method capable of measuring velocity patterns within flowing systems in a complete non-invasive way. This makes MRI velocimetry an attractive technique to study flow phenomena in many areas of research, in particular medical systems.
In this work we investigate whether flow in aneurysms can be detected my means of MR without stent artifacts. We present low field NMR imaging for the study of intra-aneurysmal velocity patterns under steady flow conditions before and after the insertion of a stent. A spin-echo pulse sequence was implemented for velocity map measurements in a portable low-field tomograph (0.22T) to study the flow behavior in phantom systems resembling arteries with aneurisms. 3D velocity maps were measured in liquids with symilar rehological properties as blood. It was posible to determine the influence of the local geometry of the artery in the internal flow of the aneurism. In absence of flow diverters, a rotational vortex in the flow pattern could be observed. The flow effects due to the insertion of the stent provoked a complete alteration of the flow patterns and a reduction of velocity magnitudes.

349 TU

EVALUATION OF THE STABILITY OF NITROXIDE- AND CARBON-BASED RADICALS FOR IN-CELL EPR

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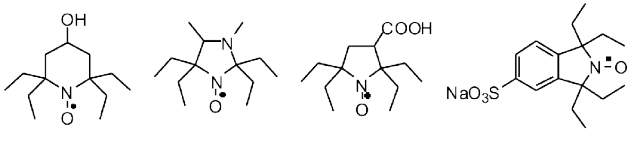
Electron paramagnetic resonance spectroscopy (EPR) is a powerful and widely used technique for studying structure and dynamics of biomolecules under bio-orthogonal conditions. In-cell EPR study is an emerging area in this field; however, it is hampered by the reducing environment present in cells, which reduces most nitroxide spin labels to their corresponding diamagnetic N-hydroxyl derivatives. Stability of nitroxide radicals depends on the size of nitroxide-bearing ring and substituents present in vicinity of the nitroxide moiety. To determine which radicals are best suited for in-cell EPR studies we systematically studied the stability of radicals in different structural contexts. In particular, we prepared piperidine-, imidazolidine-, pyrrolidine-, and isoindoline-based nitroxides, containing either methyl or ethyl substitutions adjacent to the nitroxide functional group. The kinetic and thermodynamic aspects of nitroxide reduction were studied by cyclic voltametry and the rate of reduction in the presence of ascorbate, cellular extracts and in oocytes by continuous-wave EPR spectroscopy. Our study reveals that a tetraethyl-substituted pyrrolidine-derived nitroxides are good candidates for in-cell EPR studies.



350 TH

SITE-SPECIFIC ISOTOPE LABELING FOR IN SITU MEMBRANE PROTEIN NMR ANALYSIS AND PHOSPHORYLATION QUANTIFICATION

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Isotope labeled Unnatural amino acids provided a mechanism for protein specific and site-specific isotope labeling in bacterial cells. Here, isotope labeled unnatural amino acids was incorporated to different sites of diacylglycerol kinase for further in situ NMR analysis, without protein purification. Differences of NMR chemical shift and T1 relaxation values of isotope labeled DAGK at various sites in native E. coli membrane versus in detergent micelles were observed, indicating strong influences of hydrophobic environment to membrane proteins. Isotope labeled Tyrosine was incorporated to E. coli tyrosine kinase and acquired NMR signals illustrate small portion of tyrosine phorphorylation of the kinase, in the presence of ATP and Mg2+ ions.

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Li, F., P. Shi, J. Li, F. Yang, T. Wang, W. Zhang, F. Gao, W. Ding, D. Li, Y. Xiong, J. Sun, W. Gong*, C. Tian* and J. Wang* (2013). "A Genetically Encoded F NMR Probe for Tyrosine Phosphorylation." **Angew Chem Int Ed Engl.**
(* co-corresponding author)

351 MO

A NON-INVASIVE METHOD FOR PHENOTYPING DIFFERENTIATING MESENCHYMAL DENTAL PULP STROMAL CELLS

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University of Leeds

Objectives: Human dental pulp stromal cells (HDPSCs) have the ability to differentiate into multiple tissues, offering great promise for regenerative therapies. Before such a therapy becomes clinical reality, certain scientific challenges need to be addressed. One such challenge is the need for a non-invasive method to characterise cell phenotype, ascertain the long term health of differentiating HDPSCs and understand their differentiation behaviour. Existing phenotyping methods are invasive and frequently require the sacrifice of cell samples. Our aim was to develop a non-invasive methodology for phenotyping stem cell populations seeded on to 3D scaffolds at different stages of the differentiation pathway. Methods: To achieve this, we have used NMR spectroscopy coupled with principal component analysis and statistical modelling to characterise HDPSCs in adipogenic and osteogenic culture over time. Cells were grown as monolayers or seeded on to electrospun collagen scaffolds and differentiation induced using adipogenic or osteogenic culture medium *in vitro*. NMR spectra were collected from the monolayers/constructs at various time points over a period of 6 weeks.
Results: Cells remained viable and differentiated as normal throughout. Processing the spectra provided metabolic information on cellular behaviour within the constructs. This information coupled with mathematical modelling and statistical analysis has enabled the predication of clusters and data trends for cells differentiating down different lineages, effectively phenotyping the population of cells within each construct.
Conclusion: We conclude that NMR spectroscopy may offer a non-invasive diagnostic/prognostic tool for stem cell behaviour in regenerative therapies.

Poster Presentation

352 TU

A NEW METHOD FOR AROMATIC CARBON CONTENTS (CAR) DETERMINATION OF PETROLEUM'S BY ^{1}H NMR SPECTROSCOPY

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The previous way in determining fossil fuel products in liquid state ^{13}C qNMR was the use of test method, ASTM D5292-99 (reapproved 2009). It was also used for determination of Car in gas oils, kerosenes and some other petroleum and coal distillates but not for petroleum crude and height boiling materials. This method needs additional measurements of ^{1}H NMR spectra for verification absence of olefinic carbons in such matters. Industrial applications of those connected with some problems: the need to use a stationary high sensitivity NMR spectrometer, much time (several hours) for a single measurement due to the low content of the isotope ^{13}C (≈ 1%) and the long spin-lattice relaxation time T_1 of ^{13}C (10 seconds or more). Petroleum values Car (Car=C-Cal) are concordance with content ^{1}H atom of different molecular fragments (Har+Hα + Hβ+ Hγ =H). It can be easily obtained from the ^{1}H NMR spectra using any high-resolution NMR spectrometer with an operating frequency of 30 MHz and higher.

Earlier for light petroleum fraction next semi – empirical relationship between aromatics from ^{1}H and ^{13}C spectra has been found by Cookson:

$Car=[k(((Har)^{-1}-0.01)+0.01]^{-1}$.

Analysis of the results of quantitative ^{1}H NMR spectra (600 MHz and 45 MHz) and ^{13}C (150 MHz) more than 40 petroleums and its products (from gasoline to bitums) allows us to get new universal relationship for prediction Car across ^{1}H NMR results:

$Car=-9.962(\pm1.991)+3.499(\pm0.274)*Har+0.557(\pm0.129)*H\alpha+0.157(\pm0.032)*H\beta$

$R^2=0.969$, $\sigma=0.93$

Previously published our results of ^{1}H and ^{13}C for 41 oil were used to validate this relationship. Average difference of Car measured and calculated from ^{1}H NMR spectra was found lower than 1%.

^{1}H NMR spectra which were registered by compact spectrometer showed adequacy of the results for the 45 and 600 MHz. These results allow us to recommend quantitative ^{1}H NMR spectra for the estimation of Car petroleums, using industrial commercial spectrometer with frequencies of 30 MHz and more. The limits of applicability of the new approach will be determined later.

353 TH

INVESTIGATIONS INTO ASSESSING OIL WELL DRILLING FLUID PROPERTIES BY USING ^{1}H NMR

P. Arkoudeas[1], L. Lagkaditi[1], V. C. Kelessidis[1], A. Gupta[1], M. Fardis[2], E. Karakosta[2],

G. Diamantopoulos[2], and G. Papavassiliou[2]

[1] *Texas A&M University at Qata*

[2] *Institute of Advanced Materials Physicochemical Properties Nanotechnology and Microsystems, NCSR Demokritos*

Drilling fluid properties assessment is essential for characterization of particular drilling fluid formulations and for implementation in the oil field when drilling. The drilling fluid is the blood of the oil well and performs a multitude of functions like, lubricating the bit, providing the necessary hydrostatic pressure and transferring the cuttings to surface. Most important properties are rheology, determined with simple or more advanced viscometers, and fluid loss, determined via standard API fluid loss cell. Rheology and minimization of fluid loss is controlled often with the use of bentonite or polymer additives. In this work we try to assess the capability of NMR for assessing the non-Newtonian rheology of drilling fluids and whether some information can be extracted regarding fluid loss of such drilling fluids. Specifically, ^{1}H spin-lattice T_1 and spin-spin T_2 relaxation times, as well as diffusion coefficient D measurements, were performed on water based drilling fluids of various bentonite concentrations at different temperatures and ambient pressure. The results are correlated with conventional measurements in an effort to explore the potential use of NMR for inferring rheological and fluid loss measurements of oil well drilling fluids.

Poster Presentation

354 MO

HYDRATION DYNAMICS IN TITANIA DOPED PORTLAND CEMENT A LINEAR RELATION OF ULTRASOUND VELOCITY AND ^{1}H NMR

G. Diamantopoulos[1,2], E. Karakosta[1], M. Katsiotis[3], M. Fardis[1], P. Falaras[1], M. Protopapas[2] and G. Papavassiliou[1]

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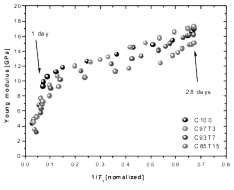
Titanium dioxide (TiO_2) is well known for its photocatalytic activity, which can impart biocidal, self-cleaning, and smog-abating functionality to cement based materials. ^{1}H Nuclear Magnetic Resonance (NMR) and ultrasound measurements were conducted to study the hydration of cement pastes doped with titanium dioxide and its effect on the hydration process. These non-destructive techniques can efficiently monitor the cement hydration, starting from the earliest few minutes after mixing with water, in a continuous manner. Specifically, the micro-structural changes resulted from the hydration of cement mixtures were monitored by measuring the ultrasound velocity of sound propagating through the material and was correlated to the porosity of the system. ^{1}H NMR T_1 spin-lattice relaxation measurements were also conducted in order to monitor the hydration process of the cement mixtures. The results indicate that ^{1}H NMR T_1 and ultrasound velocity measurements are directly correlated and show different linear dependences according to the associated hydration periods. This dependence exemplifies that both techniques can be applied complimentary for acquiring simultaneous information on both the hydration kinetics and the evolution of the mechanical properties (Young modulus) of cement based materials in a continuous and non destructive manner.



Young modulus versus ^{1}H $1/T_1$ NMR for samples containing different percentages (w/w) of cement (C) and titania (T).

355 TU

CHARACTERIZATION OF MULTIPLE EMULSIONS BY LOW-RESOLUTION T_2 RELAXOMETRY, PFG NMR DIFFUSOMETRY AND 1D-PFG NMR PROFILOMETRY

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Particle and Interfacial Technology Group (PaInT), Ghent University, Belgium

The usefulness of low-resolution NMR for W/O/W double emulsion characterization was evaluated. In order to quantify the internal and external water fractions, a low resolution NMR T_2-relaxation method was developed. In order to separate the internal and external water, a very small amount of a $MnCl_2$ solution was added to the W/O/W emulsion, which is known to reduce the T_2-relaxation time of water (Fig.1.). Hereby, only the external water is in contact with the paramagnetic Mn^{2+} ions and hence its contribution in the T_2-distribution is shifted relative to the internal water, which enables the quantification of the relative contribution of both water fractions.

Additionally, the enclosed water volume was non-destructively quantified by low resolution pfg-NMR diffusometry, which enables the discrimination between the internal and external water phase based on differences in diffusion behavior.
Finally, the creaming behavior of the multiple emulsions was investigated by 1D-pfg NMR profilometry.

Overall, the results reveal that low resolution pulsed field gradient NMR is a suitable technique for the thorough characterization of the physicochemical properties of multiple emulsions. Being an in-situ and non-destructive technique, it is also ideally suited to follow the stability over time.

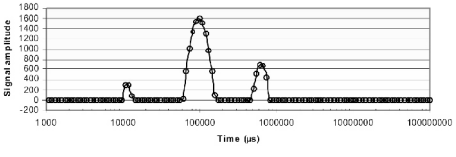

Fig.1. T_2-relaxation time distribution of a W/O/W emulsion upon $MnCl_2$ addition; from left to right, the peaks correspond to liquid oil, external water and internal water, respectively.

Poster Presentation

356 TH

NMR AND CHEMOMETRICS TO CLASSIFY PETROLEUM SAMPLES FOR LUBE BASE OIL PRODUCTION

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Base oils are the primary hydrocarbon components of industrial lubricants. They are complex petroleum mixtures composed largely of saturated hydrocarbons with carbon numbers ranging from C15 to C50. At ambient temperatures lubricating base oils are liquids of varying viscosities, with negligible vapour pressures. Base oils are produced by first distilling crude oil at atmospheric pressure to remove lighter components (e.g. gasoline and distillate fuel components), leaving a residuum that contains base oil precursors. This atmospheric residuum is then distilled under vacuum to yield a range of distillate fractions (unrefined distillate base oils) and a vacuum residuum. Removal of the asphalt components of the vacuum residuum results in unrefined residual base oils. These distillate and residual base oil fractions may then undergo a series of extractive or transforming processes that improve the base oils' performance characteristics and reduce or eliminate undesirable components.
An important goal of refinery industry is to predict the “ability” of the crude oils to produce suitable yields of base oils without the onerous and time consuming distillation and separation processes above mentioned.
This work shows a chemometric approach to classify “400+ distilled residues” of crude oils based on their ability to produce base oils by using ^{1}H and ^{13}C NMR spectra. The presence of a wide range of hydrocarbon components in “400+ distilled residues” causes a great overlapping of NMR resonances which prevents any simple and direct analysis. Chemometrics can extract chemical information and find many hidden relationships difficult to observe by “human” capacity. Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) have been chosen to analyze the NMR spectra of a series of 23 “400+ distilled residues”. The first/preliminary results show that some principal components (PCs) of NMR variables are able to separate in the scores plots the oils which can produce (PL) or not (NPL) base oils. The corresponding loadings plots of the original variables (intensities of NMR spectra) give some insights into the more influent sections of NMR spectra contributing to the more discriminating PCs, which in turn are related to the molecular features of the oils.

357 MO

NMR STUDIES ON NANOCATALYSTS FOR THE PETROLEUM INDUSTRY

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Catalytic processes are at the core of any petroleum refinery worldwide, including simple cracking reactions, alkylation, isomerization and sulphur removal. In 30 years, the industry has seen little change on the type of catalysts used, even though many novel and commercially attractive materials exist. Most exceptional is the application of nanotechnology in catalytic refining through the development of nanosized catalysts (nanocatalysts). In this work, solid-state NMR studies on two nanocatalysts developed for two major catalytic processes (cracking applications and hydro-desulphurization) are presented.
Concerning cracking applications, the acidic nature and porous structure of zeolites makes them excellent catalysts for breaking down large hydrocarbon molecules. The activity of zeolite catalysts is often associated with proton mobility between specific hydroxyl groups, known as Brønstead acid sites. In this work, the dynamic property of the protons due to these hydroxyl groups is investigated by variable High Temperature proton T_1 and T_2 relaxation measurements. It is shown that High Temperature NMR can be effectively applied to probe the different types of active sites on commercially available zeolites and nanozeolites synthesized in the lab.
Transition metal phosphides are currently of great interest in chemistry and materials sciences because they exhibit catalytic activity such as hydro-desulphurization and hydro-denitrogenation in petroleum industry. In this work, the effect of metal substitution in ternary NiCoP alloy nanoparticles is examined by variable temperature ^{31}P and ^{59}Co NMR. The correlation between the NMR response and the electronic structure of the transition metal phosphides is further investigated.

Poster Presentation

358 TU

NMR STUDY OF REORIENTATIONAL MOTIONS IN $LiZn_2(BH_4)_5$

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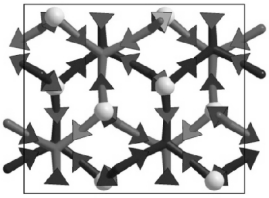

Borohydride-based materials, such as $LiBH_4$ and $Mg(BH_4)_2$, have recently come under the spotlight as promising hydrogen storage materials, due to their high gravimetric hydrogen content. However, since decomposition of these materials and subsequent release of hydrogen takes place at impractically high temperatures, there have been attempts to design new materials with more favourable thermodynamic properties.

Understanding molecular dynamics in borohydride materials is important in view of providing further insight into designing novel materials with improved thermodynamic properties – in a path to design commercially attractive systems.

Here, we present an NMR molecular dynamics study of $LiZn_2(BH_4)_5$ [1]. The system was found to have a novel structure of two identical doubly interpenetrated 3D frameworks with no bonds between them. The system contains 9.5 % of hydrogen and decomposes at 127°C. ^{1}H and ^{7}Li NMR spectra and spin-lattice relaxation was investigated in a wide temperature range, from 80 K to the decomposition temperature. The narrowing of proton spectra with increasing temperature indicates the averaging of dipolar interactions due to internal dynamics, in this case rotations/reorientations of BH_4 tetrahedra.

On the other hand, proton spin-lattice relaxation measurements reveal two components of proton relaxation – attributed to two inequivalent types of BH_4 tetrahedra in the system (B1, B2, and both B4 groups, that are close to Li atom; and B3, that is far from Li). The reorientations of tetrahedral can take place around different axes. For example, at low temperatures reorientations of B1 take place around the Zn1-Li axis, keeping the N-Zn and H-Li distances fixed. At elevated temperatures, reorientations around the axis, perpendicular to the Zn1-Li line, also become possible.

359 TH

^{1}H NMR STUDY OF HYDROGEN DIFFUSION IN DISORDERED Ti-V-Cr alloys

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During the last decades metal hydrogen systems have been extensively studied due to their ability to be used as hydrogen storage materials. For such applications information about hydrogen dynamics in the metal lattice is of great importance. The most direct way to obtain such information is to measure the hydrogen self-diffusion coefficient.

Here we report on the results of our ^{1}H NMR study of the hydrogen self-diffusion coefficient (D) in Ti-V-Cr hydrides of different compositions: $TiV_{0.8}Cr_{1.2}H_{5.29}$, $Ti_{0.33}V_{1.27}Cr_{1.4}H_{1.13}$ and $Ti_{0.5}V_{1.9}Cr_{0.6}H_{5.03}$. In the initial alloys the distribution of the Ti, V and Cr atoms over both sites of the bcc lattice is random. For $TiV_{0.8}Cr_{1.2}$ and $Ti_{0.5}V_{1.9}Cr_{0.6}$, which can uptake more hydrogen, hydrogenation induces a bcc-fcc structural phase transition [1]. We have also studied these alloys with the addition of a minor fraction of Zr_7Ni_{10}, as such materials exhibit a specific microstructure and display excellent sorption kinetics [2].

The hydrogen diffusion measurements have been performed employing the static field gradient nuclear magnetic resonance method (SFG NMR) [3] at a frequency of 100 MHz with low field gradient of 51.27 T/m within the temperature range from 293 to 403 K.

All hydrides exhibit rather slow hydrogen diffusion (D = 1÷3×10^{-11}m^2/s) at room temperature. The activation energy (E_a) strongly depends on the composition of the studied compound. For the Cr poor hydrides with fcc structure the E_a values are in fair agreement with the results obtained from our earlier relaxation studies [4]: 0.15 and 0.12 eV for $TiV_{0.8}Cr_{1.2}H_{5.29}$ and $Ti_{0.5}V_{1.9}Cr_{0.6}H_{5.03}$, respectively. However, there is a significant discrepancy in E_a values determined (from two) using the different methods for $Ti_{0.33}V_{1.27}Cr_{1.4}H_{1.13}$. According to SFG NMR measurements E_a = 0.20 eV, whereas the relaxation measurement predicts a significantly lower value of E_a 0.11 eV. The nature of (such a disagreement) this difference is not clear. (It also has been shown) Finally, it was found that addition of Zr_7Ni_{10} does not affect the hydrogen diffusion parameters.

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Poster Presentation

360 MO

STRUCTURE AND DYNAMICS OF PHOTOCHROMIC SODIUM NITROPRUSSIDE INCORPORATED IN SILICA XEROGELS

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The generation of photoinduced nitrosyl linkage isomers in $Na_2[Fe(CN)_5NO].2H_2O$ (SNP) is connected with a photochromic and photorefractive response of the material which renders it interesting for optical applications. Such applications would demand a more robust and industrial processing than growing single crystals. A potential route to applicable materials is the embedding of the complex into sol–gel based materials like silica matrices.

Our study combines total scattering and Solid State NMR (SSNMR) methods to characterize the structure of Sodium NitroPrusside (SNP) embedded in Silica xerogels. By varying the pore size of the matrix, the size of the assembling SNP particles can be adjusted from nanoparticles down to isolated molecules. It is thus possible to study the structure and dynamics of the complex as a function of particle size.

While X-Ray diffraction patterns provide estimates of the average size of the particles and give structural insight into the larger nanoparticles, analysis of Debye Function and Pair Distribution Function allows for obtaining information about the nano-scale and disordered structures. Additionally, the average distance between atoms of the host network and the guest complexes can be determined from analysis of the intermediate scattering range. NMR chemical shifts, acquired by Solid State NMR techniques, also provide structural information on the host matrix (^{29}Si, ^{1}H) and the guest complex (^{13}C, ^{23}Na). But more interestingly, SSNMR gives access to a dynamic picture of the embedded host through the measurement of anisotropic nuclear interactions and longitudinal relaxation times.

Our work shows that statistically distributed molecules as well as nanoparticles of SNP can be grown in the silica matrix while retaining their structural integrity and keeping their optical properties. Additionally, when the particles are sufficiently small, the SNP molecular complexes adopt a liquid state like behavior down to temperatures below -30°C.

361 TU

SIMPLIFYING PROTON NMR SPECTRA BY INSTANT HOMONUCLEAR BROADBAND DECOUPLING

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One-dimensional proton NMR spectra probably represent the most often acquired type of NMR data. However, ^{1}H spectra typically suffer from low resolution and severe signal overlap due to extensive scalar coupling. Homonuclear broadband decoupling, vastly increases the resolution, which in some cases corresponds to a theoretical signal dispersion of NMR spectrometers at several GHz(1).

One of the most often used approaches for homonuclear broadband decoupling uses frequency-selective pulses during a weak gradient field (2). This pure-shift method, which has recently been improved (1), relies on the acquisition of several “data chunks” in a pseudo 2D experiment and subsequent concatenation of these blocks.

Here we describe a general way to achieve homonuclear-broadband decoupling which completely eliminates the sophisticated processing scheme and the recording of a series of spectra (3). Due to the latter, the sensitivity per time is increased by a factor of 20-50 fold and it does not require the recording of series of spectra but can be acquired in a single scan. No special data processing is necessary. t also enables the acquisition of $_{2}$-decoupled ^{1}H-^{13}C HSQC spectra, which yield an extreme resolution enhancement for intrinsically unstructured proteins. A similar approach of frequency and slice-selective excitation has also been implemented to obtain rapid (relaxation-delay free) acquisition of series of proton spectra (4) and diagonal-peak free homonuclear 2D spectra (5).

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Poster Presentation

362 TH

NEW NMR ADVANCES TO PROBE HYDROGEN BONDS IN PEPTIDOMIMETIC DERIVATIVES AT NATURAL ABUNDANCE

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Hydrogen bonds (H bonds) are fundamental key features for the stability, structure, and dynamics of chemical and biological systems. In the field of natural and synthetic organic products, H bonds are less studied, since the heteronuclei usually involved, namely ^{13}C and ^{15}N, are of low naturally abundance. If the H bond is sufficiently stable, trans H bond scalar couplings occur and can be detected by NMR. However, their magnitude is often very small and tends to reduce again the sensitivity. In order to compensate the low sensitivity inherent to the low gamma nuclei and to the small trans H bond couplings, a new methodology utilizing sensitivity optimized fast repetition NMR techniques have been developed.[1] With this aim, a new set of SOFAST HMBC sequences has been implemented: H^N-CO, and H^N-N versions for detecting N-H$\cdots$OC and N-H$\cdots$N H bonds respectively. With the help of new SOFAST HMBC experiments, in combination with a 950 MHz spectrometer equipped with a cold probe, a sensitivity gain of 29 can be reached in theory, in comparison with the standard HMBC data acquired on a 400 MHz with a warm probe. The Aitken research group works on foldamers based on the trans-2-aminocyclobutanecarboxylic acid (ACBC) building block.[2] Using SOFAST HMBC, it is possible to detect the network of intramolecular H^N-CO H bonds in an ACBC octamer[3] and to confirm the conformational folding as a H_{12} helix. The H^N-CO SOFAST sequence is the first experiment able to detect all H^N-CO H bonds at natural abundance in less than 20 h of data acquisition. The technique has now been used to probe the detailed folding behavior of aza-analogues of ACBC, in which the N-terminal is a 1-aminoazetidin-2-carboxylic acid (AAzC) residue.[4]

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363 MO

100 KDA AND BEYOND: NMR OF NANODISCS

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High-resolution structural investigations of membrane proteins pose a challenge for both X-ray crystallography and NMR. In addition to the usual difficulties associacted with the production of adequate protein quantity for structural studies, NMR analyses require the stabilization of the membrane protein for a sufficiently long time to allow for the measurement of multi-dimensional experiments.

In the last several years, nanodiscs have emerged as a new tool in the NMR community that can help circumvent the stability problem. Nanodiscs are disc-shaped planar lipid-bilayers that are bound together by two copies of a derivative of Apolipoprotein A-1 which forms a belt around the disc, thus covering the hydrophobic edges of the lipid bilayer. Several advantages are inherent for nanodiscs. The aforementioned increased stability of the membrane protein allows for longer measurements that would facilitate the structure determination and/or dynamic investigation of very challenging membrane proteins, like GPCRs. A strong curvature, found in micelles, is absent in nanodiscs, simulating a more natural environment. However, the main advantage is the incorporation of lipids or lipid mixtures that allow the solution-state NMR researcher to mimic a natural environment for membrane proteins without using detegents.

The main drawback in the use of nanodiscs is their size. With around 100 kDa, their additional mass will contribute to unfavorable relaxation behavior that in some cases might offset their usefulness. Therefore, a thorough investigation of nanodiscs is required to take full advantage of this technology and minimize its drawbacks. We have embarked on the assignment and structural characterization of nanodiscs with the goals of obtaining residue-specific information on how the Apolipoprotein belt is arranged and of developing strategies to assign high-molecular weight systems with mainly alpha-helical character. Our results should facilitate future investigations of challenging systems with nanodiscs in solution-state NMR structural biology.

Poster Presentation

364 TU

THE USE OF SOLUTION-STATE NMR IN STUDIES OF STRUCTURALLY DYNAMIC MEMBRANE AND GLOBULAR PROTEIN COMPLEXES

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Proteins are important macromolecules performing vital cellular functions. In order to comprehend how proteins function in the cell and to elucidate their mechanism of action, it is necessary to identify their structural characteristics and their molecular dynamics. Nuclear Magnetic Resonance (NMR) spectroscopy is a unique and powerful tool used to study both the structure and dynamics of proteins. An interesting aspect of NMR is that it can also be used for determining structures of proteins in dynamic complexes.
With the help of novel NMR methods and cost-effective sample preparation, we plan to explore the internal dynamics of both soluble and membrane proteins. In our study we have included two important membrane proteins namely Stromal Interaction Molecule 1 (STIM1) and Presenilin Enhancer-2 (PEN-2). STIM1 is a type I trans-membrane protein, present mainly in the Endoplasmic Reticulum. It is involved in mediating Calcium ion influx, when there is a depletion of calcium ions in the ER, by gating of store operated Ca 2+ ion channels – SOCs. PEN-2 is an essential sub-unit of gamma secretase and is speculated to play an important role in the maturation of gamma secretase complex and therefore its activity. The gamma secretase itself is involved in the proteolysis of trans-membrane proteins like the Notch protein and the Amyloid Precursor Protein. The high resolution structure and mechanism of action of both proteins have not been fully understood. Our results for STIM1 indicate that most constructs gave good expression and purity. Almost all showed well defined secondary structure lacking proper tertiary fold. Due to significant line broadening for almost all resonances, backbone assignment could not be completed for STIM1. Our studies with PEN-2 showed that it is a thermally stable monomer in solution with intrinsically disordered domains. With the help of paramagnetic relaxation studies and Methyl-TROSY type NMR experiments, the global fold of the protein *in-vitro* conditions could be defined. More experiments and data analysis is needed to understand the three dimensional structure of PEN-2 and its role in the active gamma secretase complex.

365 TH

NMR-BASED APPROACH TO MEASURE THE FREE ENERGY OF TRANSMEMBRANE HELIX-HELIX INTERACTIONS

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Abstract. Energetic parameters of transmembrane helix-helix interactions are of big scientific importance as they permit to establish a structure-energy relationship for α-helical membrane domains. There exist a number of techniques developed to measure the free energies of dimerization and oligomerization of transmembrane α-helices, all having their advantages and drawbacks. In present work the methodology to determine the magnitudes of free energy of interaction between transmembrane helices in detergent micelles by means of NMR spectroscopy is proposed. The suggested approach relies on the reasonable physical model of the protein oligomerization/dimerization, taking into account the micelle-embedded state of the membrane domain. The technique has three major advantages comparing to other existing approaches – it may be used to treat both weak and relatively strong dimers/oligomers, it works well in case of complex equilibrium, when monomer, dimer and oligomer of higher order are simultaneously present and the approach can yield at the same time both structural and energetic characteristics of helix-helix interaction. The proposed methodology was applied to study processes of oligomerization of transmembrane domains of FGFR3 and VEGFR2, which allowed to measure the free energy of dimerization for both objects, to determine the populated oligomeric states of VEGFR2 and to measure the free energy of its trimerization.

Poster Presentation

366 MO

COMBINED MEASUREMENTS OF UV AND NMR SPECTRA: SOLVENT AND H/D ISOTOPE EFFECTS ON THE PROTON TRANSFER PATHWAYS IN HYDROGEN-BONDED PHENOL-CARBOXYLIC ACID ANIONS

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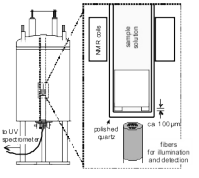
Here we present an application for the combined NMR/UV-Vis method (figure left), in which NMR and UV-Vis spectra are measured for the same sample inside the magnet of the NMR spectrometer. Heteroconjugated H-bonded anions of the type A…H…X(-), with AH = phenols and HX = carboxylic acids (figure, top right), have been studied using the aprotic solvents CD2Cl2 and CDF3/CDF2Cl. The systems were chosen to represent small molecular models of H-bonded cofactors in proteins such as the photoactive yellow protein (PYP).

It is shown that the ^{13}C chemical shifts of the phenolic residues of A…H…X(-) constitute an excellent probe for the average proton positions. These shifts correlate with the ^{1}H chemical shifts of the bridging positions, as well as with the H/D isotope effects on the ^{13}C chemical shifts. The proton transfer pathways was elucidated in a qualitative way.

Depending on the solvent polarity and the chemical structure of AH and X(-), the proton in the OHO bonds formed is located nearer to either the phenolic or the carboxylic oxygen atom. Surprisingly, for all systems the average proton position is shifted away from the phenol residue when the solvent polarity is reduced. These results are rationalized as follows: the more the charge is localized, the better the anion is solvated by polar solvent molecules (figure, bottom right).



367 TU

ANALYTE ENCAPSULATION IN PHOSPHOLIPIDIC VESICLES TO MINIMISE DIFFUSION EFFECTS IN ULTRAFAST 2D NMR

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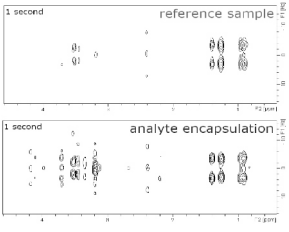
Molecular diffusion is a key factor in NMR experiments involving magnetic field gradients. This is an important drawback for some pulse sequences, particularly for the ultrafast 2D NMR methodology. The latter is capable of providing a 2D NMR spectrum within a single scan, in a fraction of second. [1] However, these powerful experiments are hampered by molecular diffusion effects leading to significant sensitivity losses and lineshape distortions. [2]
These effects are even more important as the spatial encoding duration is increased, which is the condition necessary to improve spectral resolution in the spatially-encoded dimension. As a consequence, a compromise has to be made between resolution and sensitivity. [3]
To deal with this limitation, we propose to physically reduce translational diffusion, with a new sample preparation protocol. [4] It is based on the encapsulation of the analytes inside phospholipidic vesicles (liposomes), which dramatically reduces their translational diffusion.
This protocol is fully compatible with biological samples and easy to implement. Its interest for ultrafast 2D NMR is demonstrated in the particularly diffusion-sensitive case of ultrafast J-resolved spectroscopy.



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Poster Presentation

368 TH

CAGE COBALT(II) COMPLEXES AS A PERSPECTIVE PARAMAGNETIC TAGS

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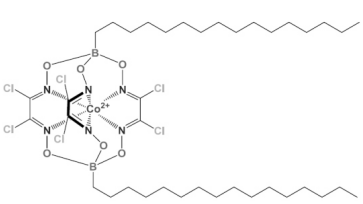

Here we report some interesting results obtained on the magnetic and structural properties of the cage complexes with an encapsulated paramagnetic cobalt(II) ion. Given that alkyl chains are typical structural units, for example, in lipids, we focused our efforts on a complex with hexadecyl substituents in the axial positions. The presence of the paramagnetic cobalt(II) ion in this complex resulted in the paramagnetic shifts of nuclei in its NMR spectrum; so the nuclei of each CH_2 group in the alkyl chain have different chemical shifts. As these nuclei are far from the cobalt ion, the interactions between them and the paramagnetic center can be described as dipole-dipolar. Thus, their paramagnetic shifts depend on their coordinates in a magnetic susceptibility tensor's frame. As an alkyl chain is a conformational flexible unit, its structure in a solid state is different from that in a solution. Therefore, one of the aims of our study was to develop a correct model of conformational behavior of the hexadecyl fragment in a cage complex in a solution. Given that the full coverage of the conformational space would be too computationally demanding, the simplified model was used that neglected a variance of bond's length and angles along the alkyl chain. Fitting the paramagnetic shifts of hydrogen and carbon nuclei within this model allowed estimating the anisotropy of the magnetic susceptibility tensor ($9.8 \cdot 10^{-32} m^3$). Note that this value is significantly larger than in most of the cobalt(II) compounds. This, together with the total chemical isolation of the paramagnetic ion and the stability of the cage ligand, allows considering these complexes perspective paramagnetic tags for structural investigations of biological macromolecules. This research was supported by the RFBR (grant 13-03-00732) and CPRF (grant MK-4842.2013.3).

369 MO

SOLVING THE HIGH-RESOLUTION STRUCTURES OF THE THREE-CONFORMER ENSEMBLE FOR THE INFLUENZA HEMAGGLUTININ MEMBRANE FUSION DOMAIN BY LEVERAGING THE INFORMATION OF KNOWN STRUCTURES

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The first 23 residues of the influenza hemagglutinin HA2 subunit form the fusion domain, which anchors into the host cell membrane and plays a pivotal role in fusing membranes of the virus and the host on viral infection. At neutral pH, the domain adopts a tight helical-hairpin amphiphilic structure that is stabilized by aliphatic hydrogen bonds and charge-dipolar interactions. We demonstrate that at low pH, where the fusion process is triggered, the fusion domain populates activated states that likely play a crucial role in forming the fusion pore, an essential structure required in the final stages of membrane fusion. We demonstrate that a conformational ensemble of at least three structures, one closed and two opened, are populated and interchange between them occurs on a microsecond timescale. A structural refinement strategy is introduced wherein the contribution from conformers of known structure is subtracted from the data, yielding new datasets that more uniquely constrain the structures of unknown conformers. With the known structure of the closed helical-hairpin as a restraint, we characterized the high-resolution structure of the three-conformer ensemble with NOEs and RDCs. This protocol can generally be applied to solving the high-resolution structures of multiconformer ensembles by leveraging known structures of a subset of conformers to help accurately define the structures of unknown conformers.

Poster Presentation

370 TU

NOVEL FOUR-DIMENSIONAL EXPERIMENTS WITH NON-UNIFORM SAMPLING FOR RNA AND PROTEINS

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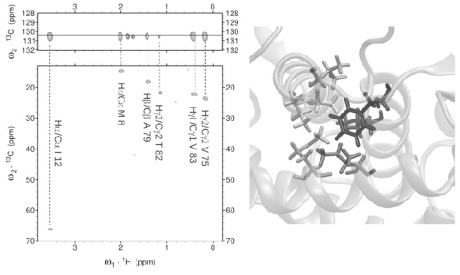
The full potential of double-$^{13}C/^{15}N$-labelling of proteins and RNAs is utilized to develop high-resolution four-dimensional experiments with non-uniform sampling. This enables to resolve ambiguities in spectra of complex biomolecules featuring poor chemical shift dispersion, e.g. non-coding RNA.

Here we present recent advances in biomolecular NMR, including 4D HNCACO-CCR experiments for measurement of cross-correlatated CSA(C')-DD(NH) and CSA(C')-DD(CaHa) relaxation rates [1], diagonal-free 4D C(ribose),C(arom)-edited NOESY for RNA [2] and 4D C(aliph),C(arom)-edited NOESY for proteins [3] (see Figure).



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371 TH

7D NMR EXPERIMENTS FOR PROTEIN BACKBONE ASSIGNMENT

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Three novel NMR experiments for a backbone assignment of proteins are proposed: HNCO(N)CACONH, HACACON(CA)CONH, (HACA)CBCACON(CA)CONH. A dimensionality of each is reduced from 7 to 5 dimensions, by the radial sampling of the underlined dimensions. Three frequencies that are encoded in a one evolution period are H, N, CO; HA, CA; N; CB, CA, N respectively. The projected dimensions are obtained using the multiple quadrature detection so that an acquisition of all three chemical shifts encoded in a common dimension is done simultaneously in the quadrature. Thus the peak frequencies are linear combinations of resonance frequencies of involved nuclei. The three chemical shifts projected into a single dimension are calculated basing on the coordinates of four peaks which leads to a system of four linear equations with only three variables which make the method even more resistant to overlap or miss of the single peak.

The main advantage of the presented approach is an unambiguous and reliable backbone assignment because of establishing inter-residual links basing on up to four chemical shifts taken from a single experiment.
The presented experiments employ non-uniform sampling that enables achieving a high resolution in the indirectly detected dimensions. 2D cross-sections were obtained using the Sparse Multidimensional Fourier Transform. The data processing were done using cleaner4d, reconstructor4d, cleaner5d and reconstructor5d. All experiments were performed using a chicken BASP (270 amino acids long IDP) at pH 2 on the Varian 700 MHz spectrometer with a standard triple-resonance probe.

Poster Presentation

372 MO

MAPPING INTERMOLECULAR INTERACTIONS IN A MEMBRANE-PROTEIN–CHAPERONE COMPLEX BY METHYL–METHYL NOES

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Integral outer membrane proteins (Omps) are transported during their biogenesis by periplasmic chaperone proteins[1]. These chaperones prevent aggregation of the highly hydrophobic Omps during their passage from the inner to the outer membrane. Binding of the substrate by the chaperone, as well as its subsequent release, occurs in absence of external energy. Skp is a trimeric 51 kDa periplasmic chaperone protein from *Escherichia coli*, belonging to the family of cavity chaperones[2]. NMR studies of Omps bound to this chaperone have revealed that the substrate adopts a compact unfolded state in the chaperone cavity, an ensemble of multiple rapidly interconverting conformers. The binding affinity is globally high but locally very weak[3]. Here, we address the role of the side chains in short-range contacts between the Skp chaperone and the transmembrane domain of its substrate OmpA (tOmpA). Selective amino-acid labeling of the methyl groups of alanine, leucine, valine and isoleucine provided suitable structural probes. We show that the chaperone–substrate interactions have a local correlation time in the range between one nanosecond and one millisecond. Just as the backbone, the isoleucine, leucine, valine and alanine side chains are rapidly averaging over multiple conformations, comparable in chemical shift dispersion to a rotamer ensemble in denaturant. Intermolecular NOEs between the alanine methyl groups of Skp and the isoleucine, leucine and valine methyl groups of tOmpA were used to map the position of the tOmpA ensemble inside the Skp cavity. This study demonstrates the feasibility to measure structurally meaningful intermolecular NOEs between a well-folded molecular chaperone and its unfolded protein substrate.

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373 TU

SPIN NOISE DETECTED HETERONUCLEAR MULTIPLE QUANTUM COHERENCE

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We present new NMR methods, which exploit transverse nuclear spin noise for indirect detection in spectroscopy and imaging. In the absence of hyperpolarization spin noise detection may be superior to classical detection schemes for minute sample amounts ($\leq 10^8$ spins), as proposed earlier. [1] With the hardware available to us, experiments with such low spin numbers is not yet possible. Hence we use large spin numbers to assess the fundamentals of spin noise detection based experiments, which bear the potential of enhanced sensitivity, once applicable to low spin numbers. We have achieved a first proof of principle for transverse spin noise detected 2D NMR spectroscopy by implementing a strategy based on the principle of CONQUEST [2] used in MRFM [3]. Instead of force detection of longitudinal magnetization fluctuations, rf-detection of transverse spin noise is employed using a high-resolution NMR spectrometer with a cryo-probe. One of the first pulse sequences developed is an HMQC experiment without ^{1}H pulses. It consists of two direct ^{1}H-noise detection periods bracketing the carbon pulses, mixing and evolutions times. In the indirect dimension heteronuclear multiple quantum coherences originating from ^{1}H spin noise evolve. When the noise blocks acquired during the two direct detection periods (with ^{13}C decoupling) are correlated, standard 2D Fourier transform can be applied. To ensure that any random phase contributions average out, while the correlated noise signal accumulate, a series of spectra processed in this manner need to be co-added. This basic principle can be extended in various ways for a multitude of spin noise detected multi-dimensional NMR methods, which - as opposed to projection-reconstruction based spin noise imaging [4] - use the multi-dimensional Fourier transform principle.

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[1] Sleator, T. et al. (1987) Phys. Rev. B36, 1969; [2] Leskowitz, G.M. et al. (2003PhD Thesis (2003), California Inst. of Technol.; [3] Mamin, H.J. et al. (2003) Phys. Rev. Lett. 91, 207604; [4] Müller, N. and Jerschow A. (2006) Proc. Nat. Acad. Sci. USA 103, 6790.

Acknowledgments: This research was supported by the Austrian Science Funds FWF (M1404 to KC), and the European Union through the EFRE INTERREG IV ETC-AT-CZ program (M00146 “RERI-uasb”) and FP7 EAST-NMR, Contract No. 228461.

Poster Presentation

374 TH

SIMULATION AIDED DESIGNING OF NEW BAND-SELECTIVE DIAGONAL-FREE 4D $^{13}C,^{13}C$-EDITED ALIPHATIC-AROMATIC NOESY EXPERIMENT

Saurabh Saxena[1], Jan Stanek[1], Michał Nowakowski[1], Katarzyna Ruszczyńska-Bartnik[2], Andrzej Ejchart[2], Krzysztof Kazimierczuk[1], Wiktor Koźmiński[1]

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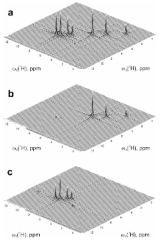

To design a new multidimensional NMR experiment, it is crucial that one must choose suitable scheme, parameters and elements in the pulse sequence. With a number of parameters that can be varied in an experiment, combined with various types of sequence elements (e.g. pulse types, gradients, decoupling schemes etc.) the task of designing and optimizing a multidimensional sequence becomes more difficult and time consuming on spectrometer. Pulse sequence simulations offer a good solution to such problem.
On the other hand, added dimensions and more number of pulse sequence elements in a multidimensional experiment still limit such procedure of prior validation on computers. Obviously, this approach can be made simpler if we try to simulate only those unconventional segments of the long pulse sequence which are crucial for the desired effect. Here we tried this approach and simulated shorter segments of multidimensional pulse sequences using Spinach[1] software library on MATLAB®. With simulations we could focus on the efficacy of multidimensional NMR experiments in terms of selectivity and sensitivity. We successfully monitored the effect of various sequence elements (pulses[2,3], gradients, delays etc.) to get the desired outcome, thereby designing a new non-uniformlly sampled band-selective diagonal-free 4D $^{13}C,^{13}C$-edited aliphatic-aromatic NOESY experiment[4].

[1] Hogben HJ, Krzystyniak M, Charnock GTP, Hore PJ, Kuprov I, J Magn Reson 208:179-194 **(2011);**
[2] Emsley L, Bodenhausen G, J Magn Reson 97:135-148 **(1992);**
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375 MO

TOWARD STABLE ISOTOPE LABELLING OF PROTEINS PRODUCED IN INSECT CELLS

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3) Plateforme de production de protéines en cellules d'insecte, Institut Pasteur, 75015 Paris, France

Most modern NMR applications rely on stable isotope labelling, from uniform enrichment of the protein in ^{15}N, ^{13}C and sometimes ^{2}H to specific introduction of $^{1}H,^{13}C$-methyl groups in an otherwise fully ^{2}H-labeled protein. Labelling is relatively straightforward to realise when the protein can be over-expressed in E. coli. Unfortunately, many eukaryotic proteins (viral and human in particular) cannot be produced this way, because they need specific chaperones to be correctly folded or specific post-translational modifications to be functional. One attractive way to produce these proteins is to use insect cells, easier to work with than mammalian cells, but able to similarly maturate the proteins. Insect cells are heterotrophic organisms only able to grow on complex media. Some of these media are sold for NMR applications but their prices remain prohibitive for most laboratories, thus greatly limiting the studies that can be performed by NMR.
We have developed a robust, cost effective solution to produce ^{15}N uniformly labeled proteins for NMR applications in Sf9 (allowing baculovirus induced transient expression) or S2 cells (allowing stable expression). We have optimised the different steps of the production protocol to reduce the production time and the volume of labelling medium. In conjunction with amino acid-type specific labelling, it makes possible at reasonable cost structural and functional studies of medium-sized eukaryotic proteins. We are currently applying this technique to the analysis of several proteins under studies in our laboratories, namely actin, the domain III of the C. elegans AFF fusion protein and an autonomously folded domain of the Hantaan virus glycoprotein Gc, with aim to gain insight into the interaction properties of actin with several peptide modulating its polymerisation and into the structural characteristics of the C. elegans and Hantaan virus proteins.

Poster Presentation

376 TU

NMR RELAXATION MEASUREMENTS FOR MEMBRANE PROTEINS: INTERNAL DYNAMICS OF THE HOMOTRIMERIC HIV-1 VIRAL COAT PROTEIN gp41 ON MULTIPLE TIME SCALES

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In the current model, HIV virus to host cell fusion is driven by a conformational change in the gp41 viral coat protein from an extended prefusion (or so-called prehairpin) intermediate during virus-to-host-cell docking to a trimer of hairpins that forms an antiparallel six-helical bundle (6HB) arrangement. Structural information on gp41 has so far been limited to individual domains, mainly in the postfusion conformation.

Here we have studied the internal dynamics of a construct of $gp41^{1-194}$ comprising the fusion peptide, N-terminal and C-terminal heptad repeat (NHR and CHR) and transmembrane region, immersed in DPC micelles. An optimized set of ^{15}N R_1, R_{1rho} relaxation and ^{15}N-{^1H} NOE measurements, using a TROSY-detection scheme, has been developed which alleviates systematic errors due to water-saturation and cross-correlated relaxation effects, particularly acute in per-deuterated systems.
^{15}N relaxation as well as PRE and SAXS data reveal a high degree of internal dynamics of gp41 on different timescales and are compatible with a prehairpin intermediate that samples a range of relative CHR vs NHR orientations, possibly in exchange with a low population of the late-fusion 6HB.

(1) Lakomek N.A., Ying J., Bax A., J. Biomol. NMR (2012), 53:209-221
(2) Lakomek N.A., Kaufman J.D., Stahl S.J., Louis J.M., Grishaev A., Wingfield P.T., Bax A., Angew. Chem. Int. Ed. Engl. (2013), 52,3911-5

377 TH

SLICE-SELECTIVE HSQMBC EXPERIMENTS

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A ^{1}H-selective HSQMBC (selHSQMBC) experiment has recently been proposed for the accurate measurement of small proton-carbon coupling constants in natural-abundance molecules. Pure-phase multiplets are obtained because J(HH) modulation during the INEPT period is avoided. In principle, a single experiment is required for each individual resonance although that the method can be successfully recorded for multiple signals using band-selection or multiple-frequency excitation.
Here we incorporate the slice-selective concept in the selHSQMBC pulse scheme in order to invert each individual resonance in a particular location along the z dimension. In this new slice-selective selHSQMBC (ss-selHSQMBC) experiment, all resonances can be monitored in a single NMR experiment without prior knowledge of existing frequencies in each sample. Several methods will be also proposed to improve the reduced sensitivity associated to slice selection.
References:

1. S. Gil, J. F. Espinosa, and T. Parella, J. Magn. Reson, 213, 145-150 (2011).
2. J. Saurí, J. F. Espinosa and T. Parella, *Angew. Chem.* Intl. Ed., 51, 3919-3921 (2012).

Poster Presentation

378 MO

GROWING COMPLEX PATTERNS IN HIGHLY MAGNETISED LIQUIDS

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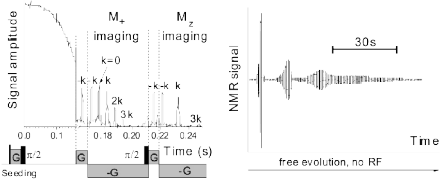
Hyperpolarisation (HP) or high magnetic fields provide high signal-to-noise ratios in liquid-state NMR but also lead to ill-controlled dynamics (precession instabilities [1]; multiple spin echoes: MSEs [2]; and multiple maser emissions: MMEs [3,4]) associated with distant dipolar fields (DDFs) and radiation damping (RD). We perform systematic low field studies with condensed laser polarised ^{3}He-^{4}He mixtures and control RD with active feedback. We report new characterisations of instabilities (without RD) and MME (with suitable RD) using MRI-based techniques. We find that instability growth rates depend strongly on the spatial wavevector (k) of the seed magnetisation pattern. Parametric amplification of the imprinted pattern and spatial harmonic generation are also observed, both in experiments and numerical lattice simulations. Our work directly probes sample size and shape effects that have previously only been inferred from MSE features in HP ^{129}Xe and ^{3}He [1], but which are expected to be important whenever strong DDFs are encountered [5]. It also opens the door to better control of DDF- and RD-dominated magnetisation dynamics.



NMR signals from dilute HP liquid He mixtures (3 mT; 1K; partly filled tube, 4-mm inner diam.).

Left: instability, with hindered RD; FID and 1D maps.

Right: MME, with enhanced RD.

[1] Nacher *et al*, J. Low Temp. Phys. **121** (2000) 743. [2] Morgan *et al*, Eur. Phys. J. D. **67** (2013) 29. [3] Marion *et al*, Chem. Phys. Chem. **9** (2008) 1395. [4] Chen *et al*, J. Magn. Reson. **208** (2011) 204. [5] Chen *et al*, J. Chem. Phys. **136** (2012) 204509.

379 TU

HIGH-RESOLUTION IN THE INDIRECT DIMENSION BY MULTIPLEXED INDIRECT SAMPLING

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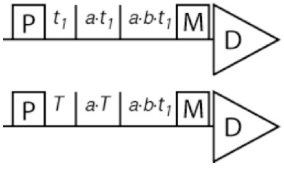
Multiplexed Indirect Sampling (MIS) is a new approach to sample broad indirectly detected dimensions. It consists in the encoding of an NMR parameter such as the carbon chemical shifts of organic compounds in an time-increment economic manner by splitting the t_1 evolution time into two or more elements.

This provides NMR spectra with the equivalent of adding minute and second hands to a watch containing only an hour hand. Precise measurements can be obtained with one to two orders of magnitude less time increments. In one approach, the t_1 evolution delays are incremented simultaneously with different steps sizes and a full quadrature results to spectra where each hand watch is shown separately. In the alternative approach, only one delay is incremented, the others being set to optimized but constant values so that the signals amplitudes of the subspectra encode the positions of the second and third hands.



In both cases, the spectra are readily suitable for an automatic reconstruction of a normal spectrum with top resolution in the indirect dimensions. A comparison of the spectra obtained with these and other methods including spectral aliasing and non-uniform sampling will be presented.

Poster Presentation

380 TH

INTERATOMIC DISTANCE MEASUREMENTS USING PURE SHIFT NOE EXPERIMENTS

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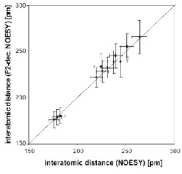
Straightforward signal volume quantification in liquid state NMR spectra is often hampered by overlap in proton dimensions. To circumvent this problem it is now common practice to increase the dimensionality of experiments, to suppress J-coupling in indirect spectral dimensions, or to resort to frequency labeling using low natural abundance nuclei. For protons, a significant reduction in spectral crowding can be obtained in the direct spectral dimension by suppressing of J-coupling multiplet structure using pure shift techniques, greatly reducing the effective signal widths typically observed.[1] This can facilitate both the analysis of homo- and heteronuclear correlation spectra, and the extraction of quantitative information from diffusion measurements.[2]
Recently, NOESY experiments with homonuclear broadband decoupling in the direct dimension have been presented and the improvements in signal separation obtainable have been pointed out.[3] As indicated in the figure shown, distance constraints can be obtained with high fidelity using such experiments, indicating their potential utility in solution structure elucidation with crowded spectra.



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[2]: P. Sakhaii, B. Haase, W. Bermel, *J. Magn. Reson.* **2009**, *199*, 192; G. A. Morris, J. A. Aguilar, R. Evans, S. Haiber, M. Nilsson, *J. Am. Chem. Soc.* **2010**, *132*, 12770.
[3]: J. A. Aguilar, A. A. Colbourne, J. Cassani, M. Nilsson, G. A. Morris, *Angew. Chem. Int. Ed.* **2012**, *51*, 6440.

381 MO

NOVEL 3D AND 4D NMR EXPERIMENTS FOR THE STRUCTURAL CHARACTERISATION OF PHENOLICS CONTAINED IN COMPLEX MIXTURES.

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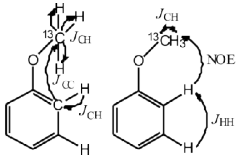
One of the frontiers of NMR spectroscopy is the structure determination of small molecules contained within complex mixtures. Examples of such mixtures include plant extracts, raw and processed food, environmental mixtures such as humic substances, or chemically/enzymatically degraded biomatter such as lignin.
These mixtures are composed of carbon- and oxygen-rich compounds containing numerous OH groups (aliphatic, phenolic, and carboxylic), which can often be classified as phenolics or polyphenols. By methylating their OH moieties with ^{13}C labelled methyl groups, we introduce an NMR active nucleus, which allows us to filter out a vast majority of resonances and to detect signals only from the immediate neighbourhood of the $^{13}CH_3O$ groups. By comparing the obtained ^{1}H and ^{13}C chemical shifts with database information we can suggest structural fragments present in these compounds.
We have developed novel 3D and 4D NMR experiments that use proton-carbon, carbon-carbon and proton-proton couplings or the NOEs to transfer the magnetisation from aromatic protons to $^{13}CH_3O$ groups of aromatic compounds as shown below.



We utilise these polarisation transfer pathways in the design of several novel NMR experiments:

3D $HcCH_3$, 3D $HCcH_3$ and 4D $HCCH_3$
3D INADEQUATE-HSQC
3D HMQC-HMBC
4D COSY-NOESY-HMQC
We illustrate the use of these experiments on a complex model mixture of phenolics.

Poster Presentation

382 TU

STUDY BY THE STD NMR TECHNIQUE OF THE INTERACTION BETWEEN PEPTIDES TARGETING THE A_VB_3 INTEGRIN AND PANC-1 CELLS

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[2] *Center for Microscopy and Molecular Imaging (CMMI), Academie Wallonie-Bruxelles, Gosselies*

Molecular Imaging by MRI is a fastly growing field of research. It involves the development of molecular contrast agents with a high affinity for a target molecule which is expressed or overexpressed in pathological conditions.
Since several years, our laboratory has chosen the strategy of grafting on MRI contrast agents small peptides with a high affinity for a target molecule. The evaluation of the association constant of the peptide with its target is thus a very important step, and NMR appears to be a very attractive tool.
Many interesting target molecules are however membrane-bound proteins, which are often difficult to deal with in solution. They indeed lose their structure and function when they are removed from their natural membrane environment. It is thus important to be able to study the non covalent interactions with these proteins directly on cells. As reported in the literature [1], the STD NMR technique is particularly appropriate.
The STD NMR technique consists in the selective irradiation of the protein NMR spectrum, which results in saturation of the protein signals and of any ligand protons interacting with the protein. This is the so-called "on-resonance" spectrum. Subtraction of this spectrum from the one collected with the selective irradiation out of the NMR spectrum of the protein and the ligand (the so-called "off-resonance" spectrum) gives the saturation transfer difference spectrum, in which only protons interacting with the protein are visible.
As a proof of concept, we have studied here the non covalent interactions between different peptides containing the RGD sequence known to interact with integrin $\alpha_V\beta_3$ and PANC-1 cells, which overexpress this integrin.
[1] Claasen, B., Axmann, M., Meinecke, R., Meyer, B. *J. Am. Chem. Soc.* 2005, **127**, 916-919.

383 TH

PROTEIN-PROTEIN AND PROTEIN-LIGAND INTERACTIONS TROUGH NMR

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Binding events of proteins with biological molecules are the key for understanding biological processes. Targeting protein–protein and protein–ligand interactions is of fundamental importance in structure-based drug design.
NMR has become a powerful and versatile tool for characterizing at an atomic level the interaction of protein partners, which represent biologically relevant targets for drug discovery. NMR has been also developed into a mature technique for the identification of small molecule ligands for macromolecular targets. The identification of high affinity ligands of target proteins usually requires an initial screening of extended libraries of low affinity compounds followed by their optimization into drugs for therapeutic intervention.
We present here an approach that combines biotechnology and advanced NMR tools for studying the interactions of "difficult" proteins involved in neurodegenerative and oncology diseases.

Poster Presentation

384 MO

RESOLUTION AND SENSITIVITY ENHANCEMENT BY REAL-TIME PURE SHIFT HSQC

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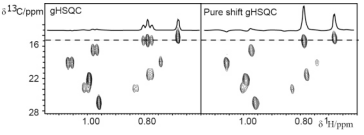
[1] *School of Chemistry, University of Manchester, Oxford Road, Manchester, M13 9PL, UK.*

[2] *Department of Chemistry, Durham University, South Road, Durham, DH1 3LE, UK.*

[3] *NMR Laboratory, Institute of Organic Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Pusztaszeri út 59-67, H-1025, Budapest, Hungary.*

[4] *University of Copenhagen, Faculty of Life Sciences, Dept. of Food Science, Rolighedsvej 30, DK - 1958 Frederiksberg C, Denmark.*

[5] *Agilent Technologies R&D a. Marketing GmbH & Co. KG, Hewlett-Packard Strasse 8. 76337 Waldbronn, Germany.*



A pulse program to acquire pure shift HSQC spectra in real time is described in which ^{1}H-^{1}H broadband decoupling is achieved using BIRD pulse trains [1,2]. FIDs for each t_1 increment are obtained in real time by windowed acquisition of chunks of data between BIRD elements in explicit sampling mode. J_{HH} evolution from one chunk to the next is refocused by a hard 180°/BIRD sequence element, and the effect of J_{XH} is suppressed with broadband heteronuclear decoupling. Homodecoupling is effective as long as the time between refocusing elements is $<<1/J_{HH}$ [3]; more frequent refocusing gives cleaner spectra, but at the expense of some line broadening due to imperfect pulses and T_2 relaxation. Here, we show that this homonuclear decoupling acquisition scheme in HSQC gives both resolution and sensitivity enhancement. The figure below shows a straight experimental comparison between standard gHSQC and real time pure shift gHSQC for the methyl region of a mixture of sarsasapogenin and stigmasterol.

[1] Garbow *et al.*, Chem. Phys. Lett. **1982**, *93*, 504-509
[2] Lupulescu *et al.*, J. Magn. Reson. **2012**, *218*, 141-146 //// [3] Aguilar *et al.*, Angew. Chem. Int. Ed. **2011**, *50*, 9716-9717

385 TU

MONITORING FAST REACTIONS BY SPATIALLY-SELECTIVE AND FREQUENCY-SHIFTED CONTINUOUS NMR SPECTROSCOPY

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Institute of Chemistry, Organic and Bioorganic Chemistry, University of Graz

The repetition rate of an NMR experiment is usually limited by the longitudinal relaxation times of the investigated molecule, which can be up to several seconds for hydrogen. Therefore NMR spectroscopy is usually used to study systems under equilibrium conditions. Nevertheless it has also been applied to investigate transient / non-equilibrium reactions in real time where it is appreciated for its broad temperature range, being noninvasive and for the level of detail of structural information.

Typically reactions can be continuously sampled down to the range of seconds. Between successive scans the system under study has to return close to its thermal equilibrium in order to avoid progressive saturation of the magnetization which would lead to intensity variations and in the worst case disappearing signals. This can be partly alleviated for example by the use of small pulse angles in general or band-selective pulses in the case NH-detected experiments of proteins. All these approaches aim at reducing the interscan delay, whose duration still often is several times the length of the actual NMR experiment.

Here we present a new technique to circumvent this shortcoming by using spatially-selective and frequency-shifted pulses to excite different nuclei in consecutive scans. Irradiation is achieved by a selective pulse applied during a weak pulsed field gradient. This allows the excitation of the whole spectrum with a single selective pulse of a few hundred Hertz, as it irradiates different resonances in different slices of the sample. After each scan the excitation frequency is shifted. Therefore, different signals are excited in a slice in each scan, while previously excited signals can start to relax. This allows for a continuous excitation and acquisition without any interscan delay. The huge increase in temporal resolution, which allows the acquisition of dozens of 1D NMR spectra per second, is traded against lower sensitivity. As an application, this continuous NMR experiment was used to follow the pH induced unfolding of myoglobin. Wagner, G. E.; Sakhaii, P.; Bermel, W.; Zangger, K. *Chem Commun* **2013**, *49*, 3155.

Poster Presentation

386 TH

FAST EXCHANGE RATE CONSTANTS OF PROTONS MEASURED THROUGH INDIRECT DETECTION VIA ^{15}N NUCLEI IN HISTIDINE BY NMR SPECTROSCOPY

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Knowledge of the exchange rates of labile protons is crucial for understanding internal dynamics of biomolecules. NMR is a unique technique since it can detect chemical exchange at atomic resolution even when the system is in equilibrium. For small molecules containing a single exchanging proton, rate constants as fast as a few thousand Hz can be determined by detecting ^{1}H signals provided that the chemical shifts of the exchanging proton and of the solvent protons are sufficiently separated. If there are several exchanging NH sites, or if the proton spectrum is crowded, the quantification of the proton exchange is impossible. In such systems, the proton exchange can be monitored indirectly on the ^{15}N resonances. The residual width of the partially collapsed lines in ^{15}N spectra can be reduced by proton decoupling. The comparison of ^{15}N spectra with and without proton decoupling gives a measure of the exchange rate constants of the attached protons. We have introduced a method to quantify the effects of scalar relaxation caused by exchanging protons, by observing the decay of ^{15}N coherence under a multiple-refocusing Carr-Purcell-Meiboom-Gill (CPMG) sequence in the presence or absence of proton decoupling. Refocusing cancels the effects of long-range couplings even in the absence of proton decoupling [1]. In the present work, we have applied this method to measure the exchange rate constants by indirect detection via ^{15}N of labile H^{N} protons in the imidazole ring and the NH_3^+ group as a function of temperature and pH in an aqueous solution of uniformly labelled L-Histidine.HCl.H_2O. These rates could be obtained although no prior knowledge was available neither about the chemical shifts of the exchanging protons nor about the short-range scalar couplings.

[1] F. Kateb, P. Pelupessy, G. Bodenhausen, J. Magn. Reson. 2007, 184, 108–113.

387 MO

COMBINATION OF NON-UNIFORM SAMPLING AND COMPRESSED SENSING: AN EFFICIENT WAY FOR ENHANCING SENSITIVITY OF NATURAL ABUNDANCE DEUTERIUM 2D-NMR SPECTROSCOPY IN ORIENTED SOLVENTS

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Natural abundance deuterium (NAD) 2D-NMR spectroscopy using chiral liquid crystals as NMR solvents is a unique and original tool and for the chemical analysis allowing e.g. the chiral differentiation between various enantio- and/or diastereoisotopomers present in the same sample (1). Recently, the non-uniform sampling (NUS) combined with covariance (Cov) processing was introduced to NAD 2D-NMR (2), improving the spectral resolution of anisotropic 2D spectra. The approach, although very effective, is however limited to symmetrical NAD 2D maps such as Q-COSY Fz 2D spectra (3). In this work, we extend the range of applications of NUS to unsymmetrical NAD 2D spectra, such as NAD Q-resolved Fz experiments (3) by using compressed sensing (CS) for the spectral reconstruction (4). The gain in sensitivity and experimental time originating from the use of relaxation-matched (5) sparse sampling is demonstrated for both symmetrical and unsymmetrical NAD 2D-NMR experiments (3) on (+/-)- -pinene dissolved in the PBLG/CHCl3 mesophase. The results show that "NUS/CS" approach can provide anisotropic NAD 2D spectra that are superior to conventional, regularly sampled data obtained in the same experimental time and processed with Fourier Transform (FT).

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Poster Presentation

388 TU

PHASE ANOMALIES IN SENSITIVITY ENHANCED HSQC SPECTRA AND 3D TRIPLE RESONANCE SPECTRA: ORIGIN OF THE PROBLEM AND A SIMPLE SOLUTION.

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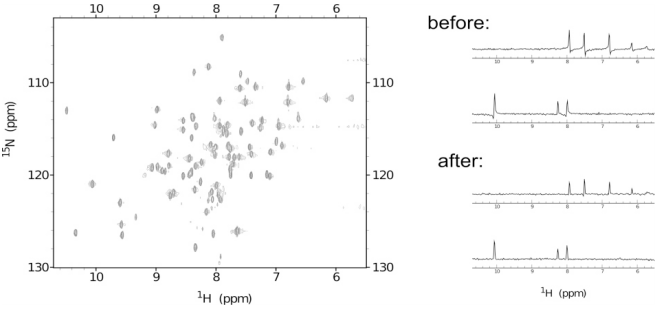
Here we describe phasing anomalies that were observed in gradient sensitivity enhanced 2D and 3D triple resonance experiments. It is shown that 15N off-resonance effects actuate undesired coherence transfer pathways in the sensitivity enhancement scheme, which leads to dispersive contributions to the observed 1H signal. As a result, the NMR spectrum "can no longer be properly phased". It appears as if traces taken at different 15N offsets require (slightly) different phase corrections along the 1H dimension. These effects are more severe for spectrometers operating at higher magnetic field, and for nuclei with larger spectral widths (e.g. 13C). We show by simulation how to effectively suppress the unwanted coherence transfer pathways using pulse field gradients, and demonstrate that this results in artefact-free, properly phased spectra in practice.



389 TH

DETERMINING THE ORIENTATION OF LIGANDS BOUND TO PROTEINS BY TRANSFERRED PARAMAGNETIC RELAXATION ENHANCEMENT NMR SPECTROSCOPY

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In the process of rational drug design it is necessary to have detailed knowledge of protein-ligand interactions. NMR spectroscopy techniques are often employed for screening compound libraries and, in cases of weak ligand-receptor interactions, for the determination of binding epitopes. We present here a new solution state NMR approach for gathering information about the orientation of a ligand in a receptor. Upon the addition of an inert paramagnetic agent to the solvent, relaxation enhancements on ligand nuclei depend on the insertion depth in the protein. If the ligand is in fast exchange between the free and bound state, these solvent paramagnetic relaxation enhancements are partly transferred to the free ligand where they can be observed with high resolution and without any size limitation of the receptor. This approach was applied to the binding of phenylbutazone to human serum albumin (HSA). Proton T_1 relaxation times were obtained from series of one dimensional ^{1}H spectra with a saturation recovery sequence at the beginning with lapses of time between 100 and 6000 ms before the 90° observe pulse. PREs were obtained by measuring relaxation times at different concentrations of the paramagnetic agent Gd(DTPA-BMA). The results are in perfect agreement with the crystal structure of phenylbutazone bound to HSA. Besides epitope mapping the solvent PRE approach gives qualitative information about how deep individual parts of the ligands insert into the receptor. This method opens up a fast route to a qualitative picture of the orientation of a ligand in a receptor without a complete structure determination, chemical modification or isotopic labeling.

Poster Presentation

390 MO

NEW METHODS FOR STUDYING PROLINE CIS-TRANS ISOMERIZATION BY SOLUTION STATE NMR

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Peptidyl-prolyl isomerization plays an important role in numerous biological processes like protein folding, gene regulation etc. X-Pro dipeptides might exist completely in either of trans or cis forms depending upon the tertiary protein structures. However, these two forms exist in the equilibrium in unfolded proteins where usually trans conformers are majorly populated. There are many peptidyl-prolyl *cis*/*trans* isomerases (PPIase) like FK506-binding proteins (FKBP), parvulins and cyclophilins that catalyze this intrinsically slow interconversion of the peptide linkage preceding a proline. (Hanoulle, Badillo et al. 2009)
The existing NMR methods based upon N_z exchange (Farrow, Zhang et al. 1994) are limited in studying the phenomenon because of the absence of amide protons in proline residues and by the intrinsically short ^{15}N spin-lattice relaxation times, T_1. Although, the catalyzed isomerization can be observed by this method on the neighboring residues which might have different chemical shifts for the cis or trans conformations of the subsequent X-Pro peptide bond. This might lead to ambiguities, especially in the proline rich regions, thus necessitating the need to develop new methods capable of observing the phenomenon directly on prolines. We have developed a new NMR method based upon the excitation of the magnetization, $C^{\alpha}_z.N_z$ during the mixing period which is suitable to observe the prolyl *cis*/*trans* isomerization of the X-Pro peptide bond directly on prolines.
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391 TU

PURE SHIFT HSQC MEASUREMENTS WITH PERFECTBIRD DECOUPLING – A METHOD TO DECOUPLE DIASTEREOTOPIC PROTONS

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The discrimination of different compounds which exhibit similar structural features, such as configurational isomers, can easily be prevented by signal overlap even when using modern high field NMR instruments. In such cases the use of signal dispersion in multiple indirect spectral dimensions is likely to be inefficient in achieving signal separation as the differences in chemical shifts observed can be quite small, possibly smaller than either the resolution of the indirect dimension or the multiplet width imposed by J-coupling.
To diminish these problems pure shift techniques hold great potential, as they can provide both high resolution and J-coupling suppression in the *direct* dimension.[1] HSQC experiments with homonuclear decoupling in the direct dimension have recently been presented using both pseudo-3D acquisition and a real-time 2D acquisition scheme.[2] The BIRD decoupling element[3] employed by the two techniques leads to a significant simplification of the spectra obtained, making them well suited for the study of complex problems. However it fails to suppress geminal scalar couplings, resulting in an "irreducible" doublet appearance of signals from diastereotopic protons.
As presented here, full homonuclear decoupling is possible even in the case of diastereotopic protons, if BIRD decoupling is combined with a perfect echo element.[4] The incorporation of the resulting perfectBIRD decoupling element into HSQC experiments, such as the F_2-coupled CLIP-HSQC experiment[5] which is suited for precise one-bond RDC-measurements, is presented.
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Poster Presentation

392 TH

NMR STUDIES OF IDPS AT NEAR PHYSIOLOGICAL CONDITIONS: EXPLOITING FAST HYDROGEN EXCHANGE TO BOOST EXPERIMENTAL SENSITIVITY

Tomáš Hošek, Sergio Gil, Zsofia Solyom, Rainer Kümmerle, Bernhard Brutscher, Roberta Pierattelli and Isabella C. Felli

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When approaching physiological conditions, solvent exchange of amide protons in intrinsically disordered proteins (IDPs) is so pronounced that it becomes one of the key features to be considered in the design of NMR experiments.
We show here how ^{13}C detected NMR experiments contribute to recovering information that would not be accessible through amide proton detection and how efficient solvent exchange can be exploited to increase experimental sensitivity. These ideas are demonstrated using a paradigmatic, well-studied IDP -synuclein. Two applications of general interest are also presented.

Gil S., Hošek T., Solyom Z., Kümmerle R., Brutscher B., Pierattelli R. Felli I.C., submitted
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393 MO

MEASURING ONE-BOND HETERONUCLEAR COUPLING CONSTANTS WITH IMPROVED RESOLUTION. UTILIZING THE POTENTIAL OF NOVEL BROADBAND PROTON-DECOUPLED HSQC-BASED METHODS

István Timári[1], Lukas Kaltschnee[2], Andreas Kolmer[2], Ralph W. Adams[3], Mathias Nilsson[3,4], Christina M. Thiele[2], Gareth A. Morris[3], Katalin E. Kövér[1]

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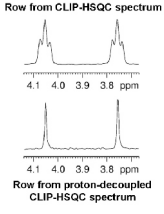

The utility of one-bond heteronuclear coupling constants for structure elucidation of small organic molecules has been growing in the recent years, particularly because of the importance of $^1D_{CH}$ residual dipolar couplings (RDC). Since the introduction of RDCs to high-resolution NMR, several methods have been developed for their measurement. One, the CLIP/CLAP-HSQC experiment[1], which is a slightly modified F_2-coupled HSQC method, provides pure absorptive in- or antiphase multiplets, allowing direct measurement of the desired couplings with good sensitivity. However, in the case of broad proton multiplets with several couplings the additional proton-proton splittings of the target heteronuclear multiplets may impede accurate measurement of one-bond heteronuclear couplings. Here we present a novel broadband proton-decoupled CLIP/CLAP-HSQC method aimed at the removal of the undesired proton-proton splittings from heteronuclear multiplets. The proposed experiments utilize ^{13}C isotope selection with a BIRD[4] pulse to refocus the effects of homonuclear *J* modulation and result in clean in- or antiphase doublets from which the desired heteronuclear couplings can be determined with ease and with high accuracy.
Based on this principle, we have developed a novel broadband proton-decoupled, BIRD pure shift HSQC method; a comparison of its performance to the decoupled HSQC experiment known from the literature[2] is also presented.
Acknowledgements: Financial support from TÁMOP-4.2.2/A-11/1/KONV-2012-0025, OTKA K 105459, Richter Gedeon Talentum Alapítvány, EPSRC grant EP/I007989, ERC and from Merck Foundation, is gratefully acknowledged.
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Poster Presentation

394 TU

HIGHLY ACCURATE MEASUREMENT OF LONG-RANGE HETERONUCLEAR COUPLING CONSTANTS WITH A NOVEL BROADBAND PROTON-DECOUPLED CPMG-HSQMBC METHOD

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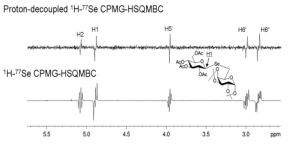

Long-range heteronuclear coupling constants are extensively used for structure elucidation of organic compounds and several methods have been developed for their measurement. One of them, our CPMG-HSQMBC experiment,[1] has found widespread application because of good sensitivity and straightforward determination of the desired coupling constants. However, when proton-proton and heteronuclear couplings are comparable, distortions can occur in the target CPMG-HSQMBC multiplet due to partial signal cancellation. Computer-aided multiplet fitting is of limited use to treat this problem. Here we report a novel broadband proton-decoupled CPMG-HSQMBC method for the removal of the unwanted proton-proton splittings from the heteronuclear multiplets. These experiments are based on the pure shift principle of Zangger and Sterk[2] and result in clean antiphase doublets from which the desired heteronuclear couplings can be determined with ease. The proposed proton-decoupled 1D/2D CPMG-HSQMBC pulse schemes have been tested on diglycosyl-selenides and other carbohydrate derivatives, as well as on (di)nucleotides for the measurement of $^nJ(^{77}Se,^1H)$, $^nJ(^{13}C,^1H)$ and $^nJ(^{31}P,^1H)$ values, respectively.

Acknowledgements: Financial support from TÁMOP-4.2.2/A-11/1/KONV-2012-0025, OTKA K 105459, Richter Gedeon Talentum Alapítvány, and from EPSRC grant EP/I007989 is gratefully acknowledged.

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395 TH

SPATIALLY ENCODED NMR SPECTROSCOPY ON A STRIPLINE PROBE

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Stripline NMR probes are a recent advance towards sensitive, chip-integrated NMR analysis. The term 'stripline' refers to how signals are detected by electromagnetic induction not in a resonant coil, but a linear resonator deposited (lithographically) upon a glass substrate, which may play host to microfluidic or capillary channels as desired. The linear design results in an efficient filling factor to counteract the low signal-to-noise of mass- and volume-limited samples and can comfortably supply rf fields on the order of several hundred kHz. The flat, highly symmetrical structure ensures low susceptibility broadening. Stripline devices are scalable between nanolitre- an microlitre volume samples with a typical single-scan detection limit of approximately 10^14 proton spins (c. 1 nanomole), depending on size.

In this work we explore options for spatially encoded NMR experiments on striplines. We use a 'tapered' stripline, where the width of the resonator varies across the strip length. This deliberate inhomogeneity in the setup translates into a linear B_1 gradient for spatial encoding. In hardware terms the effort is trivial; the only modification is a change in the width of the strip. Facility for pulsed field B_0 gradients is much more difficult at present. The B_1 encoding is typically used to monitor ultra fast chemical reactions in situ with a time resolution of less than 100 ms.

We show the ability to measure liquid-state molecular self-diffusion coefficients and diffusion-ordered spectra using a tapered stripline tuned at 600 MHz. We also discuss possible options for fast 2D (correlation) spectroscopy using B_1 gradients rather than switched B_0 gradients.

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Poster Presentation

396 MO

SIMULTANEOUS MEASUREMENT OF THE MAGNITUDE AND THE SIGN OF MULTIPLE HETERONUCLEAR COUPLING CONSTANTS IN SMALL MOLECULES

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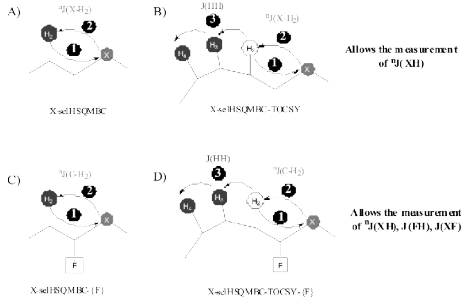
We have recently reported a suite of powerful selective HSQMBC experiments for the accurate measurement of proton-carbon coupling constants. Here we make extensive these experiments to other heteronuclei than 13C. We show that the magnitude and the sign of multiple heteronuclear coupling can be simultaneously measured from the analysis of a single cross-peak. We also discuss the enhanced effects to include TOCSY editing or the presence of passive spins and their complementarities with the IPAP and E.COSY techniques. Finally, the success of analogue time-shared experiments are exemplified for the simultaneous measurement of proton-carbon and proton-nitrogen coupling constants in nitrogen-congaing derivatives.



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397 TU

DETAILED INSIGHT INTO QUINONE-TYPE LIGAND BINDING BY THE SODIUM ION-TRANSLOCATING NADH:QUINONE OXIDOREDUCTASE FROM *V. CHOLERAE*

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The sodium ion-translocating NADH:quinone oxidoreductase (Na^+-NQR) from *Vibrio cholerae* is a respiratory membrane protein complex that couples the oxidation of NADH and the reduction of membrane-bound quinone to the transport of Na^+ across the bacterial membrane. The Na^+-NQR (216 kDa) is composed of six subunits NqrA-F and contains at least five redox-active cofactors. How electrons are transferred between NADH and quinone, as well as the entire mechanism of sodium translocation is still enigmatic and constitutes the major objectives of this project.

Our NMR investigations are focused on the soluble NqrA subdomain of Na^+-NQR as it was suspected that quinone could bind to the NqrA during the last electron transfer step. Protocols for expression in *E.coli* and purification of unlabelled and various labelled forms of NqrA were developed and improved. Considering insufficient stability of full length NqrA a truncated construct with 377 amino acids was also developed.

By saturation transfer difference NMR (STD NMR) spectroscopy we could show that ubiquinone-1 (Q_1) and the inhibitors HQNO (2-heptyl-4-hydroxychinolin-N-oxide) and DBMIB (2,5-dibromo-6-methyl-3-isopropyl-1,4-benzoquinone) bind to NqrA. Later STD NMR studies with HQNO and DBMIB in the presence of Q_1 showed an allosteric behaviour indicative of the presence of two individual binding sites within the NqrA subunit. Surface plasmon resonance (SPR) and fluorescence titration experiments also corroborate this hypothesis. Furthermore by detecting Interligand Overhauser Effects (ILOEs), we could then show that Q_1 and DBMIB/HQNO bind to an extended binding pocket in direct vicinity to each other. Subsequent STD NMR studies with Q_1 and DDM-solubilised holo-Na^+-NQR complex in the presence of DBMIB showed similar behaviour as with the isolated NqrA subunit which is indicative of functional importance of our findings. A high quality ^1H-^{15}N TROSY NMR spectrum of fully deuterated ^{15}N-labelled NqrA-377 was obtained. Addition of DBMIB to the sample perturbs specific signals in the spectrum. To identify the DBMIB binding pocket, a perdeuterated ^{13}C-^{15}N-labelled NqrA-377 sample was expressed and purified the backbone assignment of which is currently in progress. We were already able to locate the binding site of DBMIB from the chemical shift perturbations.

Poster Presentation

398 TH

59^CO NMR INVESTIGATION OF COBALT-CONTAINING NANOCOMPOSITES

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NMR-in-magnetics as a variant of NMR technique is widely used for investigation of magnetic nanostructures especially of cobalt-containing ones. Here we represent some results of studying of nanocomposite materials which were consisted of cobalt clusters in/on various diamagnetic matrices. Parameters of NMR signals allow one to inspect if the studied material is ferromagnetic at local level, to define local magnetic structure of nanoparticles, to estimate a percentage of cobalt in the metallic phase for different preparation methods etc. Hence, NMR-in-magnetics technique can be used as an effective method of testing, characterization and certification of magnetic nanomaterials. In this connection we discussed in brief various ways of obtaining and representation of 59^Co NMR spectra in magnetic materials, in particular in nanoparticles.
A few systems have been analyzed in more detail. First, we considered bimetallic Fe-Co nanoparticles on the surface of Teflon granules. A rather narrow and symmetric 59^Co observed spectrum arose owing to existence of metallic-cobalt core inside each particle. At the same time Mossbauer 57^Fe measurements showed that iron presented only in oxide forms, both as Fe(III) and Fe(II). Together NMR and Mossbauer results allowed one to conclude that studied nanoparticles had core-shell structure and consisted of a core of metal cobalt and the patch shell of iron oxides.
As the second example we analyzed a structural transformation of cobalt clusters in SiO_2 matrix with time (aging effect) and its dependence on method of the material preparation. For one type of preparation NMR spectra and local magnetic properties changed fast enough, and in some months no metallic cobalt with regular structure was detected. It means that used preparation technique led to a material with more or less free access of air oxygen to cobalt core, and this kind of preparation could be useful for catalytic applications. On the other hand, nanoparticles prepared by another method showed significantly higher stability namely, single-line NMR spectrum did not change during a year. Nevertheless, after a few years the spectrum also changed its shape, and the second, lower-frequency line appeared.

399 MO

LOCAL MAGNETIC PROPERTIES IN CR_8, CR_7CD AND CR_7NI MOLECULAR RINGS FROM ^{19}F-NMR

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A detailed experimental investigation of the ^{19}F nuclear magnetic resonance (NMR) is made in the homometallic Cr_8 antiferromagnetic (AFM) molecular ring and in the heterometallic Cr_7Cd and Cr_7Ni rings at low temperature and as a function of the magnetic field . Since the F^- ion is located midway between two magnetic metal ions in the ring the ^{19}F-NMR spectra have a complicated field dependent structure, due to both isotropic transferred hyperfine contact interactions and anisotropic dipolar and pseudo-dipolar interactions. In Cr_8, where the ground state is a singlet with total spin S=0, the ^{19}F-NMR spectra at 1.6 K and low external magnetic field show a single narrow line, proving that the local spin density in the ground state is zero as expected for a molecular singlet state. By increasing the magnetic field towards the first level crossing field (H_c about 7 Tesla) a structure appears in the ^{19}F-NMR spectrum whose evolution as a function of temperature and external magnetic field proves that the thermal excitation to the first magnetic excited state generates a statistical distribution of molecules in the ground state and excited state each with fixed values for the local moments at the Cr sites. This is a novel and unexpected result. On the other hand in Cr_7Cd and on Cr_7Ni. the ground state is magnetic with a non uniform distribution of the local spin density [1]. This leads to a ^{19}F-NMR spectrum with a shifted line attributed to the ^{19}F nuclei that are located midway between a Cr^{3+} ion and a Cd^{2+} or Ni^{2+} ion, thus allowing the determination of the transferred hyperfine constant F^--Cr^{3+} and F^--Ni^{2+} for the specific site. The values of the hyperfine constants are compared to the ones known for F^--Mn^{2+} in $KMnF_3$ and MnF_2 , for F^--Ni^{2+} in $KNiF_3$ and NiF_2 and for F^--Cr^{3+} in K_2NaCrF_6.

Poster Presentation

400 TU

QUANTUM-CRITICAL SPIN DYNAMICS IN QUASI-ONE-DIMENSIONAL ANTIFERROMAGNETS

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Low-dimensional quantum antiferromagnets in a magnetic field are remarkable model systems for studying the field induced exotic phases, e.g., Bose-Einstein condensation [1], and the related quantum critical points (QCPs) where the continuous quantum phase transition occurs at zero temperature. In the vicinity of QCPs the physics is complex but universal, i.e., insensitive to the microscopic properties of the system, and scale invariant, with temperature setting the only energy scale.

We present here the first clear experimental demonstration of the quantum-critical behaviour in magnetic insulators [2]. By means of nuclear spin-lattice relaxation rate T_1^{-1}, we have followed the spin dynamics as a function of the applied magnetic field in two gapped quasi-one-dimensional (quasi-1D) quantum antiferromagnets: the anisotropic spin-chain system $NiCl_2$-4SC$(NH_2)_2$ and the spin-ladder system $(C_5H_{12}N)_2CuBr_4$. In both systems, spin excitations are confirmed to evolve from magnons in the gapped state to spinons in the gapless Tomonaga-Luttinger-liquid state. In between, T_1^{-1} exhibits a pronounced, continuous variation, which is shown to scale in accordance with quantum criticality. We extract the critical exponent for T_1^{-1}, compare it to the theory, and show that this behaviour is identical in both studied systems, thus demonstrating the universality of quantum-critical behaviour.

The T_1^{-1} data and analysis presented in Ref. [2] correspond to a quasi-1D critical behaviour, which is not yet covered by any theory. For this regime we have extracted the experimental scaling function, to serve as a reference for future theoretical descriptions. The data have been further extended to a 3D region at lower temperatures in $NiCl_2$-4SC$(NH_2)_2$, and to the true 1D region at higher temperatures in $(C_5H_{12}N)_2CuBr_4$. We discuss the results in both of these limits, where a comparison to the existing theories is possible.

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401 TH

HIGH MAGNETIC FIELD STUDIES ON SINGLE CRYSTALS OF THE $S = 2$ 1D HEISENBERG ANTIFERROMAGNET $MnCl_3(bpy)$

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$MnCl_3$(bpy) (bpy = $C_{10}H_8N_2$) (Mn^{3+}, $S = 2$) is one of the rare compounds regarded as the $S = 2$ one-dimensional (1D) Heisenberg antiferromagnet [1]. In a previous study [2], this compound showed no magnetic long-range order down to 30 mK and the transition fields H_c (~1.5 T) caused by the closing of an energy gap between the singlet ground state and the first excited triplet state were evaluated. A high field magnetization curve of a powder sample of this compound was well fit to the calculated magnetization for the $S = 2$ 1D Heisenberg antiferromagnet with a small anomaly at around 25 T, but the ESR results indicate typical antiferromagnetic resonance modes with biaxial anisotropy in a long-range antiferromagnetic ordered phase [3]. Therefore, we have performed magnetization and electron spin resonance (ESR) measurements on single crystals of $MnCl_3$(bpy) in high magnetic fields. We observed a spin flop transition at around 25 T in the magnetization curve for H // c (chain) at 1.3 K, while no such transition was observed for $H \perp c$. The frequency versus resonance fields plot indicates similar antiferromagnetic resonance modes with biaxial anisotropy. These results imply that a long-range order occurs above H_c where the magnetism recovers.

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Poster Presentation

402 MO

SPATIALLY RESOLVED MAGNETIZATION IN THE BOSE-EINSTEIN CONDENSED STATE OF $BA_2CUSI_2O_6$: EVIDENCE FOR IMPERFECT FRUSTRATION BY HIGH FIELD NMR

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Low dimensional, weakly coupled antiferromagnetic (AF) spin systems offer a huge variety of interesting physical phenomena that can be controlled, amongst others, by an external magnetic field *H*. For instance, field induced quantum phase transitions between different magnetic ground states can be observed [1]. As an example, we will present here the Han purple compound $BaCuSi_2O_6$, which is considered to exhibit a prototype magnetic field induced Bose-Einstein Condensation (BEC) of triplet excitations (hard core bosons) on a lattice. $BaCuSi_2O_6$ consists of AF coupled spin-1/2 Cu^{2+} dimers that are arranged in stacked planar square lattices. Their $H = 0$ ground state is a collective spin singlet state separated from the first excited triplet state by a magnetic excitation gap. For *H* larger than the critical magnetic field H_{c1} this energy gap is closed and a canted XY AF ordered state occurs, which is described as a BEC of hard core bosons. Its hallmark is the field dependence of the transition temperature T_{BEC} (H-H_{c1}), where = 2/*d* = 2/3 in the standard 3D ($d = 3$) case. However, in $BaCuSi_2O_6$ T_{BEC} varies linearly with (H-H_{c1}) between 40 mK and 700 mK and this system has thus been claimed to exhibit a dimensional reduction of the BEC from 3D to 2D due to frustrated interlayer coupling.
In order to understand the nature of the 2D BEC phase in $BaCuSi_2O_6$, we performed detailed ^{63}Cu and ^{29}Si NMR above the critical magnetic field $H_{c1} = 23.4$ T [2]. Our study reveals two substantially different local magnetizations close to H_{c1}. They can be attributed to different alternating layers present in the system due to a structural phase transition occurring at 90 K [3]. On one layer, the magnetization is very weak, and its size and field dependence are highly sensitive to the nature of inter-layer coupling. Its precise value could only be determined by ^{63}Cu NMR at the site of the electron spin. Our data are fully reproduced by a model of interacting bosons, in which the perfect frustration associated to tetragonal symmetry is slightly lifted, leading to the conclusion that the population of the less populated layers is not fully incoherent but partially condensed.
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403 TU

ELECTRON SPIN RESONANCE STUDY OF ANISOTROPIC EXCHANGE IN SPIN LADDERS

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We report on electron spin resonance (ESR) studies of the magnetic spin-ladder materials $(C_5H_{12}N)_2CuBr_4$ and $BiCu_2PO_6$ (hereafter referred to as BPCB and BCPO, respectively). The former is known as one of the best realizations of a spin-ladder model in the strong-coupling limit ($J_{rung} \sim 4J_{leg}$), while the latter has comparable J_{rung} and J_{leg} and is characterized by a substantial frustration along the ladder. Our experiments provide direct evidence for the presence of anisotropy in BPCB, which is in contrast to a fully isotropic spin-ladder model employed for this system previously. It is argued that the anisotropy is caused by spin-orbit coupling, specifically by the symmetric anisotropic interaction. Our observations are consistent with results of calculations [Furuya *et al.*, Phys. Rev. Lett. 108 (2012) 037204]. Furthermore, the angular dependence and frequency-field diagram of the ESR transitions in BCPO are analyzed employing a simple spin-1/2 dimer model taking into account the symmetric anisotropic exchange interaction. In BCPO, we report on the observation of a number of ESR modes; the data are compared with results obtained from inelastic neutron-scattering experiments [Plumb *et al.*, arXiv:1301.5324v1]. The peculiarities of the ESR excitation spectrum in BCPO are discussed.
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Poster Presentation

404 TH

THE ORDERING PHENOMENA AND COEXISTENCE OF THE MAGNETIC AND PARAELECTRIC SUBSYSTEM IN Li_2ZrCuO_4 STUDIED BY ^7Li NMR

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Coexistance of the interpenetrating sublattices of frustrated quasi-one-dimensional S=1/2 quantum Heisenberg magnetic Cu^{2+} chains and frustrated quantum Ising electric sublattice of Li_i ions is an intrinsic feature of the Li_2ZrCuO_4.
Incommensurate spin order occurs in this compound at T_N=6K. The low temperature ^7Li NMR spectra of oriented powder samples show that the peculiar NMR lineshapes are determined by the specific distribution of the Li_i ions in the glassy ordered electric sublattice.
The analysis of the NMR data in the framework of a spin-spiral model gives evidence for a remarkable interaction between magnetic and electric degrees of freedom in -Li_2ZrCuO_4 which suggests it as promising model compound for fundamental studies of complex magnetoelectric phenomena.

405 MO

IMPURITY EFFECTS IN A S=1/2 HEISENBERG SPIN CHAIN PROBED BY ^{63}Cu NMR

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We present ^{63}Cu NMR measurements on undoped, Ni doped and Mg doped $SrCuO_2$ single crystals. $SrCuO_2$ is a good realization of a one-dimensional S=1/2 Heisenberg spin chain. This is confirmed by the theoretically-expected temperature independent NMR spin-lattice relaxation rate T_1^{-1}. Doping with Ni, which can be regarded as a S=1 impurity, has a major impact on the magnetic properties of the spin chains. On the one hand, this is manifested by unusual features in the NMR spectra below 100 *K*, revealing the existence of an impurity-induced local alternating magnetisation. On the other hand, exponentially decaying spin lattice relaxation rates towards low temperatures indicate the opening of a spin gap similar to Ca doped $SrCuO_2$ [1]. Mg doping (S=0) has, however, no influence on the magnetic properties of the spin chains. Neither the NMR spectra nor the spin lattice relaxation rates differ from those measured on pure $SrCuO_2$. While the different impact of Ni and Mg doping on the spin chains could be explained by their different impurity spins, the opening of a spin gap in case of Ni doping is totally unexpected and not yet understood.
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Poster Presentation

406 TU

MONITORING THE TRACE OF DYNAMIC AND STATIC CHECKERBOARD POLARONS IN COLOSSAL MAGNETORESISTIVE MANGANITES. A ^{139}LA AND ^{55}MN NMR STUDY

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Charge carriers in low doped Mott insulators might induce lattice distortions and trap themselves forming "small polarons". By increasing doping, polarons correlate and form short range species, extending over a few lattice constants (large polarons), while by further increasing doping they might form a long range ordered structure characterized by spin, charge and orbital ordering. An archetypal family exhibiting this kind of behavior is the manganese perovskite family $La_{1-x}Ca_xMnO_3$ (LCMO). The mother system $LaMnO_3$ is a Mott insulator, where e_g orbitals of the Jahn-Teller active Mn^{3+} form a staggered arrangement with ferromagnetic coupling of the Mn ions in the MnO layers and antiferromagnetic inter-planar coupling. Doping this system with holes leads to intriguing arrangements where orbital, charge and spin order interact strongly. One such example is the spin, charge and orbitally ordered insulating phase (known as CE-phase) at half doping x=0.5, whereas at intermediate doping (around x=1/3) frustration results in a ferromagnetic metallic ground state, which in the vicinity of Tc exhibits the famous Colossal Magnetoresistance (CMR) effect (significant decrease of the electrical resistivity by applying a magnetic field). Remarkably, right above Tc, in the PM phase, frozen CE type short range correlated polarons with correlation length 15Å have been detected, as evidenced in the diffuse elastic or quasielastic neutron diffraction peaks, observed in the vicinity of $q_{CE}=(1/4,1/4,0)$[1]. Below Tc ferromagnetic fluctuations rapidly melt the local CE correlations, leading to a ferromagnetic metal, with however poor conductivity and small carrier scattering length. Only recently inelastic neutron scattering measurements have shown that CE polarons survive as fluctuations deep into the FM phase of $La_{1.2}Sr_{1.8}Mn_2O_7$[2]. Here, by using ^{139}La and ^{55}Mn NMR as local probes, we unveil the dominant role of CE-type static and dynamic polarons in the electron spin and transport properties of LCMO at optimal doping x=1/3, in the temperature range 3K – 500K[3,4].

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407 TH

ZERO BOIL-OFF MAGNET
- SOLUTION FOR HELIUM CRISIS -

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2 JEOL Resonance Inc.
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Nuclear magnetic resonance systems (NMR, MRI, etc.) require the existence of high magnetic field with good homogeneity. It should be noted that such magnetic field is practically obtained only by the liquid helium cooled superconducting magnet.
In the meanwhile, due to the volatile supply situation of liquid helium, together with resulting cost increase, a demand for an advanced helium conservation system has become more stringent.
As a solution to such a demand, JEOL Resonance developed a novel NMR System which does not require liquid helium refill.
Key technologies which enabled this product development involve those to suppress noise issues possibly caused by the application of cryocooler as well as those to extend helium hold time in case of cryocooler stoppage due to possible power disruption.

Poster Presentation

408 MO

T_2 RELAXIVITY OF SUPERPARAMAGNETIC FERRITE NANOPARTICLES MFE_2O_4 (M= MN, CO, NI)

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Superparamagnetic spinel ferrites MFe_2O_4 (M = Mn, Fe, Co, Ni) are currently considered among the most successful magnetic nanoparticles since they find applications in various research fields spanning from technological applications (e.g. density magnetic storage) to biomedical applications. Biomedical applications include the contrast enhancement in magnetic resonance imaging (MRI), drug delivery and hyperthermia against cancer. Bio-applications require magnetic nanoparticles such as ferrite nanoparticles with well-defined composition and morphology, narrow size distribution, and high saturation magnetization values.
In particular, MRI is one of the most powerful *in vivo* imaging technologies in which metal ferrite (MFe_2O_4) nanoparticles demonstrated to act as negative contrast agents. The degree of the T_2 contrast effect is typically represented by the transverse (or spin–spin) relaxivity R_2 ($R_2=1/T_2$), where higher values of R_2 result in a greater contrast effect. These properties of the magnetic nanoparticles are dependent on the chemical and physical characteristics of the particles as well as their surfaces. Moreover, the role of surfactant can be considered crucial as it influences the morphology and surface chemistry and as a consequence it meets the specific demands for bio-applications. In the present study, we present the synthesis, characterization and the functionalization of a series of ferrite nanoparticles with the formula MFe_2O_4 where M = Mn, Co, Ni. Truncated MFe_2O_4 nanoparticles of different sizes have been isolated in the presence of long aliphatic chains, such as oleylamine (OAm) and octadecylamine (ODA). In order to reveal the possibility of application of the MFe_2O_4 nanoparticles as MRI contrast agents, transverse relaxation time, T_2 of hydrogen proton of water molecule in presence of the MFe_2O_4 nanoparticles and at various concentrations, has been studied using NMR spectrometry (11.7 T). For the T_2 measurements, a multi-spin-echo CPMG (Carr-Purcell-Meiboom-Gill) sequence was employed. Furthermore, relaxivities (r_2) ($mM^{-1}s^{-1}$) were extracted.

409 TU

COBALT FERRITE NANOPARTICLES AS CANDIDATES FOR MRI PROBES AND BIOLOGICAL APPLICATIONS

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Spinel Cobalt Ferrite nanoparticles ($CoFe_2O_4$ NPs) have vast potential for application in many different areas of biomedicine, from diagnostics to treatment of diseases. The superparamagnetic properties as a result of the nanoscale in combination with the high anisotropy of cobalt and the adjustable surface coating make them efficient candidates as MRI probes. Optimization of contrast enhancement agent properties includes: enhancement of magnetic properties, dispersion stability in aqueous medium and *in vivo* biocompatibility. Key factors for these properties are considered: the particle size, surface morphology, surface coating, crystallinity, composition and finally cation distribution of spinel structure.

In the present study, hydrophobic $CoFe_2O_4$ NPs have been prepared via solvothermal method, in a range of sizes (8-18 nm), using as surfactant long-chain amines of similar molecular weight. Structural characterization of the samples (XRD, TEM, IR) showed that the products consist of a single spinel cobalt ferrite phase. The presence of the amine on the surface of the NPs was confirmed (IR, ^{1}H-NMR, TGA) and morphological characterization (TEM, SEM) gave valuable information about the shape and distribution of size for each sample. Composition analysis (EDS, ICP-OES) determined the stoichiometry of $CoFe_2O_4$ NPs. The magnetic properties were measured by VSM and SQUID magnetometers. Saturation magnetization values were found to be close to the bulk value (~80 emu/g). The enhancement of magnetization is attributed to high crystallinity of nanoparticles due to the solvothermal conditions. Furthermore, $CoFe_2O_4$ NPs were functionalized in order to become water soluble and T2 relaxation time measurements could be carried out by an NMR spectrometer (11.7 T). The effects of different sizes, magnetic properties and surface coating on relaxivity are discussed. Additionally, the amine coated $CoFe_2O_4$ NPs were functionalized with a fluorescent bioconjugate compound (FITC-Bovine Serum Albumin) and their behavior *in vitro* was observed by confocal microscopy. The cytotoxicity tests confirmed that $CoFe_2O_4$ NPs toxicity lies within tolerable limits.

POSTER PRESENTATION

410 TH

NMR SPECTROSCOPY OF INDOMETHACIN MOLECULES EMBEDDED WITHIN THE MESOPORES OF SILICATES AND METAL-ORGANIC FRAMEWORKS

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Solid-state NMR spectroscopy provides a unique tool for studying the structural properties of the mesoscopically confined drug, and for studying the drug-drug and drug-matrix interactions. We demonstrated this in model drug-delivery systems prepared from non-functionalized and functionalized SBA-15 mesoporous silicate matrices [1,2], Cr-, Fe-, and Al-based MIL-101 metal organic frameworks, and Al-based MIL-53 metal organic frameworks, loaded with different amounts of indomethacin molecules. In the SBA-15-based drug-delivery systems 1H MAS and 1H-13C CPMAS NMR spectroscopy indicated that only when concentration of indomethacin within the mesopores becomes sufficiently high (when the mass fraction of indomethacin within the sample exceeds 0.15), hydrogen bonds between the drug molecules become abundant. Nitrogen sorption analysis and comparison of 1H spin-lattice relaxation times in progressively loaded SBA-15 matrices suggested that at low loading concentrations indomethacin forms a layer on the silicate walls of the mesopores, and that at moderate or high loading concentrations rigid nanoparticles that extend throughout the entire mesopore cross-section are formed. 1H-13C CPMAS NMR spectrum of indomethacin embedded within the mesopores of SBA-15 closely resembled the spectrum of the bulk amorphous indomethacin and did not allow to draw firm conclusions about the molecular conformation and the packing of the drug molecules within the pores. On the contrary, variable-temperature 1H spin-lattice relaxation measurements showed that the mesoscopically confined indomethacin is significantly different from the bulk amorphous indomethacin. It does not become rubbery and it exhibits a solid-solid transition at 363 K that is similar to the phase transition of the crystalline indomethacin solvate with tetrahydrofuran. In MIL-101- and MIL-53-based drug-delivery systems, in addition to the structural and dynamical information about the incorporated indomethacin molecules, 1H MAS and 1H-13C CPMAS NMR experiments provided a very convenient way for the determination of the amount of the loaded drug.

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411 MO

FERROMAGNETISM IN ANNEALED $Ce_{0.95}Co_{0.05}O_2$ and $Ce_{0.95}Ni_{0.05}O_2$ NANOPARTICLES

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This paper reports an investigation on the role of transition-metal ions in producing ferromagnetism in CeO_2 nanoparticles by electron paramagnetic resonance (EPR). Several samples of CeO_2 nanoparticles annealed at 200, 300, 400, and 500° C, doped with 5% Ni and 5% Co ions, characterized by X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), thermogravimetry analysis (TGA) and mass spectroscopy (MS), investigated by X-band EPR at 4, 10 and 300 K, and by magnetometry at 300K. Magnetic properties and EPR/FMR (Ferromagnetic Resonance) spectra of these nanoparticle samples were found to depend strongly on the annealing temperature (T_A), oxygen stoichiometry, and dopant-ion species. Different behavior of saturation magnetization in the samples with the dopants, Co and Ni, is found to be due to different - inward and outward - surface diffusion of these impurity ions, respectively, during annealing. Most notable features of the present study are as follows. (i) A detailed simulation of EPR/FMR spectra of isolated Co and Ni ions carried out here provides in-depth details on the role of the doped ions and oxygen (O^-) defects played in the observed magnetic properties. More details on the spins and the corresponding spin-Hamiltonian parameters for the transitions metal ions Ni and Co present in the samples are found. This, in turn, helps to understand the role of the doped ions and oxygen (O^-) vacancies on the observed magnetic properties. (ii) The various mechanisms of occurrence of ferromagnetism in Co- and Ni-doped CeO_2, as well as coexistence of ferromagnetic and paramagnetic phases in these samples have been unraveled. (iii) A model for the formation of superparamagnetic state in the sample of CeO_2, doped with 5% Ni, has been proposed here. (iv) EPR and TGA-MS data provide evidence for increased formation of oxygen vacancies as the annealing temperature, T_A, increases. This plays a major role in the magnetic properties of the annealed samples. (v) Different behaviors of saturation magnetization in the samples doped with Co ions as compared to those with Ni ions, from 200 to 400 C, are interpreted to be due to different -- toward and outward surface diffusions, respectively, of these impurity ions during annealing.

POSTER PRESENTATION

412 TU

INVESTIGATION OF MIXED-MONOLAYER-PROTECTED NANOPARTICLES BY NMR: THE USE OF LANTHANIDES FOR MULTI-COMPONENT DISTRIBUTION MAPPING*

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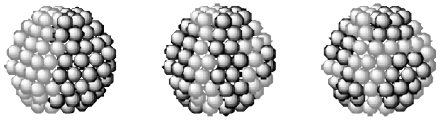
Monolayers of organic molecules coating nanoparticles (NPs) are known to produce different patterns (1) depending on the chemical nature of the grafted molecules (Figure: (a) Janus, (b) stripes, (c) patches). In principle, if such patterns could be turned into specific motifs, they may result in cooperation of functional groups to obtain molecular recognition in every field where NPs are applied, from nanomedicine to catalysis and materials development. Opposite to the nanocrystal core, however, there exist few experimental methods that can provide information on the monolayer structure itself. In this challenging framework, we exploit lanthanide-based NMR spectroscopy to investigate the properties of mixed monolayers.



(a) (b) (c)

When Gd^{3+} ions bind to nanoparticles coated with mixed monolayers, the signals arising from the different coating molecules experience a different paramagnetic relaxation enhancement (PRE) depending on their distance from the binding site. As a consequence, observation of the patterns of signal broadening provides direct information on the monolayer organization (2).

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413 TH

ON THE LOCAL STRUCTURE AND MAGNETIC BEHAVIOUR OF ANATASE $CO_XTI_{1-X}O_2$ NANOPARTICLES

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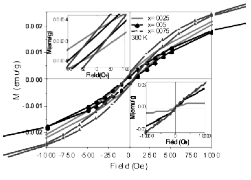
Recent research efforts on developing new room temperature ferromagnetic dilute magnetic oxides, like TiO_2, have become the subject of intensive interest, because of the possibility to manipulate both charge and spin degree of freedom in a single semiconductor material. Ferromagnetism observed has been established to have close link with oxygen vacancies [1]. However, the magnetic properties still remain a controversial issue, even in the case of Co-doped TiO_2, while the observed magnetic behaviour appears to be strongly dependent on the preparation methods, and intrinsic disorder [2].
In this contribution, a study of local structure and magnetic properties of hydrothermal synthesized anatase $Co_xTi_{1-x}O_2$ nanoparticles (0x0.1) was performed by EPR and magnetic measurements, as well by XRD and EDS analyses. One present that cobalt is partially inserted in the lattice as Co^{2+} ions, located on substitutional (octahedral coordination with hyperfine structure resolved) and interstitial (tetrahedral coordination) sites. The fraction of diluted Co is limited to 3 at. % for concentration higher that x0.05 [3]. The Q-band EPR spectra show that the largest contribution comes from the interstitial Co^{2+} ions. Paramagnetic and room temperature ferromagnetic behaviour is observed in all samples. Hysteresis loops for samples with x=0.025, 0.05 and 0.075 are shown in the figure below. The existence of coercive field (64 Oe for x=0.025) up to 380 K is assigned to a long-range ferromagnetic ordering. Paramagnetic contribution to the sample magnetism dominates at higher Co content due to decrease of oxygen vacancies concentration and formation of paramagnetic Co_3O_4 clusters.



[1] Tomasz Dietl, Nature Materials, Vol. 9, DEC 2010
[2] M.N. Grecu *et* al. Phys. Status Solidi b 248, 2927 (2011)
[3] M.N. Grecu *et al.*, Appl.Phys Lett. 102,161909 (2013)
Acknowledgements: We acknowledge financial support from UEFISCSU, Romanian project IDEI 4/2010, code ID-106

POSTER PRESENTATION

414 MO

SYNTHESIS AND CHARACTERIZATION OF PEO-BASED STAR POLYMERS AS MODEL SYSTEMS FOR DRUG AND NUCLEIC ACIDS DELIVERY

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Nucleic acid (NA) therapy has been an emerging field in pharmaceutical sciences due to its potential therapeutic applications targeting genetic disorders. Delivered NAs such as plasmid DNA (pDNA) and short interfering RNA (siRNA) can be used either to express or silence the targeted protein or enzyme in the cell. Consequently, they make possible the therapeutic treatment of several diseases including cancer, and other genetic disorders. Recent studies are focused on finding such a delivery vehicles that prevent electrostatic repulsion between cell membrane and NAs leading to increase of NAs cell permeability.

Atom transfer radical polymerization (ATRP), one of the most robust controlled radical polymerization (CRP) techniques, is used to prepare polymers with diverse architectures (e.g., nanogels and star polymers) enabling innovative functionalities to be introduced. The goal of this research is to evaluate efficient, biocompatible polymeric carriers for NA delivery. Promising candidates in this field are star polymers with multiple of polymer arms joined to a centrally located core, having 3-dimensional globular compact architecture and functionalities either on the periphery or in the core. The study includes the polymerization of poly(ethylene glycol) (PEG)-based star polymers, with a cationic (methacrylate N, N-dimethylamino acetate (DMAEMA)) degradable core, by an "arm-first" ATRP method. The comparison of their structural properties with linear PEO and simple PEO stars, determined by means of DSC and NMR methods, will be presented.

1. Makrocka-Rydzyk, M., Wegner, K., Szutkowski, K., Kozak, M., Jurga, S., Gao, H., Matyjaszewski,K.; *Zeitschrift fur Physikalische Chemie* 2012, **226** (11-12), pp. 1271-1291
2. Cho, H. Y., Averick, S. E., Paredes, E., Wegner, K., Averick, A., Jurga, S., Das, S. R., Matyjaszewski, K., *Biomacromolecules*, 2013, **14** (5), pp 1262–1267

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415 TU

LEAD-FREE RELAXOR FERROELECTRIC MATERIALS INVESTIGATED BY ^{23}NA SOLID-STATE NMR

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Due to their high dielectric permittivity and piezoelectric coefficients, relaxor ferroelectric materials find application as dielectrics in capacitors and actuators in fuel injection systems, among others. It is thought that these materials' enhanced electric properties are related to polar nanoregions of a size of about 10 nm and hence are not easily accessible to diffraction techniques. Solid-state NMR, on the other hand, can probe the local structure of these materials because of the short range nature of interactions between the probed nucleus and its environment.

Although lead-based relaxor ferroelectrics have been widely employed as commercial piezoelectric materials, research on lead-free compositions has been stimulated by increasingly restrictive environmental legislation, with promising results for $(Na^{+}_{0.5},Bi^{3+}_{0.5})TiO_3$ (NBT) based materials, more specifically for its solid solutions with barium, (1-x)NBT-xBT (BT: $BaTiO_3$).

It is assumed polar nanoregions do not grow to macroscopic ferroelectric domains due to random electric fields present in the material, which may origin by virtue of chemical disorder. Structural disorder is achieved in the investigated compositions by the presence of 3 aliovalent cations occupying the same crystallographic site, namely Na^{+}, Ba^{2+} and Bi^{3+}. An open question regarding this system is whether A-site occupancy happens in an ordered or random fashion, and if there is any segregation of cations at specific regions.

Bearing these questions in mind, compositions of (1-x)NBT-xBT with $x < 0.15$ were analyzed by means of static and MAS ^{23}Na NMR spectra, in which the position of the 2nd order quadrupole perturbed centerband showed a marked dependence on Ba^{2+} content, with the detection of two distinct sodium environments for some compositions. The analysis of these spectra with respect to Quadrupolar and CSA parameters may deliver some insight on the local environment of ^{23}Na nuclei as well as on the distribution of Ba^{2+} throughout the structure, and also on the degree of disorder as a function of its content.

Poster Presentation

416 TH

XENON CAPTURE ON SILVER-EXCHANGED ZEOLITES: ^{129}XE NMR AND ADSORPTION STUDY

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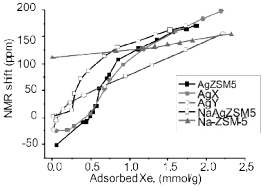
The global monitoring of radioactive isotopes of xenon in the atmosphere is requested by the Comprehensive Nuclear Test Ban Treaty. On-site sampling and measurement automatic system developed for this purpose by French Atomic Energy Commission uses a selective membrane and carbon based adsorbents. The development of adsorbents with large heat of adsorption of xenon will result in the shortening of the separation chain and thus simplification of the hardware design of xenon analyzer.
In this communication we report the results of characterization of adsorption sites and the quantification of their number and strength for a series of partially and fully silver exchanged faujasite and ZSM-5 zeolite. The modelling of xenon adsorption isotherms allows one to discriminate two types of adsorption sites depending on the nature of zeolites and silver loading. For ZSM-5 strong adsorption sites with the enthalpies of adsorption as high as 50 kJ/mol have been attributed to binuclear silver clusters basing on the ratio between the amount of loaded silver and the number of such sites.



Variable pressure ^{129}Xe NMR spectra of xenon adsorbed on the zeolites (MFI and FAU) exhibit two types of chemical shift dependence on pressure – with and without plateau at low xenon coverage region. Appearance of such plateau correlates with the presence of strong adsorption centres of xenon. Two types of strong adsorption centres can be differentiated from ^{129}Xe MAS NMR spectra.

417 MO

DETAILED STUDY ON CARBONATES BY SPECTRAL AND IMAGING TECHNIQUES

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Carbonate reservoirs present a very heterogeneous formation. The majority of oil reservoirs in the Middle-East are carbonate reservoirs. To develop more accurate and successful Enhanced Oil Recovery techniques, it is essential to study in depth the pore structure of these carbonate reservoirs from a few micro-meters to wellbore scale and develop a model that underlines these scaling relationships.
The scope of this study is to create a digital three-dimensional model of a carbonate reservoir and study its behavior under different conditions. This three-dimensional model will be used as reference in future studies for both research and industrial purposes.
An outcrop at the North-Eastern coast of Qatar was chosen in order to model the carbonate reservoir. The experimental procedure includes: on site permeability measurements at various locations, lab measurements of porosity and permeability on the collected samples, micro- X-ray Computed Tomography Scanning, Scanning Electron Microscopy, X-Ray Energy Dispersive Spectroscopy, thin sections, Atomic Force Microscopy and Nuclear Magnetic Resonance.
The imaging techniques allow us to extract information about mineralogy, porosity and flow pathways. Based on these results we try to make a comparison between the pore structures indicated by each of these techniques. Additionally, we present a comparison between the porosity measured at the lab and the porosity obtained by Nuclear Magnetic Resonance. We also compare the permeability measured at the lab with the onsite measurements of permeability (measured with a probe permeameter) and with the estimation of permeability which results from the Nuclear Magnetic Resonance measurements. Based on that, we try to develop a porosity-permeability correlation. Also, pore size distribution gives us vital information about the pore structure and mineralogy and confirms the presence of micro porosity which is commonly found in carbonates. With all this information we will be able to develop a solid petrophysical-properties database for our future model.

Poster Presentation

418 TU

SELECTIVE SIGNAL DETECTION IN SOLID-STATE NMR USING DIPOLAR DEPHASING FILTERED-INADEQUATE FOR LIGNOCELLULOSE AND CHEMICAL SHIFT COMPARISONS WITH SOLUTION NMR

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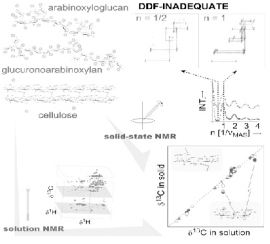
Solid-state dipolar dephasing filtered (DDF)-INADEQUATE, where dipolar dephasing was used as a signal filter to remove signals derived from cellulose, allowing for the hemicellulosic signals in lignocellulosic mixture to be detected. The maximum filtering efficiency was obtained when the dephasing time was synchronized with half of rotor period ($1/2n_{MAS}$). This indicated that hemicelluloses had 10^{-4}-10^{-5} order molecular motions which significant faster than those in cellulose. In the DDF-INADEQUATE spectrum with a dephasing time of $1/2n_{MAS}$, the chemical shifts of b-D-xylopyranose (Xyl*p*) and a-L-arabinofuranose (Ara*f*) in glucuronoarabinoxylan, which is the major hemicellulose in the secondary cell walls of the gramineous plant were assigned in ^{13}C-labeded lignocellulose from corn (*Zea mays*). ^{13}C chemical shifts assigned in solid-state NMR were compared with those in solution NMR. ^{13}C and ^{1}H chemical shifts in solution state were assigned using multidimensional NMR experiments of ^{13}C-labeled lignocelluloses dissolved in DMSO-d_6/pyridine-d_5 (4:1(v/v)). Chemical shift differences in cellulose between solution and solid-state NMR were agreed with conformational specific chemical shifts determined by quantum-chemistry calculation.[1] The comparisons indicated that heterogeneous structures of lignocellulose where structural and crystalline cellulose, and unstructural and mobile hemicelluloses existed together.



[1] Suzuki *et al.*, J. Mol. Struct. 921, 219.

419 TH

NMR AND THERMODYNAMIC CHARACTERIZATION OF THERMORESPONSIVE COPOLYMERS AND NETWORKS

Lenka Hanykova[a], Julie Stastna[a], Jiri Spevacek[b]

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It is well known that thermoresponsive polymers show in aqueous solutions a lower critical solution temperature (LCST). They are soluble at lower temperatures but heating above the LCST results in phase separation. When these polymers are chemically crosslinked their hydrogels undergo around this temperature a volume phase transition (collapse). Their thermosensitivity makes thermoresponsive polymers interesting for miscellaneous biomedical and technological applications. ^{1}H NMR techniques combined with differential scanning calorimetry (DSC) experiments are employed to investigate the temperature-induced phase transition in aqueous poly(*N*-isopropylmethacrylamide-*co*-acrylamide) (P(IPMAm/AAm)) solutions. Formation of globules results in a marked line broadening of a major part of polymer segments in NMR spectra. From temperature dependences of high-resolution ^{1}H NMR spectra it is possible to obtain temperature dependences of the phase-separated fraction p and subsequently also thermodynamic parameters H and S characterizing the phase transition. NMR and DSC experiments reveal that the increasing fraction of hydrophilic AAm units in the copolymer significantly influences the transition characteristics. The rather high mobility of AAm units suggests that they extensively interact with solvent molecules. This fact results in dynamic heterogeneity of copolymer chains in mesoglobules where AAm sequences and surrounding IPMAm sequences are hydrated and mobile. This conception is consistent with results of NMR relaxation experiments.

Combination of NMR and DSC was also used to study collapse phase transition in hydrogels of interpenetrating networks poly(*N*-isopropylmethacrylamide)/poly(*N*-isopropylacrylamide) (PIPMAm/PIPAAm) with various PIPMAm content. A single transition at temperatures between transition temperatures of neat components was revealed in most hydrogel samples, indicating enhanced mutual intertwining of PIPAAm and PIPMAm chains.

Acknowledgement: Support by the Czech Science Foundation (project 13-23392S) is gratefully acknowledged.

POSTER PRESENTATION

420 MO

DETAILED INFORMATION ON ETHYLENE POLYMERS AS REVEALED BY ^{1}H AND ^{13}C NMR

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DSM Resolve, Netherlands

^{13}C NMR is extensively used for the characterization of homo- and copolymers of ethylene. It is used for the determination of the co-monomer composition, sequence distribution and short and/or long chain branching. Minor structural features or small amounts of additives present in polyethylene are often not accessible using ^{13}C NMR though. Because of the increased sensitivity of ^{1}H NMR and the resolution provided by the high field strengths of modern NMR spectrometers, we have used ^{1}H NMR for this purpose and proved it to be extremely powerful.
In this work we have looked at a variety of polyethylene samples, including low density polyethylene (LDPE), linear low density polyethylene (LLDPE) and metallocene based ethylene polymers (m-PE). A number of industrially relevant problems have been addressed: type and amount of chain transfer agents (CTA), additives (primary and secondary antioxidants, acid scavengers, slipping agents etc.), unsaturations, co-monomer composition and distribution. Our study proves once again that ^{1}H and ^{13}C NMR are extremely valuable complementary techniques for the characterization of these types of polymers.

421 TU

^{7}LI AND ^{1}H SOLID-STATE NMR INVESTIGATIONS OF IMPROVED MATERIALS FOR LI BATTERIES

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2. *Imperial College, Department of Materials, SW7 2AZ, London (UK)*

Li-based batteries are considered as one of the most promising advanced energy storage systems. However, their successful implementation in emerging technologies is a significant challenge because energy requirements, system and component safety, and manufacturing costs are subject to more stringent demands than in current commercial batteries. Therefore, optimization of battery components such as electrode materials and electrolytes is a key step for the rational development of advanced battery systems. In this work we present ^{7}Li and ^{1}H solid state NMR studies of promising materials for the implementation of new Li-based batteries. We show how information obtained by solid state NMR can help to understand and improve the performance of such materials. We will present examples of materials suitable to replace metallic lithium electrodes anodes, and of ceramic materials with promising characteristics for solid-state electrolytes.
Due to their high specific theoretical capacities, metals that form alloys and Zintl' salts with lithium show great promise for next generation Li-based batteries. For example: Sn (994 $mAhg^{-1}$), Al (993 $mAhg^{-1}$), Ge (1600 $mAhg^{-1}$), Si (4200 $mAhg^{-1}$), (vs. commercially-used carbon, 372 $mAhg^{-1}$). However, repeated alloying and de-alloying during battery cycling can produce up to 300% volume expansion which leads to degradation, mechanical failure by pulverization, and loss of electrical contact within a few charge-discharge cycles. In this work we present the characterization by solid-state NMR of the Sn-Li alloy formation in a novel hybrid organic-inorganic composite of polycarbonate-based polymer and Sn particles which have demonstrated enhanced cycling stability in Li half-cells.
The development of highly ion-conductive ceramics with improved electrochemical performance and increased safety and stability is of paramount importance for the commercialization of Li-ion secondary batteries. $Li_{3x}La_{2/3-x}\square_{1/3-2x}TiO_3$ has been reported to have the highest bulk Li-conductivity in a ceramic material with 10^{-3} S/cm at 25 C with x = 0.11 ($Li_{0.33}La_{0.56}TiO_3$). However, the high grain boundary resistivity of this system decreases the total conductivity to values around 10^{-5} S/cm, which is too low for battery applications. Solid state NMR was used to monitor the grain boundary degradation and to design an effective strategy to improve the grain boundary conductivity by a factor of 5.

Poster Presentation

422 TH

^{2}H NMR STUDY OF SELECTIVELY LABELLED LIQUID CRYSTAL ELASTOMERS

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Liquid Crystal Elastomers (LCEs) are very interesting materials which combine orientational properties of the liquid crystalline component and the elasticity of the polymer network. They are very good candidates for different technological applications, such as artificial muscles, smart surfaces, MEMS, and NEMS [1]. The local orientational order of the polymer network components can be determined via ^{2}H quadrupole-perturbed nuclear magnetic resonance, since the measured frequency splitting is directly proportional to the nematic order parameter.
In this work we investigate the orientational order of selectively deuterated side-chain liquid single crystals elastomers. Two samples with selectively ^{2}H-labeled mesogen and crosslinker, respectively, were prepared. We detected differences in the temperature behavior of nematic order *S* and orientational order of nematic domains (Fig. 1). Specifically, at the transition from the high-temperature paranematic phase into low-temperature nematic phase, the crosslinker exhibits a significantly stronger orientational disorder than the mesogen. The results are discussed in view of different local molecular mobilities. Taking into account the proportionality between $S(T)$ and the macroscopic strain temperature profile (T), we also compare the NMR results with the measurements of the thermomechanical response.

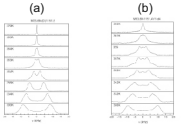

Figure 1: Series of ^{2}H NMR spectra (^{2}H Larmor frequency of 76.7 MHz) recorded from the isotropic to the nematic phases on a monodomain LSCE sample, deuterium labelled on the phenyl ring: deuterated mesogen (a) and deuterated crosslinker (b).
Reference: [1] V. Domenici, M. Conradi , M. Remškar , M. Viršek , B.Župančič, A.Mrzel , M. Chambers, B. Zalar.New composite films based on MoO_{3-x} nanowires aligned in a liquid single crystal elastomer matrix. *J. Mater. Sci.,* 46: 3639–3645, 2011

423 MO

LINKING CHEMICALLY SPECIFIC STRUCTURE INFORMATION TO PHYSICAL PROPERTIES OF POLYOLEFINS

Lavinia Utiu, Vipin Agarwal, Victor Litvinov, Matthew Parkinson, Markus Gahleitner and Arno P.M. Kentgens

Radboud University Nijmegen

In this study, a detailed analysis of the physical and chemical structures of five commercial grades of polypropylene-based polymers was performed. The studied samples, isotactic polypropylene homopolymer (iPP), three random propylene-ethylene copolymers (PPR) (with ethylene concentration less than 8 mol %) and a heterophasic propylene-ethylene copolymer with 23 mol % of ethylene units (IEPC23), were prepared using heterogeneous Ziegler-Natta catalysis. Solution-state NMR was used to identify the chain microstructure and a combination of WAXS, SAXS and ^{1}H time- and frequency-domains solid-state NMR was employed to study the semi-crystalline morphology and phase composition of the five samples over a wide temperature range and under loading conditions. Results show that in undrawn/drawn samples the crystallinity, melting temperature, long-period and crystal thickness decrease with ethylene content and temperature. A complete description of the phase composition (including chain dynamics) of undrawn iPP and PPR requires a four-phase model (rigid/crystalline, semi-rigid interfacial, soft-amorphous and mobile-amorphous). In comparison, for analysis of drawn iPP and PPR we focused on FID data only and restricted the analysis to three-phase model. Changes in morphology and chain dynamics of uniaxially stretched PP were investigated as a function of temperature and drawing ratio using ^{1}H solid - state NMR at low field. Drawing causes an increase in crystallinity and a large decrease in molecular mobility in the amorphous phase.
1D and 2D high-resolution solid-state ^{13}C NMR methods were employed to investigate distribution of chain microstructures in the least- and most-mobile fractions of these polymers [complex-rigid (CR) and complex-amorphous (CA) fractions, respectively]. The fractions were discriminated based on the strength of dipolar coupling, i.e., non-averaged vs. averaged dipolar couplings for the CR and the CA fractions, respectively. From ^{13}C NMR magic angle spectrum it was evident that isolated ethylene units (E) are present in the CR fraction, while nine different chemical moieties corresponding to triad sequences were identified in the CA fraction. The partition coefficient for distribution of ethylene units in the CR fraction had indicated that about 90% of isolated ethylene units are rejected from the CR fraction at 50°C.

Poster Presentation

424 TU

HUMAN SERUM 1H NMR METABOLIC PROFILES AND ISCHEMIC HEART DISEASE

Min-Ji Shin [a], Ulrich L. Günther [b], Christian Ludwig [b], Risto Tanner [a], Margus Viigimaa [a, c], Marika Pikta [c], Galina Zemtsovskaja [c], Ago Samoson [a,d]

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b. HWB-NMR, School of center Sciences, University of Birmingham, Birmingham, B15 2TT, UK
c. North Estonia Medical Centre, J. Sütiste tee 19, 13419 Tallinn, Estonia
d. Department of Physics, University of Warwick, Coventry CV4 7AL, UK

Ischemic heart disease is the most common cause of death in the world. We investigated the perturbed metabolic pattern in serum of ischemic heart disease patients and sought to identify associated metabolic biomarkers.
Metabolic profiling of serum from ischemic heart disease patients and healthy individuals were studied using one dimensional proton nuclear magnetic resonance spectroscopy coupled with multivariate statistical analysis, such as principal components analysis and partial least squares analysis.
Multivariate statistical analysis showed a significant distinction between patients and healthy individuals. The ischemic heart disease patients was characterized by the increased concentration of 3-Hydroxybutyrate, acetoacetate, three as yet unassigned metabolites peaks, and by the decreased concentration of dimethyl sulfone, N-acetylcystein and some of amino acids.
These data demonstrate that a metabolomics approach may be useful for the early diagnosis of ischemic heart disease and for the further understanding on this category of diseases.

425 TH

UNDERSTANDING OF METABOLOMICS COMPARTMENTALIZATION IN CANCER BY NMR BASED EXPERIMENTS

Mei G. Chong - Daniel A. Tennant - Christian Ludwig - Ulrich L. Günther

School of Cancer Sciences, University of Birmingham, United Kingdom

A pivotal part of the transformation of normal cells to cancer cells is a fundamental reprogramming of metabolism. However, our understanding of the nature of these changes and the magnitude of change is poor and requires detailed study. In addition, the metabolic networks are further complicated by the dynamic exchange of metabolites between compartments – a process which is even less well understood. To dissect out some of these complex interactions, profiling of the metabolic flux of nutrients entering the cell is required using labelled compounds and sophisticated analysis methods.
The use of stable isotope tracers coupled with NMR is a robust tool for investigating complex metabolic networks. Such system enables the unambiguous tracking of individual atoms throughout metabolic networks. In our present study, 13C labelled glucose and 13C labelled glutamine precursors are employed for understanding the metabolic reorganization of breast cancer cell lines and mice breast tumour tissue. We used 2D-1H,13C-HSQC and 2D-TILT-TOCSY-HSQC experiments to reveal the 13C-label distribution and to explore the independent incorporation of multiple 13C nuclei into the same molecule. The 2D-TILT-TOCSY-HSQC experiment not only assists the assignments of metabolites but provides the insights to interplay with/between metabolic pathways.
From NMR results, we have identified major metabolic phenotypes of cancer cells. Moreover, by de-convolute labelling patterns from HSQC and 2D-TILT-TOCSY-HSQC, our data suggest a role for substrate channelling of metabolites in cancer cells, in which there is a preference for using pentose-phosphate derived carbon in the tricarboxylic acid cycle over reduction to lactate. This strongly supports previous data suggesting channelling of NADPH produced from oxidative pentose phosphate pathway activity to lipogenesis.[1]

[1] DeBerardinis, R. J., A. Mancuso, et al. (2007). PNAS, 104(49): 19345-19350

Poster Presentation

426 MO

REAL TIME METABOLISM IN LIVING CLL CELLS REVELS HYPOXIA-RELATED PLASTICITY

Katarzyna Koczula[1#], Christian Ludwig[1], Rachel Hayden[2], Guy Pratt[1], Chris Bunce[2], Farhat Khanim[2], Ulrich Gunther[1]

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Chronic lymphoid leukaemia (CLL) is a multi-compartment disease with tumour cells circulating between the blood and solid tissues including lymph nodes where the cells proliferate and receive environmental cues promoting drug resistance. The ability to transit these diverse compartments is likely to require CLL cells to adjust their metabolism. Understanding this metabolic plasticity may create new avenues for therapeutic intervention.

We have developed a nuclear magnetic resonance (NMR) based system for measuring real time metabolic changes in living CLL cells caused by a transition from normoxia to hypoxia. Primary peripheral blood CLL cells were suspended in semisolid media with restricted oxygen supply and NMR metabolomics readings taken over 24hr, after which cells remained viable and were harvested for further analyses. One–dimensional proton NMR allows to identify ~30 metabolites including glucose, glutamine, glutamate, pyruvate, lactate, alanine, 3-hydroxybutyrate and hypoxanthine, which decreased or increased over the time of measurement. We calculated kinetics of all the metabolites showing such changes. The pH inside the NMR tube was monitored using histidine chemical shifts.
Oxygen levels during the NMR experiment were measured during the NMR experiment using an oxygen sensor. In parallel experiments hypoxia inducible factor (HIF-1α) protein expression could be demonstrated as a robust hypoxic response observed within 1-2 hrs of the transfer of cells to NMR tubes. We demonstrated that CLL cells metabolism was not affected by the multiple circulation between normoxic and hypoxic environment.
These observations indicate a high degree of metabolic plasticity in CLL cells that underpins their physiological agility. The timing of the changes in oxygen level together with changes in HIF-1α and the kinetics of the cells' altered metabolism indicate that rapid modulation of HIF-1α targets may be responsible for facilitating this response which is likely to be of significant biological importance for cells migrating between different compartments.

427 TU

FAST SPATIALLY-ENCODED 3D NMR STRATEGIES FOR METABOLIC FLUX ANALYSIS

Renaud BOISSEAU[1], Benoît CHARRIER[1], Stéphane MASSOU[2], Jean-Charles PORTAIS[2], Serge AKOKA[1] and Patrick GIRAUDEAU[1]

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The measurement of site-specific ^{13}C enrichments in complex mixtures of ^{13}C-labeled metabolites is a powerful tool for metabolic flux analysis. One of the main methods to measure such enrichments is homonuclear ^{1}H 2D NMR.[1] However, the major drawback of this technique is the acquisition time which can amount to a few hours. It strongly affects the quantitative performance of 2D experiments, and is ill-suited for high-throughput analysis. We recently designed an ensemble of methods for measuring specific ^{13}C-enrichments in a very fast and accurate way, by using experiments based on ultrafast 2D NMR.[2] This approach is capable of providing 2D correlations in a single scan. Strategies based on ultrafast heteronuclear J-resolved spectroscopy and ultrafast COSY, [3] all of them characterized by excellent analytical performances.
However, these experiments are still limited by overlaps due to ^{1}H-^{13}C splitting, thus limiting the metabolic information accessible for complex biological mixtures. To circumvent this limitation, we propose to tilt the ^{1}H-^{13}C coupling in a third dimension. For that, we designed a fast 3D NMR method, [4] based on ultrafast spatially-encoded NMR because the conventional 3D NMR needs a very long time of acquisition. The 3D spectrum is recorded in a few minutes with a hybrid conventional-ultrafast strategy which provides an original way to acquire indirect dimensions. Two experiments were designed and carefully optimized to reach an optimum analytical performance: UF-Jres-COSY and UF-COSY-Jres.

The analytical potentialities of both methods were evaluated on a series of ^{13}C-enriched samples (5, 10, 25, 50, 75, 90%) and on a biomass hydrolyzate obtained from *E.Coli* cells. Our optimized pulse sequences were characterized by a trueness of *ca*. 3 %, a precision of 4 % and an excellent linearity.

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428 TH

WHAT NMR ON SENILE FLY JUICE CAN TELL US ABOUT ALZHEIMER'S DISEASE NMR METABOLOMICS OF HEADS AND BODIES OF AB-EXPRESSING *DROSOPHILA*

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We use NMR to study the metabolite responses to different types of environmental and genetic factors in *Drosophila melanogaster,* and to assess disease models in *D. melanogaster*.

The specific target in this case is a model of Alzheimer's disease (AD). AD is characterised microscopically by the accumulation of two distinct protein amyloid deposits, neuritic plaques that are extracellular deposits of the amyloid β peptide (Aβ), and the intracellular tangles composed of the tau protein.

We use NMR metabolomics of *D. melanogaster* heads and bodies to see how expression of the so called arctic (E22G) mutant of Aβ with a propensity to cause AD at an early age, affects the changes in metabolite concentration that occur with aging. These results are used to identify specific AD-related responses in nervous and other types of tissue.

429 MO

QUANTITATIVE DETERMINATION OF THE COMPONENTS OF NATURAL AND PROCESSED CASHEW NUT-SHELL LIQUID BY NMR

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The cashew tree (*Anacardium occidentale* L) is mainly known for the production of cashew nuts. Of less commercial value are the side products cashew apple juice and cashew nut shell liquid (CNSL). CNSL has many industrial applications in which it is important to know the ratio in which the different components are present.
In the nut shell the oily liquid consists mainly of anacardic acids, which are upon high temperature processing converted to cardanols. These compounds are alkylphenols with alkyl chains of varying length and number of double bonds. Previously methods for the quantification of the individual components have been reported using HPLC and GC-MS, but these methods are rather elaborate.
We developed a method in which ^{1}H NMR can be used for the accurate characterization of the CNSL, providing not only the ratios of anacardic acids, cardanols and cardols, but also the insaturation percentage of the side chains and the eventual presence of impurities, such as triglycerides.
To get accurate quantitative data we investigated the use of our recently developed software ARNSED (Automatic Reduction of NMR Spectra to Essential Data), in which the spectra are automatically deconvoluted to provide automatic correction for peak distortions or peak overlapping.

POSTER PRESENTATION

430 TU

NOVEL NMR METHODS FOR THE ANALYSIS OF COMPLEX MIXTURES

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The analysis of several important constituents in natural products, food chemistry and biotechnology demands sample pretreatment procudures and / or chemical derivatization of constituents. Herein, the following NMR methods for the analysis of complex mixtures without any isolation or derivatization steps will be presented:
I. 1D and 2D ^{1}H-^{13}C HSQC and HMBC NMR experiments for the determination of phenolic acids, triterpenoids [1] and geometric conjugated linoleic acid isomers of lipids.
II. A novel strategy which is based on the deshielded signals between 8 to 14 ppm attributed to the hydroxyl protons of flavonoids [2- 7].
III. Diffusion-Ordered Spectroscopy (DOSY) which is based on the resolution enhancement of the resonances of the –OH protons [8].
IV. In situ direct monitoring of dynamic changes of constituents of plant extracts and rapid analysis of enzymatic reaction products [9].
Acknowledgments: This research has been co-financed by the European Union (European Social Fund-ESF) and Greek national funds through Operational Program "Education and Lifelong Learning of the National Strategic Reference Framework (NSRF)-Research Funding Program: Heracleitus II" & the Cyprus Research Promotion Foundation.

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431 TH

METABOLIC PROFILING BY ^{1}H NMR SPECTROSCOPY OF PLASMA AND DIALYSATE FROM PATIENTS UNDERGOING HEMODIALYSIS

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^{1}H NMR observation of plasma and dialysate from hemodialysis (HD) patients was subjected for profiling of metabolites. Blood sample collection is limited in HD patients because of anemia. Then we used dialysate, which is non-invasive for patients and without limitations of collection. Recently, we have verified dialysate as a surrogate for blood in measuring small metabolites during HD, by quantitative ^{1}H NMR. In analytical aspect, plasma samples were less reproducible because plasma is heterogeneous mixture of proteins, lipids, organic acids and other small metabolites. On the other hand, dialysate has better reproducibility for it is composed of only small metabolites, and ensures adequate quantification of metabolites by ^{1}H NMR.

In this study, 480 dialysate samples from 16 patients were collected in time course during HD sessions, and measured 1D single-pulse spectra by 600 MHz NMR (ECA, JEOL Ltd.) spectroscopy. The main metabolites were quantified by their peak integrations on the spectra. These concentrations in time course revealed to have unique pattern to patient in every HD session. The finding has potential information for future personalized therapy.

In all of patients, creatinine exhibited monotonous decay and valine showed plateau toward the end of the session. While HD patients derived from chronic-glomerular-nephrites exhibited significant increment of lactate together with alanine and pyruvate at the middle during HD sessions, which indicated rather amount of productions from the body. HD treatments rapidly remove electrolytes, water, and small molecules including bioactive necessities and nutrients as well as uremic toxins from the blood. As the response to the HD stimuli, compensative reaction to maintain homeostasis may occur followed by their productions into blood. The increment of lactate, pyruvate and alanine suggested accelerated glycolysis and/or disturbances of metabolic pathway at the entrance to TCA cycle.

We will discuss the relation between metabolic profile in each patient and one's etiology.

Poster Presentation

432 MO

METABOLOMICS OF TRADITIONAL CHINESE MEDICINE PLANTS

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In the last years, many studies have been implemented to investigate the characterisation and toxicity of traditional chinese medicine (TCM) plants.
Next to gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS) or capillary electrophoresis (CE), NMR-based techniques offer a useful diagnostic tool to analyse TCM plants.[1,2] Such studies can give a metabolic snapshot to qualify and quantify different compounds in biological systems.[3]
In our studies, we did the first step to identify a significant metabolite pattern for a selection of the 38 different types of the genus rheum, which mainly exists in China.
To date, for five types (*Rheum officinale*, *Rheum palmatum*, *Rheum tanguticum*, , and *Rheum australe*) we find characteristic patterns which seem to classify not only the genus *Rheum*, but also allow to distinguish the five types from each other.

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433 TU

METABOLOMICS OF PLANT EXTRACTS

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Metabolomics with Nuclear magnetic resonance (NMR) spectroscopy can be used for the analysis of organisms under certain conditions, which gives an instantaneous snapshot of biological systems. In the last years NMR spectroscopy has received increasing interest for studies of metabolites of whole organisms, tissue samples, or cells. Simple sample preparation, reproducibility and short analysis time are the major advantages why NMR spectroscopy is a powerful tool for metabolic studies. Here, we have analyzed plant extracts of tobacco cell suspension cultures and rice plants to compare the metabolite profiles of different developmental stages or in response to environmental cues.
Plant secondary metabolism with an estimated $5 \times 10^4 - 10^5$ specific compounds bears great potential for therapeutical use. Tobacco is a rich source of secondary metabolites. Two tobacco cell lines, VBI-0, which is not responding to light, and VBI-3, a light-responsive cell line[1] were isolated. This provides the unique possibility to compare the metabolomes of both cell lines in the dark and under light conditions and to identify light responsive metabolic pathways as a first step towards light-switchable molecular farming.
High salinity of soil is an increasing problem in agriculture all over the world. Rice is one of the most important crops and it is highly sensitive to the stress factor of soil salinity. Plant hormonal cascades contribute to the signaling occurring in response to increased salinity. We want to investigate the function of one major plant stress hormone, jasmonic acid, in the response to high salt concentration by comparing metabolite profiles in wild type and jasmonate-deficient mutants (*hebiba* and *coleoptile photomorphogenesis 2* (*cpm2*)).[2,3,4]

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434 TH

DIFFERENTIAL BRAIN METABOLITE LEVELS DURING VISUAL SEXUAL AROUSAL IN PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN: FUNCTIONAL MRS

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The purpose of this study was to compare the brain metabolite changes between premenopausal and postmenopausal women during visual sexual stimulation with erotic video clips using functional magnetic resonance spectroscopy (fMRS).

Each 20 premenopausal (mean=40.6) and postmenopausal (mean=56.2) women underwent to fMRS exams on a 3.0T MRI scanner (Magnetom Tim Trio, Siemens Medical Solutions) with the following parameters: TR/TE= 2000/30 ms, NA= 96 and voxel size= 1.8×2×2 cm^3. The brain metabolite levels were measured from a localized voxel on the anterior cingulate gyrus which is one of the most important areas associated with sexual arousal. The fMRS data were acquired from during rest period and activation period with erotic video clips. The brain metabolic changes between the two groups were analyzed by ANCOVA test.

During the rest period, the postmenopausal women showed significantly lower levels of both the β·γ-Glx/Cr and Lip/Cr compared with the premenopausal group, while during the activation period (p<0.05) only the β·γ-Glx/Cr was reduced. The percentage changes of the metabolite levels of β·γ-Glx/Cr and Lip/Cr during the activation and rest periods were significantly different in two groups (p<0.05): the premenopausal group showed 8.2% increase of β·γ-Glx/Cr, while the postmenopausal group showed 6.5% decrease; the premenopausal and postmenopausal groups showed increased Lip/Cr levels by 1.9% and 17.8%, respectively.

In conclusion, the fMRS was able to evaluate the differential brain metabolite changes during visual sexual arousal in premenopausal and postmenopausal women. These findings would be helpful to understand the neural mechanism on the visual sexual arousal in conjunction with the brain metabolite variation following menopause.

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435 MO

NMR-BASED METABONOMICS REVEALS COMPLEX METABOLIC NETWORKS IN PATIENTS SUFFERING FROM ACUTE LUNG INJURY

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Pulmonary dysfunctions, with development of acute lung injury (ALI), are common complications in patients undergoing elective coronary artery bypass grafting (CABG) with the use of cardiopulmonary bypass (CPB). The underlying pathological mechanisms are not completely understood and there are no reliable biomarkers that can predict early progression into ALI after CABG. High-resolution 1H nuclear magnetic resonance (NMR) spectroscopy–based metabonomics has been applied to investigate the metabolic fingerprints in serum of patients undergoing CABG, aiming for a better understanding, and earlier diagnosis of this disease.

Serum samples collected 16 hours after surgery from left atrium and pulmonary artery were investigated using NMR spectroscopy, univariate and multivariate statistical analyses such as ANOVA, Kruskal-Wallis, and partial least squares discriminant analysis (PLS-DA).

Modelling of NMR data allowed patients that progressed into mild or severe ALI to be discriminated from patients with no signs of pulmonary dysfunction.

Significant metabolites involved in sample grouping were identified and could be related to different metabolic pathways. Furthermore, by using a Monte-Carlo validation, it was possible to predict with high specificity and sensitivity the progression into ALI from samples collected 16 hours after CABG.

Poster Presentation

436 TU

HR-MAS NMR-BASED METABOLOMIC APPROACH TO STUDY THE EFFECT OF FUNGICIDAL SEED TREATMENT ON WHEAT SEED GERMINATION

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The treatment of various seeds with fungicides for the prevention of diseases is a common routine. However, it has been observed that environmental stress (such as fungicide application) can have phytotoxic effects. This study thus aimed to investigate the effect of a fungicidal growth environment on the germination rate of wheat seeds using HR-MAS NMR spectroscopy. The effect of both systemic and non-systemic fungicides was investigated. Multivariate statistical analysis was performed using Partial Least Square Determinant Analysis (PLS-DA) and key metabolites identified and related to specific metabolic pathways. NMR metabolic analysis shows that retardation of seed germination occurs by fungicide application and that retardation occurs to a greater extent by non-systemic fungicide than by systemic fungicide. It is demonstrated that HR-MAS NMR spectroscopy can be successfully utilized to distinguish between metabolic responses of wheat seeds germinating under different growth conditions.

437 TH

DETECTION OF ADULTERATED COFFEE BY ^{1}H-NMR AND CHEMOMETRICS METHODS

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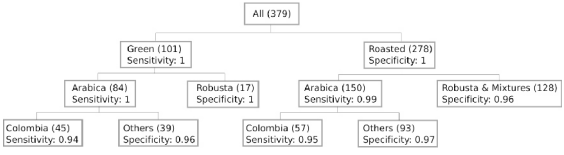

The production of coffee in Colombia has decreased during the last decade, but its quality has maintained and even progressed. Colombia thus imports large quantities of coffee for its internal consumption, since it obtains high prices for its coffee on the international market. This situation makes imperative the implementation of methods able to detect frauds, i.e., adulterated coffees.

Here we present an expert system that runs in a fully automatic manner and enables to discriminate Colombian coffee beans from beans from other countries, including from neighbouring countries on the same continent. Since Colombian coffee is exclusively Arabica, the problem can be recast into discriminating Colombian Arabica vs. other Arabica coffees.

Our system has been designed for coffee extracts obtained in non-deuterated solvents, thus ensuring lower operational costs, and consists in a cascade of binary partial least squares discriminant analysis (PLS-DA). The discrimination power is very high; here we report sensitivities and specificities ranging from 94% to 100% using a real data set of over 500 samples from 25 countries and 3 continents (train/test: 295/84 samples).

Finally, upon detection of non 100% Arabica coffee, a PLS regression is used to quantify the ratio Arabica / Robusta. Again, the results showed that quantification is possible with a high accuracy, although the effects of roasting schemes have to be studied with more care.

438 MO

CELLULOSIC BIOMASS CHARACTERIZATION AND ITS DEGRADATION PROCESS FOLLOWED BY SOLID AND SOLUTION NMR

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Plant biomass is now highlighted by view points both basic and applied science such as biorefinery materials [1,2]. It consists polysaccharides and lignin, both are so difficult biomacromolecules in terms of tackling by NMR. Major difficulties are attributed to their low solubility, mixture complexity and low spectral dispersion. In order to "escape" the problem for mixture complexity, firstly we tried NMR experiments using 13C labeled cellulose. Several solid-state NMR measurements (VPCP, 2D-INADEQUATE and DARR), as well as solution-NMR (2D-HSQC and HSQC-NOESY) were employed for ionic-liquids solubilized and regenerated samples, then statistical multivariate analysis were useful approach to monitor structural variations [3-5]. Next subject should be tackling to monitor its degradation process, namely in the mixture complexity, such as microbiota. Therefore we applied multi-variate and statistical correlation analysis for degraded metabolite mixture NMR spectra observed in the microbiota. Namely, time-dependent analysis of cellulosic degradation in methane fermentation media [6,7], paddy soil [8], as well as termite symbiotic system [9] will open a new field for environmental science using NMR.

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439 TU

SIMULTANEOUS NMR AND UHPLC-HRMS METABOLOMIC ANALYSIS FOR THE IDENTIFICATION OF SIGNIFICANT BIOMARKERS IN DIFFERENT RESISTANT *VITIS* CULTIVARS

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As for other disciplines, NMR and LC-MS platforms are routinely utilised in plant metabolomics satisfying different applications. In the current study we exploit the power of both HRNMR and UHPLC-HRMS in order to develop a strategy for the development of a plant metabolomics workflow. As a case study, woods of *Vitis vinifera* cultivars exhibiting different susceptibility to pathogens were used, one cultivar resistant to *botrytis* (BR), one to downy mildew (DMR) and a susceptible to both pathogens (Cont). The wood samples were divided in 18 groups (6 per cultivar) and extracted separately using EtOAc. Two different sample preparation protocols were developed for the NMR (600 MHz) and UHPLC-HRMS analysis of the extracts. For the LC-HRMS analysis the same samples were subjected to an UPLC-TOF and an UHPLC-Orbitrap. Multivariate data analysis (PCA PLS-DA) was applied to all different datasets (NMR, LC-TOF & LC-Orbitrap). A clear distinction between the three groups was observed in all data matrices while a high convergence regarding the discrimination patterns between NMR and UHPLC-MS data was obtained and significant biomarkers were revealed. In order to go further in identification of significant metabolites SHY was utilized for the correlation of both NMR and UHPLC-HRMS data. A filtering step regarding the HRMS data was performed and only the common significant features deriving from both platforms were used to construct the SHY matrix. A 1D NMR pseudospectrum for each significant LC-MS loading was generated. For enrichment of structure information, STOCSY was used at the NMR data using different peak drivers in order to reveal peak correlations. The generated correlation spectra from STOCSY in combination with SHY yielded to the identification of several compounds significant for *Vitis* resistance. Following, this work-flow the potential of both NMR and LC-MS could be exploited simultaneously resulting to more complete and accelerated identification of critical metabolites. To our knowledge, this is the first time that such correlation tools are utilised in a plant metabolomics study.

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Poster Presentation

440 TH

EFFECT OF SUPPLEMENTATION WITH OLIVE LEAF EXTRACT, ENRICHED IN OLEUROPEIN, USING NMR AND MS METABOLOMICS ANALYSIS

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Oleuropein is a natural phenolic antioxidant, present in elevated concentration in olives, olive oil and olive tree leaves, which is related with the beneficial health effects of olive oil and the Mediterranean diet. The aim of the present study is to provide a holistic evaluation of oleuropein effect on human physiology by detecting alterations in the metabolic profile, through urine and blood serum metabolomics, in order to gain better insight into oleuropein mechanism of action.
Nine healthy volunteers received olive leaf extract, enriched in oleuropein, or placebo for one week in a randomized, balanced, and double-blind manner. Two weeks of washout followed and then each participant received the alternate supplement (crossover design) for an additional week. Urine and blood samples were collected before and during supplementation. In total, 124 urine and 115 serum samples were collected.
^{1}H 1D and 2D, as well as ^{1}H-^{13}C 2D NMR spectra of urine and serum samples were acquired on a 600 MHz spectrometer according to experimental protocols and sequences optimized for metabolic studies. In order to maximize the information from the NMR spectra, Principal Component Analysis (PCA) was performed to evaluate statistical significant changes that might be related to olive leaf extract administration. Mass spectrometry-based metabolomics were carried out using an LTQ-Orbitrap discovery instrument capable of delivering a resolution of 30000 in both positive and negative ion mode. An Accela UHPLC with 1.9 μm particle diameter column was used and the results were processed by XCMS, MZMINE, R and Simca. Clustering of individual dosed and not dosed subjects has been observed and key metabolites cycles and up and down metabolic pathways recognized. The most affected metabolic pathways were the ascorbate – aldarate, citrate and arginine-proline pathways. Alterations observed in metabolites, such as hippuric acid, require further investigation.

441 MO

SPECTROSCOPIC SELECTION IN PULSE EPR DISTANCE MEASUREMENTS

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We present a new EPR methodology for selective distance measurements on biomolecular samples labeled in a site specific chemo-selective way with Ln(III) ions and nitroxide radicals. New double electron-electron resonance (DEER)-based[1-3] (Gd(III)-nitroxide pairs) and relaxation enhancement (RE)-based[4-7] (Dy(III)-nitroxide pairs) distance measurement approaches have been developed in our group. Combining Ln(III) ions and nitroxide radicals allows measuring distances in different spin pair types in the same sample.[2,8-10] With this strategy one can handle multi-spin systems and simultaneously measure several intramolecular distances[2] or, alternatively, detect intramolecular distances while monitoring biomolecule aggregation or association/dissociation processes.[9,10]
Nearly perfect spectroscopic selectivity allows for further experiments to characterize local environment of particular spin labels at specific sites without a need of additional singly labeled samples.[9] The new measurement schemes work both on water soluble[3,7] and on membrane protein samples.[6,9,10]
To build a basis for this methodology, both Gd(III)-nitroxide DEER and Dy(III)-nitroxide RE experiments were analyzed in detail experimentally and theoretically. We discuss essential features of spin dynamics and dipolar couplings for the high-spin Gd(III) centers as well as orientation averaging, multiple pathway relaxation and distance extraction procedure for the Dy(III)-nitroxide RE experiments. A range of examples from synthetic model compounds to membrane protein complexes is used to illustrate the new methodology.
We acknowledge collaboration with several groups from the universities of Bielefeld, Wageningen, York, UCLA, ETH Zurich and Basel and the financial support of SNF (Grant Nr. 200021_121579).
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Poster Presentation

442 TU

A MICROFABRICATED XENON HYPERPOLARIZER

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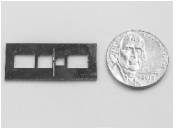

Chemical sensors utilizing hyperpolarized ^{129}Xe as a sensitive reporter of binding and association have become useful for targeted detection of chemical species[1]. Related chemical and molecular imaging experiments depend upon large and immobile instrumentation, such as superconducting magnets and gas polarizers, which limit their application in portable analytical devices. To overcome this limitation, we have developed a microfabricated xenon gas polarizer which produces hyperpolarized ^{129}Xe at a rate optimized for use in microfluidic "lab on a chip" devices. Here, we present a prototypical device which accomplishes both the production and optical[2] remote detection[3] of hyperpolarized ^{129}Xe gas in a NMR imaging system at low magnetic fields. When coupled with Xe-based chemical sensor technology, such a device could find use in low-field NMR chemical sensor systems.

We demonstrate transfer of polarization from pumping chamber to probing chamber with polarization lifetimes greater than 1 second. We present a theory which predicts the possibility of polarizations two orders of magnitude greater than experimentally achieved with longer lifetimes. Finally, we propose fabrication methods for increasing both polarization magnitude and lifetime.

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D.J.K., S.J.S, H.L.R., V.S.B., and A.P. were supported by the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. R.J-M., E.A.D., S.K., and J.K. were supported by the National Institute of Standards and Technology.

443 TH

ULTRAFAST 2D NMR WITH EASE

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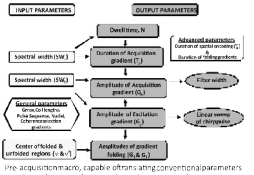

Pre-acquisition macro, capable of translating conventional parameters into UF-specific ones to facilitate the implementation of UF experiments

Ultrafast (UF) NMR makes it possible to record 2D spectra in a fraction of a second. [1] In the last few years, we improved the performance of this methodology and we applied it to a variety of situations, from the monitoring of organic reactions [2] to hyphenated techniques [3] and quantitative analysis. [4] Still, in spite of its remarkable performance, **UF NMR remains underutilized**. To date, less than ten research groups have mentioned its use in the literature. This is mainly due to the numerous acquisition and processing parameters and procedures governing UF experiments which limit their implementation by non-specialists.

In order to make UF NMR more accessible, we developed a user interface, accessible on the web, offering a number of **user-friendly tools to facilitate its implementation**. It includes a detailed implementation protocol, a troubleshooting procedure, as well as **a macro capable of translating conventional parameters** (SWs and transmitter frequencies) **into specific UF parameters** (gradients and chirp pulses). We demonstrated the efficiency of our routines on a variety of experiments and samples. [5] We hope it will unlock a new door towards the routine implementation of UF 2D NMR.

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Poster Presentation

444 MO

DISSOLUTION DNP COMBINED WITH PARALLEL RECEIVER NMR

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Dynamic nuclear polarization (DNP) boosts the otherwise low sensitivity of NMR spectroscopy. There are now several implementations of DNP, where dissolution DNP yields the highest sensitivity enhancements by polarising at low temperature and thus combining a sizable temperature factor with the actual polarisation. We have used the commercial Hypersense™ implementation in combination with a parallel receiver console.

Dissolution DNP suffers from the need to transfer the sample within the longitudinal relaxation time of the nuclei from the polariser to the NMR magnet. Losses of sensitivity are mainly caused by field inhomogeneity and air bubbles arising from the rapid pressure change. These factors have significantly limited the feasibility of fast two-dimensional spectra.

Here we present a newly designed dissolution device to overcome these problems. A custom-build microcontroller operated device is keeping the dissolved material under constant pressure, avoiding sample de-gassing and subsequent air bubble formation, leading to greatly enhanced lineshape, stability, reliable water dissolution, reduced shuttling time and reduced T_2^* relaxation caused by sample inhomogeneity.

Applications of the new dissolution device include the parallel acquisition of ^{13}C and ^{31}P spectra and improved options to acquire multi-dimensional dissolution-DNP enhanced NMR spectra.

445 TU

PRE-POLARIZATION OF NUCLEI WITH ALTERNATING LOW-FREQUENCY MAGNETIC FIELD (FOR DETECTION OF NMR IN THE EARTH FIELD)

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Saint-Petersburg State University, Faculty of Physics

The NMR sensitivity strongly depends on the magnitude of static magnetic field B_0. The experience shows that the registration of NMR signals is possible even in the Earth magnetic field (~$0{,}5 \cdot 10^{-4}$ Tl) . To compensate the decrease of the NMR signals in weak magnetic fields, including Earth magnetic field, the following techniques are used: the increase of a sample volume; the preliminary polarization of nuclei with an additional strong magnetic field; the use of coils of complex geometry which allow the enhancement of the ratio of a signal to external electromagnetic hindrances; the signal digital processing; the accumulation of NMR signals. In this work some peculiarities of the polarization of nuclei with an additional strong magnetic field have been considered. In particular, the polarization of nuclei with an alternating magnetic field at a very low frequency F (but $F >> 1/T_1$, where T_1 is the spin-lattice relaxation time) has been investigated. It is convenient and useful to turn the additional magnetic field on the perpendicular direction relatively B_0. We have been shown that in this case it is possible to fulfil the conditions of the adiabatic change of the direction and magnitude of the summary field. The description of the process can be carried out on the basis of the Bloch equations using the "shaking" frame.

The application of the alternating magnetic field can help to decrease transient processes in the NMR sensor if the polarization current is switched off at the certain phase. The amplitude of damping transients can be approximately in 50 times less than in the case of switching continuous current off. Using the NMR in the Earth magnetic field, it is possible to detect liquid (including explosive) substances in big corked or sealed containers (bottles, flasks, cans, etc.). The liquid objects can be disposed in closed containers with walls which made from non-magnetic materials: glass, plastics, etc., and even thin metal (≤0.1 mm).

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Poster Presentation

446 TH

FIRST HIGH-RESOLUTION PORTABLE MR ANALYZER FOR SOLIDS AND LIQUIDS: APPLICATIONS TO FOOD PRODUCTS

Anne Soleilhavoup[1], Cédric Hugon[2], Javier Alonso[1], Angelo Guiga[1], Dimitris Sakellariou[1,3]

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Permanent magnets can be used to produce moderate magnetic fields (up to about 2 T), usually with very poor field uniformity. Therefore their applications have been limited in studies of relaxation or diffusion. Recent developments in magnet design allowed us to build, characterize and shim a portable permanent magnet having a longitudinal field orientation and homogeneity, similar to the ones found in superconducting magnets.
High resolution experiments have been reported but only on liquids or on small size samples. Here we present, for the first time, high resolution 1H MAS spectra of solid and semi-solid samples. After a brief description of the magnet configuration, the homogeneisation protocol and the MAS instrumentation, we will present our results obtained on large solid sample volumes (ground seeds inside a 7mm rotor) that have been accessible thanks to a large sub ppm homogeneity of the magnetic field.
On ground colza seeds sample, the broad static spectrum of 10 ppm linewidth is reduce to 0.4 ppm resolved peaks under spinning (i.e a gain of 25 times compared to the static resolution). The resolution enhancement allows us to quantify and study relaxation behaviours of six different components of the lipidic solid part at low field consistent with the results obtained at high field. This type of direct spectroscopic information that was previously unavailable in low-field portable systems opens the way for new high-performance analytical tabletop MR instruments.

447 MO

A NEW APPROACH TO SEPARATE CHEMICAL SHIFT AND SCALAR COUPLING OF 1D PROTON SPECTRA

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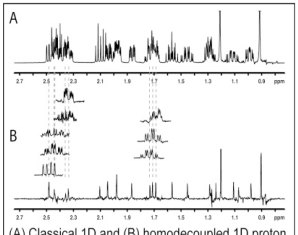
In ^{1}H spectra, the multiplet structure due to homonuclear scalar coupling makes it difficult to properly analyze 1D spectra in the case of complex samples with severe signal overlap. Obtaining 1D pure shift proton spectra is therefore a quite interesting decades-old challenge [1-4]. We propose here a new approach based on spacial encoding to quickly obtain high-resolution 2D spectra leading to homodecoupled 1D spectra after a simple processing. The experiment is based on the Zangger-Sterk element [5] and combined with spectral aliasing to increase the resolution in the indirect F1 dimension. The resulting 2D spectra show only diagonal signals with a multiplet structure only along the F2 dimension. A simple manipulation of the spectrum cleanly separates chemical shift and scalar coupling.



(A) Classical 1D and (B) homodecoupled 1D proton spectra of 4-Androstene-3,17-dione. Cross-sections are taken along the F2 dimension.

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Poster Presentation

448 TU

FANTASTIC FOUR: THE 2ND GENERATION A PLUG 'N' PLAY SET OF OPTIMAL CONTROL PULSES FOR ENHANCING NMR SPECTROSCOPY

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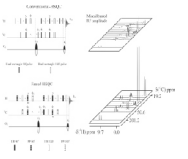
The steady increase in the magnetic field strength of NMR spectrometers improves the sensitivity and resolution of the resulting NMR spectra. However, hard pulses, the commonly used workhorses of most NMR pulse sequences, cannot always excite spins effectively over this increased bandwidth. This is especially problematic for nuclei with large chemical shift ranges such as ^{13}C, ^{15}N, ^{19}F, and ^{31}P. Additionally, hard pulses do not compensate well for radio frequency (RF) field inhomogeneity/miscalibration. We present a set of highly robust optimal control-based [1-4] shaped pulses designed to replace all 90° and 180° hard pulses in a given pulse sequence, such as 2D-HSQC, for improved performance. Special attention was devoted to ensuring that the pulses can be substituted in a one-to-one fashion for the original hard pulses without any additional modification of the existing sequence (see figure). First, we review the original Fanta4: 1st generation pulses for ^{1}H and ^{13}C nuclei [5]. The set of four pulses for each nucleus consists of 90° and 180° point-to-point (PP) and universal rotation (UR) pulses of identical 1 ms duration. They provide uniform performance over resonance offsets of 20 kHz (^{1}H) and 35 kHz (^{13}C), and tolerate reasonably large RF inhomogeneity of 15% (^{1}H) and 10% (^{13}C), making them especially suitable for NMR of small-to-medium sized molecules (for which relaxation effects during the pulses are negligible) at an accessible and widely utilized spectrometer field strength of 600 MHz. Second, we present new Fanta4: 2nd generation pulses for ^{1}H and ^{13}C nuclei. The set of two pulses for each nucleus consists of 90° and 180° UR pulses of identical 500 µs duration, making them suitable for NMR of larger molecules that can be subject to considerable relaxation during long pulses. The new pulses are extremely easy to implement on any given spectrometer. We will focus on experimental results.



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449 TH

DISTINCT CONFORMATIONAL STATES OF THE ALZHEIMER AMYLOID PEPTIDE Ab CAN BE DETECTED BY HIGH PRESSURE NMR SPECTROSCOPY

Hans Robert Kalbitzer, Claudia Elisabeth Munte, Markus Beck Erlach, Werner Kremer, Joerg Koehler

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Amyloid fibrils in the brain consisting of the amyloid peptide (Aβ) are a lead marker of Alzheimer's disease. A deeper understanding of the mechanism of fibril formation may help to design drugs for preventing the deposition of amyloid, therefore the existence of specific conformations of monomeric Aβ is potentially important. Aβ-monomers in aqueous environment are usually assumed to occur in a disordered state. In contrast, by high pressure NMR spectroscopy we detect two main conformational states at atmospheric pressure, a compactly folded state 1 and a partly unfolded state 2 with relative populations of 0.7 and 0.3, respectively.
Pure random-coil like structures were not be detected. The pressure response indicates an ordered structure between amino acids 16 to 24 and 30 to 37 in state 1. Aβ-fibrils depolymerise at high pressure, the dissociation constant of monomers from the fibrils increases by two orders of magnitude at 200 MPa and 283 K. The partial molar volume of the monomer unit changes by 101 ml/mol with binding. The thermodynamic data suggests that state 1 is responsible for fibril elongation.

Poster Presentation

450 MO

SPIN NOISE NMR: TWO DIMENSIONAL SPECTRA AND NON-LINEAR EFFECTS

[1]Norbert Müller, [1]Chandra Kousik, [1]Martin Nausner, [1]Judith Schlagnitweit, [2]Vilko Smrecki, Christian Wohlschlager[3]

[1]Institute of Organic Chemistry, [3]Virtual Reality Services, Johannes Kepler University Linz, Austria; NMR Center, [2]Rudjer Boskovic Institute, Zagreb, Croatia

We introduce 2D spin noise NMR methods that exploit ^{1}H transverse nuclear spin noise for indirect detection. The sensitivity of these experiments depends in an unexpected way on the sin concentration due to non-linear behaviour caused by radiation damping. This non-linearity is also detectable in ^{13}C noise NMR spectra. [1]
In the absence of hyperpolarization spin noise detection may be superior to classical detection schemes for small sample amounts ($\leq 10^{8}$ spins), as estimated using an approach by Sleator and Hahn [2]. While signal power scales with the square of the number of spins for polarization based experiments, it scales linearly for noise detection. Currently experiments with such low spin numbers are not in our experimental reach. Therefore we use large spin number samples and state-of-the art hardware (e.g. cryo-probes) to develop noise detection based experiments, which bear the potential of enhanced sensitivity, once applicable to such low spin numbers. As a proof of principle we present a transverse spin noise detected HMQC experiment. By contrast to the earlier spin noise imaging, which is based on projection-reconstruction [3], then new spectroscopic approach is a form of 2D Fourier transform NMR spectroscopy.

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Acknowledgments
This research was supported by the Austrian Science Funds (projects I01115000 to NM and M1404 to KC), and the European Union through the EFRE INTERREG IV ETC-AT-CZ program (project M00146 “RERI-uasb”) and FP7 EAST-NMR, Contract No. 228461.

451 TU

SUB-THZ MICROWAVE COUPLING FOR DNP EXPERIMENTS

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Dynamic nuclear polarization (DNP) is an experimental technique where the high electron spin polarization is transferred to nuclear spins leading to a dramatic increase of the NMR signal intensity. One of the prerequisites is the partial or full saturation of the EPR transition during the NMR measurement. Due to the large difference in the nuclear and electron gyromagnetic ratio this technique is particularly challenging in mid- and high-field NMR (400 - 800 MHz) where the electron Larmor frequency is in the sub-THz range (263 – 527 GHz) where high-power sources are expensive.
Hence, an efficient coupling of the available microwave power to the sample is the prerequisite to obtain a maximum NMR signal enhancement and/or to allow the use of low-power sources.
In this presentation, we will discuss the details of the coupling between the sub-THz microwave field and the sample in different DNP setups that have been successfully used in the recent years. Namely, we will focus on (i) routine solid-state MAS DNP experiments at temperatures around 90 K, and (ii) on liquid state Overhauser DNP around room temperature.
For the special case of solid-state MAS systems we present results from a simulation study where the refraction, diffraction and scattering of the incident microwave beam by the coil and the MAS rotor was studied. We will address alternative microwave coupling strategies that could be used to obtain a more homogeneous microwave field distribution in the sample volume and to reduce the necessary microwave power.

In contrast to solid-state DNP experiments, Overhauser DNP is particularly challenging due to the high absorption rate of liquids at sub-THz fields and requires a different strategy of microwave coupling. For this type of experiment we have developed a dedicated probe based on a microfluidic chip and we present RF and microwave field simulations and experimental DNP data.

Poster Presentation

452 TH

AN OPTIMAL SET OF MULTIDIMENSIONAL EXPERIMENTS FOR RESONANCE ASSIGNMENT OF INTRINSICALLY DISORDERED PROTEINS

Anna Zawadzka-Kazimierczuk, Katarzyna Grudziąż, Wiktor Koźmiński

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Intrinsically disordered proteins feature high conformational dynamics, which causes averaging of local magnetic field and consequently – low chemical shift dispersion. Therefore studying of disordered proteins usually requires performing high-dimensional experiments (≥4D) to solve the problem of peak overlap. Within last few years we have proposed several such experiments, dediacted for resonance assignment [1-3]. They were successfully applied to intrinsically disordered proteins [4-7]. The data can be processed using the Sparse Multidimensional Fourier Transform [8] and analyzed automatically using the TSAR program [9]. Here we report the relative senstivities of the experiments and give guidelines for choosing an optimal set of experiments for a particular protein sample.

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453 MO

REAL-TIME MULTIDIMENSIONAL NMR WITH NON-UNIFORM SAMPLING: APPLICATION TO DEUTERIUM EXCHANGE AND PHOSPHORYLATION

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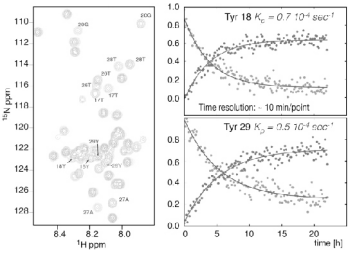
During the recent years a remarkable progress was made in developing of fast multidimensional NMR acquisition techniques for studying chemical and biochemical process kinetics in the real-time. Unfortunately the “curse of dimensionality”, i.e. rapid increase of acquisition time in multidimensional NMR spectroscopy, prohibits application of experiments with more than two spectral dimensions in real-time measurements. Here we introduce a new general approach to overcome this problem. By applying non-uniform sampling and MDD co-processing we were able to follow both fast and slow kinetic process together in a single 3D NMR spectrum. In contrast to the conventional approach, where sacrifice in spectral resolution is inevitable to improve time resolution, the new approach allows recording of 3D spectra with high resolution both in frequency and time.



The new methodology is demonstrated with 3D HNCO experiment by following in real time hydrogen-deuterium exchange in Ubiquitin and tyrosine phosphorylation in intrinsically disordered cytosolic domain of CD79b belonging to the B- cell receptors. While the Ubiquitin experiment serves for validation of the method and probing its limits, use of the 3D spectra in the latter case was essential due to heavy overlap in the 2D HSQC of the disordered CD79b domain.

Poster Presentation

454 TU

MAGNETIC SUSCEPTIBILITY OF MATERIALS USED IN MICRO MR, MR ENGINEERING AND BEYOND

Matthias C Wapler (1), Jochen Leupold (2), Iulius Dragonu (2) , Dominik von Elverfeldt (2), Maxim Zaitsev (2) and Ulrike Wallrabe (1)

University of Freiburg, IMTEK, Laboratory of Microactuators (1) and University Hospital, Department of Radiology (2)

With decreasing scales of MR devices, increasing resolution in imaging and more accurate spectroscopy, it becomes increasingly important to optimize magnetic homogeneity in the design of new devices, and hence to know the susceptibility of the involved materials. For pure elements and simple compounds, there exists a comprehensive database [1], but for complex materials like polymers and technical glasses, there are only various scattered sources of data available [2].
In this contribution, we present a systematic measurement of the magnetic susceptibility of a wide range of materials used in MR engineering as well as a more detailed study of polyurethane (PU) as a casting material and as a substitute for PDMS and of glass types that may be potentially used as substrates. The susceptibility values were obtained at 9.4T by measuring the distortion of the magnetic field by small cylindrical or rectangular bar-shaped samples in purified water as a reference liquid. The resulting field maps were fitted slice-by-slice to a three-dimensional finite element simulation, accounting also for an inhomogeneous background and a shift and tilt in the sample position. For samples in a range of susceptibilities up to 2ppm, the measurements were very accurate, typically to 1%, with 4ppb as a limit due to temperature fluctuations.
Many of the 33 polymers are within a range of +- 0.5ppm compared to water, which can be considered to be “acceptable”. One particularly suitable material, PU, is available as a rigid casting resin, glue and elastomer. Hence, we investigated the dependence of the susceptibility on the curing parameters, mixtures and additives. Spectral evaluation showed that its MR signal is close to zero at echo times > 1 ms and hence it is suitable as an MR-compatible substitute for PDMS.
Glass and composites, in contrast, have a very large spread in the susceptibility, up to 5ppm compared to water. In a survey of 14 different glasses, however, we identified some types significantly below 0.5ppm, and made predictions for other types that may be below 0.1ppm.
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455 TH

MODIFIED ADIABATIC INVERSION SWEEPS FOR ENHANCED MRFM-SIGNAL

Sebastian M. Schnoz, Ivan T. Tomka, Rosmarie Joss, Beat H. Meier

Magnetic resonance force microscopy (MRFM) was proposed two decades ago (1). Since then, MRFM could prove that it can be used as a microscopic technique with a high sensitivity (2), a high resolution (cryo-MRFM ~10 nm (2), room temperature MRFM ~100 nm (3)) and a high chemical selectivity (4-7).
During MRFM detection, the spins must be periodically inverted at the mechanical resonance frequency of the cantilever. Recent work has experimentally shown that optimized constant adiabatic inversion sweeps (CAIS) can be superior to conventional linear read-out sweeps. Signal to noise enhancement by a factor of 6 was obtained for a polymer blend containing 20% PTFE and 80% PEEK (8). Up to now it is not completely clear why the enhancement factor depends so strongly on the type of chemical compound used as sample.
The presented work will further characterize the performance of CAIS compared to linear inversion sweeps, using samples with different structure (amorphous/crystalline) and relaxation times(T_1/T_1ρ).

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Poster Presentation

456 MO

PROSPECT TO EXCEED 1.5 GHz FOR SOLUTION NMR SPECTROMETERS; WORLD'S FIRST NMR MEASUREMENTS WITH A (RE:RARE EARTH)$Ba_2Cu_3O_{7-\delta}$ HIGH TEMPERATURE SUPERCONDUCTING NMR MAGNET OPERATED AT 400 Mhz

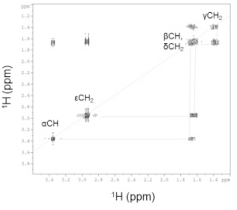
Y. Yanagisawa[1,2], R. Piao[1,2], X. Jin[1], H. Nakagome[2], K. Kominato[3], M. Hamada[3], S. Matsumoto[4], T. Kiyoshi[4], H. Suematsu[5], M. Takahashi[1], and H. Maeda[1]

[1]RIKEN, [2]Chiba University, [3]JASTEC, [4]NIMS, [5]JEOL RESONANCE

The current density of a high temperature superconducting (HTS) inner coil for ultra-high field NMR magnets is limited by the conductor hoop stress due to Lorentz force. The 2nd generation (RE)$Ba_2Cu_3O_x$ (REBCO, RE: rare earth materials such as Y) HTS conductors tolerate hoop stress of >700 MPa, while the 1st generation $Bi_2Sr_2Ba_2Cu_3O_x$ (BSCCO) HTS conductors ~200 MPa. Thus, a REBCO coil is preferred over a BSCCO coil to achieve a higher NMR magnetic field with a smaller magnet. The use of REBCO inner coils enables NMR magnets to exceed 1.5 GHz, if we replace a few low temperature superconducting (LTS) inner coils of the 920 MHz NMR magnet by REBCO coils. However, an NMR magnet using a REBCO coil has never been developed so far. This paper presents the world's first successful 400 MHz NMR spectrometer using a REBCO inner coil and LTS outer coils, as the first step toward a REBCO NMR spectrometer exceeding 1 GHz. The major problem regarding REBCO coils is screening current induced in the coil, deteriorating the stability and the homogeneity of the magnetic field. In this work, we suppressed the effect of the screening current and succeeded in solution NMR measurements.

The innermost coil of a conventional 600 MHz LTS NMR magnet was replaced developed a LTS/REBCO NMR magnet. The magnet was operated at 400 MHz in driven power supply. The evaporated liquid helium was recondensed using a cryocooler for h temporal field-drift due to relaxation of the screening current was suppressed by a currer of the magnetic field generated by correction coils due to the screening current resul inhomogeneity. The error harmonics were corrected by ferromagnetic shims. The peal sample spinning for 1% $CHCl_3$ in acetone-d_6 were 0.24 Hz, 10.7 Hz and 11.0 Hz, respecti for Lysine was successfully obtained as shown in Fig. 1.

Fig. 1 COSY spectrum for Lysine



457 TU

DNP USING PHOTO-EXCITED TRIPLET STATES: HIGH PROTON SPIN POLARIZATION IN PENTACENE: NAPHTHALENE CRYSTALS AT MODERATE MAGNETIC FIELD AND TEMPERATURE

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Dynamic nuclear polarization (DNP) is a powerful tool to greatly enhance spectroscopic signals from nuclear spins beyond thermal limits and its potential has become a driving force in different fields of nuclear magnetic resonance (NMR) applications.
In DNP experiments the high polarization of an electronic spin system is transferred by suitable microwave irradiation to the nuclear spin system. In usual classical schemes ground state paramagnetic centers are introduced into the sample as dopants and their electron spin polarization is determined thermally. This requires cooling the sample to temperatures of 1K or lower and applying a magnetic field of several Tesla to achieve high nuclear polarizations. As an alternative, short-lived photo-excited triplet states of aromatic molecules can also provide the electron spins for the DNP process, which relieves the drawbacks of conventional DNP. In the photo excitation process the population of one triplet level is strongly favored. Thus neither low temperatures nor high fields are prerequisite for a high electron polarization. However, the short triplet state lifetime of a few tens of microseconds requires a fast polarization transfer. Classical DNP processes, thermal mixing or solid effect, driven by weak cw microwave irradiation are too slow and one has to resort to pulsed DNP methods using strong microwave fields which resonantly transfer the polarization of electron spins to nuclear spins in a Hahn-Hartmann type experiment.
Here we discuss the concepts of triplet DNP and present a versatile experimental setup to perform pulse DNP experiments in bulk samples. In single grown pentacene:naphthalene crystals we achieve, at 100 K and 0.35 T, proton polarization build-up rates that exceed one percent per minute and result in a proton polarization level above 50%, corresponding to an enhancement factor of more than 5 orders of magnitude [1].

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458 TH

CRYOGEN FREE MAGNETS FOR MULTI-FREQUENCY AND SWEPT-FIELD NMR AND DNP STUDIESD

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Cryogenic Ltd.

Pulsed NMR methods have evolved over many decades for the study of chemical structure and dynamics. Multi-dimensional pulse sequences are applied at high magnetic field < 20 Tesla to acquire high resolution spectra and resolve the structure of complex molecules. Meanwhile the magnet is run in persistent mode and the receiver is tuned to detect the response to pulses of RF energy. But several magnets are required to study the field dependence of NMR spectra.
Since the 1990s, Cryogenic Ltd. have been developing experimental platforms based on cryogen free magnets and variable temperature inserts for research at high magnetic field < 20 Tesla and low temperature > 30 mK. These systems are now finding application in the field of magnetic resonance. During the 2000s, cryogen free EPR magnets at 9 to 14 Tesla helped launch the field of mm-wave EPR spectroscopy [1] [2]. In 2007, a cryogen free 600 MHz magnet was built for static NMR studies. Last year, a compact cryogen free 1.5 Tesla pre-clinical MRI magnet was installed [3]. In this contribution, we show how the homogeneity of magnets may be optimized without shimming and describe temperature-stabilized supplies that hold the main field with high temporal stability in swept-field mode. A Japanese group have demonstrated a novel method of elemental analysis using a small desk-top magnet [4]. In France, the 600 MHz magnet has been used to measure quadrupolar-broadened lines spanning tens of MHz [5]. With the goal of enabling high resolution MAS and DNP studies over a broad span of magnetic fields and at low temperature, we present NMR spectra that demonstrate the potential of this technology.

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459 MO

SLOW-SPINNING LOW-SIDEBAND HR-MAS NMR SPECTROSCOPY FOR DELICATE STUDY OF BIOLOGICAL SAMPLES

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CNRS INCS UPR 2301; Aix Marseille Université UMR 7313 ISM2; Bruker France

High-Resolution Magic-Angle Spinning NMR spectroscopy (HR-MAS) has become an extremely versatile analytical tool to study heterogeneous systems endowed with liquid-like dynamics. Spinning frequencies of several kHz are typically required to obtain NMR spectra that display resolution approaching that of purely isotropic liquid samples. An important limitation of the method is the large centrifugal forces that can damage the structure of the sample.
In this communication, we show that optimizing the geometry and the filling factor of the sample chamber of the HR-MAS rotor leads to high-quality low-sideband NMR spectra even at very moderate spinning frequencies, thus allowing the use of well-established solution-state NMR procedures for the characterization of small and highly dynamic molecules in the most fragile samples, such as live cells and intact tissues.

Poster Presentation

460 TU

APPLICATIONS OF SIGNAL FEEDBACK IN NMR AND MRI

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Tuned pick-up (PU) coils with high quality factors *Q* are used in NMR and MRI for high-sensitivity and low-noise detection. However they limit the signal bandwidth (BW) and the enhanced currents cause strong radiation damping (RD) effects in the samples. Signal feedback (FB) can be used to actively control these currents and adjust the BW and RD without resistive losses and noise penalty [1,2]. Here we report on successful implementations of signal FB in two experiments. We use an inductive coupling scheme to feed a suitably amplified phase-adjusted signal back into the PU coils of our low-field NMR systems. One system is used for NMR studies of distant dipolar field effects in highly polarized liquid ^{3}He [3] without or with RD. The moderate intrinsic *Q*-factor (~9) can be strongly reduced by negative FB (down to ~1) or increased by positive FB (up to ~100). This is used, e.g., to control transient maser oscillations. The other system is used for MRI, with a pair of Litz-wire PU coils separately tuned with an intrinsic *Q*~150 and placed around the sample. High impedance amplifiers matched our PU coils introduce significant uncontrolled FB, possibly by internal capacitive coupling. This affects the measured *Q* and results in unstable operation at high positive gain, but appropriate negative FB prevents such instability. Negative FB also efficiently broadens the detection BW (with an adjustable effective *Q*) and decouples the detected signals by reducing the currents in the coils. This elementary coil-array is used for parallel acquisition in MRI with a field of view (FoV) smaller than the object. Data acquired at 87kHz (2mT) from a water sample are used for SENSE image reconstruction [3]. The artefact-free final (unfolded) images demonstrate the efficiency of the FB coil decoupling scheme for arbitrary coil geometries.

V.V. K.'s work is supported by the FPGG Foundation.
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461 TH

BRUKER MILLIMETER WAVE EPR SYSTEM ELEXSYS E780: DESIGN AND PERFORMANCE

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The ELEXSYS E700/E780 EPR spectrometer has recently been developed by Bruker Biospin. The spectrometer utilizes the latest achievements of millimeter wave technology in combination with the well-proven Bruker intermediate frequency (IF) concept. The IF bridge operates at X-band frequencies for excitation and detection in both CW and pulse EPR modes. The quasi-optical front-end converts the IF bridge frequencies up to 263 GHz for excitation and down converts the resulting reflection or induction mode signal to X-band frequencies for quadrature detection.

An innovative cryogen-free sweepable 12 T magnet has been specifically designed. Its main and high-resolution ±0.15 T sweep coils provide ultimate and fast control over magnetic field. The cryogen-free approach simplifies magnet maintenance considerably and eliminates of extra helium boil off during main coil sweeps. The spectrometer is controlled via the Xepr software package.

ELEXSYS E700/E780 features two detection modes: reflection and induction. In reflection mode, the response signal, which is co-polarized to the excitation wave, is recorded. In induction mode, the cross-polarized wave is observed. In both detection modes, the signal is detected in quadrature providing absorption/dispersion signals in CW operation and real/imaginary signals in pulse operation.

The E780 wide sample space probe head is based on a tapered corrugated transmission line and works in a non-resonant and resonant configuration. The cylindrical sample area is up to 5 mm in diameter. The samples are mounted in a Teflon cylinder which is then attached to a sample holder for vertical positioning of the sample. Alternatively, the samples in standard EPR tubes could be used as well. The TE_{011} ENDOR probehead has similar transmission line ended with taper to rectangular waveguide. It has variable frequency tuning and coupling. Moreover, the probe incorporates both ENDOR and CW modulation coils and, optionally, optical access for sample illumination. Both probeheads are suitable for helium temperature operation.

Further development of the system includes implementation of the 10 watt EIK amplifier and arbitrary microwave pulse shape control.

Poster Presentation

462 MO

SYNTHESIS OF EPR PULSE SEQUENCES ON A FAST ARBITRARY WAVEFORM GENERATOR

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On a traditional pulsed EPR spectrometer, pulse shaping is limited to switching on and off a set of microwave channels. Since fast arbitrary waveform generators (AWGs) with timing resolution below 1 ns became available in the past few years, the amplitude and phase of microwave pulses can be controlled precisely. Pulse shaping, as applied in NMR with great success, can therefore be adopted to specific problems in EPR spectroscopy. Due to the fact that an AWG opens up several new ways to design an experiment, the interface between the user and the experiment gains in complexity.
Here, we report on our approach of using an AWG to synthesize pulse sequences as a whole. In this concept, the AWG generates all involved excitation pulses as well as synchronization triggers for external hardware. The whole pulse sequence is therefore sliced into a set of digital waveforms, each complemented with playback instructions, such as for instance the number of individual waveform loops.
Before starting an experiment, the user provides the pulse sequence as an event-based data structure, which defines all intended timings in nanoseconds. Hierarchical parameter variations on this data structure facilitate complex pulse sequences. Based on this data structure and a set of AWG- and spectrometer-specific constraints, the digital waveforms are calculated. Subsequent transfer to the AWG avoids any reprogramming during an experiment. Once an experiment is started, control is based on counting acquisition events on the detection device.
We discuss some of the limitations of this procedure and demonstrate preliminary experimental results using pulse sequences with traditional rectangular pulses. The experiments were acquired on a basic spectrometer employing a heterodyne transceiver.

463 TU

DYNAMIC NUCLEAR POLARIZATION AND ^{1}H-^{6}LI CROSS POLARIZATION AT 6.7 T AND 4.2 K

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Dynamic nuclear polarisation (DNP) at low temperatures is a prerequisite for dissolution experiments where the NMR polarisation of low-gamma nuclear spins such as ^{6}Li can be enhanced by four to five orders of magnitude, compared to the Boltzmann equilibrium at room temperature after dissolution [1].
We have developed a probe for cross polarization from ^{1}H to ^{6}Li that is compatible with a home-built CP-DNP polarizer operating at 6.7 T and in the temperature range 1.2 – 4.2 K [2]. The new ^{6}Li CP probe can deliver 90° pulses of 4.5 and 6 microseconds on the ^{1}H and ^{6}Li channels respectively, using amplifiers providing no more than 100 W. Cross polarisation from ^{1}H to ^{6}Li was achieved under DNP conditions at 4.2 K in a 2 M solution of lithium acetate (with ~7.4 % ^{6}Li in natural abundance) in a 1:1 mixture of D_2O and deuterated ethanol with 50 mM TEMPO as a free radical. The design of the probe is compatible with the requirements of rapid dissolution after CP [3]. The probe also features a coil for longitudinally detected electron spin resonance (LODESR). Both CP-DNP and LODESR data will be presented.
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464 TH

FINDING A NEEDLE IN A HAYSTACK: COMBINING NMR AND NANOPARTICLES FOR THE IDENTIFICATION OF ORGANIC MOLECULES IN COMPLEX MIXTURES*

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e present a new sensing protocol for the detection and identification of organic molecules in complex mixtures based on the combination of ligand-screening NMR techniques and molecular recognition abilities of gold nanoparticles (NPs).
Detecting and identifying analytes in complex mixtures is still a challenge: latest NMR methods, coupling diffusion-ordered spectroscopy (DOSY)[1] with the use of a "stationary phase"[2-5], still suffer of resolution and applicability limitations. In our approach, the concept of "chromatographic NMR" is revised: the separation is based upon the affinity of the analyte towards a tailored nanoparticle (stationary phase), and the detection is entrusted to NOE-based sequences, such as NOE-pumping [6] or Saturation Transfer Difference (STD, [7]).
Proof of concept was demonstrated by the detection of sodium salicylate in solutions of similar organic salts. Secondly, we compared the striking selectivity of TEG-functionalized thiols with other monolayers, and deduced some insights on how the structure of the coating affects the targeting. Finally, as a physiological application, we revealed the presence of salicylate from a sample of urines. In conclusion, our method overcomes previous limitations, is easily implemented and also versatile, since the nanoparticles coating can be tailored for different classes of analytes.

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465 MO

COMBINED EPR/NMR/DNP COMPACT MULTIPURPOSE EQUIPMENT

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Introduction
A new compact combined EPR/NMR/DNP equipment is developed both for research and for analytical applications. The instrumentation described can be characterized by low cost, reliability and simplicity, small size and high level of measurement automatization, a wide choice of functional modules and units. The instrumentation features are based on the latest developments in electronics, computer technology, original design and scheme solutions.
Specifications of the EPR part of equipment:
sensitivity - $2x10^{10}$ spin/G, operating frequency -(9,0…9,6) GHz, maximum microwave power -0,15 W, maximum induction of magnetic field - 0,5 T, maximum ampoule diameter - 10 mm.
Specifications of the NMR part of equipment:
operating frequency - 5…20 MHz, RX sensitivity - better than 1 μV (when S/N ratio = 3), adjustable TX output power - up to 500 W, three selectable bandwidths of analog filter - from 100 Hz up to 1 MHz, pulse sequence length - up to 64 K events with resolution 100 ns, - probe tube diameter - up to 40 mm
Original/Custom instrumentation
Among original instrumentation developments there are:
compact high-stability microwave units of homodyne and threshold type, low-noise Gunn oscillator being used as a microwave oscillator;
handheld magnet systems based on electromagnets and permanent magnets with high uniformity of magnetic field;
digital systems for recording and processing the EPR and NMR data.
For DNP measurements at 0.35 T an electromagnet and special cylindrical microwave cavity with TE 011 mode have been used. Also the portable DNP system based on Halbach type permanent magnet has been developed.
Applications
EPR/NMR instrumentation are used in stationary research laboratories and in industrial conditions

466 TU

TWO-DIMENSIONAL NMR SPECTROSCOPY ON A DESKTOP SPECTROMETER

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Two-dimensional (2D) NMR spectroscopy is a general concept that enables chemical information to be encoded into a second dimension making use of spin-spin interactions like the *J*-coupling. It is particularly useful to simplify 1D spectra with overlapping signals and to identify coupled chemical groups. While 2D techniques are routinely used at high field they are not exploited on desktop spectrometer. One of the main reasons for this is the high stability required to sample the data along the indirect dimension. Frequency or phase instabilities between scans result in stripes along the t_1 direction also known as t_1 noise. Furthermore, the acquisition of meaningful 2D spectra is only possible if the spectrometer provides enough resolution and high signal-to-noise, otherwise the acquisition time for a 2D spectrum can become excessively long. In this work we demonstrate the performance of 2D NMR on a 1 Tesla permanent magnet. The magnetic field homogeneity of the magnet can be finely shimmed to achieve sub Herz resolution by means of shim coils up to order three, and high field stability is achieved by means of an external lock system. From the large variety of available 2D pulse sequences we tested the performance of *J*-resolved spectroscopy, correlation spectroscopy (COSY), and double quantum filtered COSY. The results presented here demonstrate that 2D NMR is of great assistance at low field where the spectrum of small molecules may appear crowded due to the strong coupling limit.

467 TH

ON-LINE MEASUREMENT OF CHEMICAL REACTIONS BY A COMPACT NMR SPECTROMETER

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On-line nuclear magnetic resonance (NMR) spectroscopy analysis of chemical reactions provides important information for process development and optimization. However, the use of conventional high-field NMR spectrometers for this purpose is limited because the large size of this equipment and the required maintenance makes it difficult to install them in the chemistry labs where the reactions are carried out. For this application desktop spectrometers based on permanent magnet technology appear as a powerful alternative. Examples of the use of low-field medium-resolution NMR spectrometers for the on-line monitoring of chemical reactions have been presented in the past. However, the reported performance was always far from the one required for industrial applications or in routine chemical process analysis to study relevant chemical reactions.
Technically relevant chemical reactions performed in industry using microreactor technology like Knoevenagel condensations, Suzuki reactions, or transfer hydrogenations are run in the low concentration limit and involve a number of chemical components with complex spectra. Small sample concentrations are typically available, either due to economical reasons, or in order to have control over the reaction kinetics, which can be altered by highly exothermic effects. To allow high quality understanding of such reactions high spectral resolution and sensitivity are required. In this work we present real time ^{1}H NMR spectra measured under the fume hood with a new compact 1 T system offering improved resolution and sensitivity. In combination with a flow-through setup, different chemical reactions, such as the transfer hydrogenation of acetophenone to 1-phenylethanol or the base-catalyzed transesterification of vegetable oils with a short chain alcohol to produce biodiesel were studied in batch mode. Reaction yields and kinetic constants are calculated for different reaction conditions. Additionally, we have studied the effects of flow rate, cell characteristics, and tubing lengths on the accuracy to measure concentrations of a flowing mixture with the aim of optimizing the flow settings.

Poster Presentation

468 MO

A DESIGN CONCEPT FOR LOW-ORDER SHIM ARRAYS WITH NON-CIRCULAR ELEMENTS

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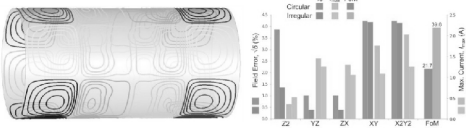
Correcting for inhomogeneity in the primary magnetic field of an NMR/MRI device is typically achieved using a set of dedicated shim coils that are responsible for generating spherical harmonics fields [1]. However, due to space constraints, these systems are restricted to low-order field correction. More recently, an alternative has been proposed that uses an array of small circular coil loops that may be driven independently to afford more flexible field modelling and higher-order correction [2]. In the present work, we investigate whether an improvement in field accuracy and efficiency can be obtained by considering array elements with non-circular geometry.
The design method involves the optimization of a distributed surface current density over a set of subregions that represent the array elements. A set of component arrays are designed for generating low order spherical harmonics and these are combined to produce composite arrays with an optimum figure of merit involving low field error, high efficiency and low power dissipation. Theoretical designs for first and second-order shim arrays are provided as a proof of concept and show significant gains when compared to equivalent arrays of circular elements (see Figs. 1-2).

Fig. 1: Second-order shim array of 24 elements.
Fig. 2: Comparison to circular array.



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469 TU

STUDYING PROTON EXCHANGE TO ASCERTAIN THE LIFETIME OF *PARA*-WATER

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Para-water has two proton spins in a singlet state that is antisymmetric under permutation. Its spin angular momentum vanishes so that no NMR signal can be directly detected from this system. Since the intramolecular dipole-dipole interaction is not an effective relaxation mechanism for a singlet state, its relaxation to equilibrium has a time constant T_{para} that might be many times longer than the conventional longitudinal relaxation time constant T_1. Our strategy to observe this "forbidden fruit" relies on creating an imbalance between the populations of triplet and singlet states using dynamic nuclear polarization at T = 1.2K, where a proton spin temperature of 3 mK can be achieved prior to the transfer of the sample to an NMR spectrometer at room temperature.
A chemical addition onto an aldehyde such as chloral allows one to monitor the relaxation of the singlet state of the hydroxyl protons in chloral hydrate through its influence on the signal of the remaining proton.
In this scenario, it is fundamental to slow down all mechanisms that can contribute to the relaxation of *para*-water. Since one of the most crucial mechanisms is chemical exchange of protons belonging to different water molecules, the aim of this study is to determine the rate of this process in different organic solvents, in order to find the favourite one where the lifetime of *para*-water could be extended as far as possible.
Using a 1D EXSY sequence, we were able to determine exchange rate constants for systems composed of H_2O, D_2O and HDO diluted in various organic solvents in the millimolar range.
Chemical proprieties of the solvents, such as their dipole moments, polarities and dielectric constants, may influence the rate of the exchange process. In fact, it can be slowed down by an order of magnitude, simply by choosing the most appropriate solvent as reaction medium. Once *para*-water is made accessible to NMR, a wide range of experiments that are currently impossible would become feasible. Studying processes like chemical exchange, transport and diffusion on longer time scales are just a few of possible exciting new applications.

Poster Presentation

470 TH

SPIN TRANSITION ANTICOOPERATIVITY INDUCED BY WEAK INTERMOLECULAR INTERACTIONS IN COBALT(II) CLATHROCHELATES: NMR, EPR AND X-RAY DIFFRACTION STUDY

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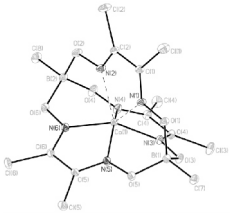

As a classical example of molecular bistability, spin transition (ST) paves the way towards molecular switches based on energy difference between two spin states of a transition metal ion. One of the main challenges in this field is pursuing the cooperativity, which allows switching the spin states of all molecules in a given crystal simultaneously. Such a cooperativity is currently considered as a result of "chemical pressure", implying that in its origin are the changes in molecular volume upon ST, or, taken to the molecular level, as a consequence of weak intermolecular interactions in a crystal. It is, however, not clear if the rational design of molecular switches should be based on the meticulous analysis of crystal packing, or only the volume difference between high- and low-spin complexes matters.

In our study, we showed that very weak intermolecular interactions can cause a significant anticooperativity of an ST. Thus, the ST of cobalt(II) clathrochelates (which do not change the molecular volume upon ST) in solution (followed by NMR and EPR spectroscopy) was more abrupt than in solid state, and this was shown to be the result of very weak (1.7 kcal/mol) π…Cl intermolecular interactions, stabilizing one of the Jahn-Teller distorted forms and thus leading to the more gradual ST.

Our results clearly demonstrate that the standard model of elastic interactions is not universally applicable, and the successful design of molecular switches is not possible without thoughtful analysis of intermolecular interactions, however weak they seem to be. This research was supported by the RFBR (grant 13-03-00732) and CPRF (grant MK-4842.2013.3).

471 MO

CONFORMATIONAL ISOMERISM INDUCED PARAMAGNETISM IN DITHIOLE AND THIAZOLE DERIVATIVES

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In this work we report the synthesis, structure and magnetic characterization of a new class of purely organic biradicals based on N-(1,3-dithiol-2-ylidene)pyrimidin-2-amine and other purely organic analogues leading to materials that exhibit different magnetic behaviour. It has been proved experimentally and supported by a simple theoretical model derived from high-level electronic structure calculations that a fraction of the molecules in the materials are in a triplet ground state (S = 1). In some of the reported materials, the origin of the observed magnetic properties is in micro domains that present cooperative effects giving place to a ferromagnetic behaviour even at ambient temperature. We have found that the existence of these unconventional biradical structures is associated to specific conformers of molecules containing the basic central >C-N=C< structural unit and is more frequent than expected from conventional valence bond theory commonly used to rationalize the electronic structure of organic molecules. The implications of these biradical structures, triplet or open shell singlet, are expected to be of fundamental significance to understand the nature of the ground state and reaction mechanisms involving this kind of molecules in organic and bioorganic chemistry. Also, the possibility to control the biradical character of these organic molecules opens the door to a new family of organic magnetic materials active in a very wide range of temperatures.

472 TU

PARASSIGN – PARAMAGNETIC NMR ASSIGNMENT OF PROTEIN NUCLEI ON THE BASIS OF PSEUDOCONTACT SHIFTS

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The use of paramagnetic NMR data for the refinement of structures of proteins and protein complexes is widespread. However, the power of paramagnetism for protein assignment has not yet been fully exploited. PARAssign is software that uses pseudocontact shift data derived from several paramagnetic centers attached to the protein to obtain amide and methyl assignments. The ability of PARAssign to perform assignment when the positions of the paramagnetic centers are known and unknown is demonstrated.
PARAssign has been tested using synthetic data for methyl assignment of a 47 kDa protein, and using both synthetic and experimental data for amide assignment of a 14 kDa protein. The complex fitting space involved in such an assignment procedure necessitates that good starting conditions are found, both regarding placement and strength of paramagnetic centers. These starting conditions are obtained through automated tensor placement and user-defined tensor parameters. The results presented herein demonstrate that PARAssign is able to successfully perform resonance assignment in large systems with a high degree of reliability. This software provides a method for obtaining the assignments of large systems, which may previously have been unassignable, by using 2D NMR spectral data and a known protein structure.

473 TH

STUDY OF MAGNETIC ANISOTROPY OF HEPTACOORDINATE Ni(II) COMPLEXES

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The study of magnetic anisotropy of Single-Molecule Magnets is attracting a lot of interest, as this parameter governs their properties. As a consequence many efforts are also devoted to the understanding and control of magnetic anisotropy in single ion complexes which are the building units for SMM.
Here we present High-Frequency/High-Field EPR spectroscopy powder studies performed, at several frequencies, on mononuclear Ni(II) complexes. These complexes with pseudo pentagonal-bipyramidal geometries (Fig. 1) are found to exhibit rather large axial magnetic anisotropies associated to moderate rhombic terms. With the help of ab-initio theoretical calculation, the relation of the Zero-Field Splitting terms with the d-orbitals and the molecular structures will be displayed.

Poster Presentation

474 MO

INVESTIGATIONS IN ASYMMETRIC LANTHANIDE CHELATING TAGS

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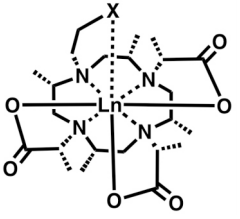
One of the main fields in modern NMR spectroscopy is protein structure determination in solution. Besides protein structure determination, protein-protein and protein-ligand interactions are challenging fields.[1,2] Long range structural information is a crucial prerequisite to solve such problems. Especially paramagnetic relaxation enhancement (PRE) and pseudo contact shifts (PCS) can provide such long range information. To gain such information a paramagnetic metal has to be attached rigidly to a protein. Binding pockets or lanthanide chelating tags are used to attach metals in a rigid way to proteins. Starting from DOTA-M8 we investigated new asymmetric DOTA-M7 derivatives. DOTA-M7 has one specific new side chain, which contains different hetero atom to investigate the electronic influence of donating atoms to the magnetic properties. DOTA-M8-Ln (4 times N, 4 times O donor) was used as symmetric reference system. Different PCS between the paramagnetic DOTA-M8-Ln and DOTA-M7-X-Ln were obtained.



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475 TU

MAGNETO-STRUCTURAL CORRELATIONS IN TRIAZOLO-BRIDGED DINUCLEAR COPPER(II) COMPLEXES

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The magnetic properties of paramagnetic transition metal ions incorporated in various coordination compounds have been the subject of interest over many years. Particular interest has been directed towards the study of dinuclear copper(II) complexes as model systems to study magneto-structural correlations. Systematic investigations of coordination chemistry of 3-substituted [1,2,4]triazolo[4,3-a]pyridines resulted in a variety of coordinating modes featured in complexes of ligands with copper(II) ions. The 3-(2-pyridyl)-substituted derivative prefers a mononuclear complexation mode[1] while 3-(2-pyrazyl)-substituted derivative forms readily a 2D network.[2] Only after introduction of a methyl group in 6-position of the pyridine substituent sterical stress imposed on the coordination sphere of a complex allows a dinuclear double bridging coordination mode. Controlling molar stoichiometry of ligand-to-metal ion two dinuclear complexes with a very different local geometry of copper(II) ions have been synthesized. Magnetic measurements revealed antiferromagnetic coupling in both complexes, however, of very different strengths. Electron paramagnetic resonance (EPR) spectroscopy has been applied to investigate magnetic properties of the complexes in detail. Experimental findings have been supported by “broken symmetry” DFT calculations. Systematic magneto-structural correlations are discussed.

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Poster Presentation

476 TH

STRUCTURAL INVESTIGATION OF AN ARTIFICIAL METALLOENZYME BY PSEUDO CONTACT SHIFT ^{19}F-NMR SPECTROSCOPY

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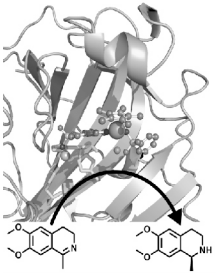

Artificial metalloenzymes combine the broad reaction scope of organometallic catalysts with the stereoselectivity of enzymes. Iridium complexes anchored to human carbonic anhydrase type II (hCA-II) by means of a sulphonamide ligand, have been shown to perform stereoselective transfer hydrogenation reactions. The enantioselectivity can be improved by single point mutations of the protein in the vicinity of the Ir-center.[1] However, to further improve the system towards even higher selectivity, structural data of the protein ligand complex in solution are crucial to identify promising mutation sites. Therefore we investigated the structure of such an artificial metalloenzyme by PCS ^{19}F-NMR spectroscopy. Four different mutants of hCA-II were prepared, all bearing single cysteine mutations at different positions on the protein surface for the attachment of [Tm(DOTA-M8)][2] as a paramagnetic label. Additionally a fluorinated derivative of an active Ir-complex[1] was synthesised to enable the observation of pseudo contact shifts (pcs) by simple one-dimensional ^{19}F-NMR experiments. Based on the pcs data, initial structural calculations were performed which are currently undergoing further refinement.

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Acknowledgements: We gratefully acknowledge T.R. Ward for his expertise on hCA-II and R.A. Byrd for providing us with a sample of tetramethylcyclen.

477 MO

PSEUDOCONTACT SHIFTS OF INVISIBLE MOLECULAR STATES MEASURED WITH PARAMAGNETIC NMR RELAXATION DISPERSION SPECTROSCOPY

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NMR relaxation dispersion techniques provide a powerful method to study protein dynamics by characterizing lowly populated conformations. Paramagnetic NMR is a versatile tool for investigating the structure and dynamics of proteins. It is demonstrated that these two techniques can be combined to obtain accurate and precise pseudocontact shifts of lowly populated conformations. In this way, valuable long-range structural restraints can also be obtained for non-ground state conformations of macromolecules, being in solution, and while in the process of dynamic exchange with the ground state. Such lowly populated states are often thought to be required in functional processes, e.g. enzyme catalysis.

The results also unveil a critical problem with the lanthanide tag used to generate paramagnetic relaxation dispersion effects in proteins, namely that the motions of the tag can interfere severely with the observation of protein dynamics. The two-point attached CLaNP-5 lanthanide tag was attached to the adenylate kinase. From the paramagnetic relaxation dispersion only motion of the tag is observed. The data can be described accurately by a two-state model in which the protein attached tag undergoes a 25° tilting motion on a time-scale of milliseconds.

Plausible mechanisms for this motion are proposed.

Poster Presentation

478 TU

DUAL MODE EPR SPECTROSCOPY OF COMPLICATED PARAMAGNETIC SYSTEMS

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In systems containing complicated paramagnetic species, the EPR spectra recorded under conventional conditions (perpendicular mode, usually X-band) often consist of multiple signals whose assignment is ambiguous. Due to this complexity, the information which can be obtained is inevitably limited and cannot be evaluated appropriately. On the other hand, EPR spectra recorded in parallel mode are much simpler and the assignment of the signals is, in many cases, straightforward. To this end, application of dual mode EPR spectroscopy is extremely useful in identifying the paramagnetic species present in composite systems.
In the present work we present results applying dual mode EPR spectroscopy in the study of the properties of paramagnetic systems including:

a. Systems with large hyperfine interactions.
b. Integer spin systems (Fe^{2+}(S=2), Mn^{3+}(S=2), exchange coupled systems)
c. Half integer spin systems with |D|< hv (Mn^{2+}, Gd^{3+})
d. Weakly interacting dimers.

479 TH

STRUCTURAL BASIS OF ABERRANT ANDROGEN RECEPTOR ACTIVATION IN LATE STAGE PROSTATE CANCER

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The androgen receptor (AR) is a 919-residue nuclear hormone receptor responsible for the development of the male sexual phenotype. Its N-terminal transactivation domain (NTD) is intrinsically disordered and contains activation function 1 (AF1), the main activation function of AR, which is a potential therapeutic target for castration resistant prostate cancer (CRPC) for which there is currently no cure. However, due to its size and intrinsically disordered nature, this activation function has remained little studied.
We have studied the structural properties of the NTD of AR at atomic resolution using Nuclear Magnetic Resonance (NMR) spectroscopy to gain a better understanding of the molecular mechanisms by which this domain regulates the function of the AR and causes disease.
We have in addition studied the interaction of this domain to one of its cognate ligands, the RAP74 subunit of human transcription factor (TF) IIF, and have found that the interaction takes place at a key motif for AR transcriptional regulation in CRPC. In addition, we observed how binding induces the formation of structure in this otherwise disordered domain.
We have designed mutants to further characterize the interaction between AR and RAP74 and its role in AR transcription activity in CRPC patients. The results obtained *in vitro* by NMR are validated by *in vivo* experiments, including functional assays. Furthermore, we are studying the binding of small molecules that are drug candidates to the NTD. By identifying the binding site and by monitoring the conformational changes observed in the AR upon binding the small molecules, we are working towards a better understanding of the mode of action of these compounds.

Poster Presentation

480 MO

COOPERATIVE INTERACTION OF COLLAGEN TYPE I WITH HUMAN FIBRONECTIN

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Fibronectin (FN) and collagen are two key components of the extracellular matrix and together control a variety of cellular processes, including adhesion, migration, growth and differentiation. Despite its obvious biological importance, this interaction has not been well characterized on a structural level. Here we present a model on how the full-length gelatin binding domain (GBD) of FN and the most common collagen type interact on a molecular scale. This work was carried out using an integrated structural biology approach, employing a variety of methods, such as NMR, X-ray crystallography, fluorescence and SAXS. We show that collagen binding on GBD is mostly mediated by the $^{8\text{-}9}$FnI sub-fragment, which interacts with sites on D-period 1 (1/10 site) and D-period 4 (3/4 site) of both collagen type I chains. All four binding sites contain a core consensus sequence with a conserved leucine at position 2 and an arginine at position 9 (1). However, the binding affinity of these four sites differs by up to 400 fold. Our analysis suggests that, in select cases, the remaining GBD sub-fragment, ^{6}FnI$^{1\text{-}2}$FnII^{7}FnI (2), can act to reduce this disparity. For the first time we show directly a collagen interaction that engages all GBD modules in a cooperative manner. We speculate that the final physiological result of this additional association is the creation of four broadly equipotent FN binding sites on type I collagen. Ensemble SAXS analysis of the GBD alone and in complex with the cooperatively bound collagen epitope shows that collagen can stabilize a preformed, monomeric GBD conformation in solution. Our findings demonstrate how FN fragments form unique functionally competent multi-domain units, that build a versatile protein interaction hub in the extracellular matrix. 1. Erat, M. C., Slatter, D. A., Lowe, E. D., Millard, C. J., Farndale, R. W., Campbell, I. D., and Vakonakis, I. (2009) *Proc Natl Acad Sci U S A* **106**, 4195-4200. 2. Erat, M. I. C., Schwarz-Linek, U., Pickford, A. R., Farndale, R. W., Campbell, I. D., and Vakonakis,I. (2010) *Journal of Biological Chemistry* **285**, 33764-33770.

481 TU

A MOLECULAR MODEL OF KNOB AND PfEMP1 LOCALIZATION ON THE ERYTHROCYTE SURFACE

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P. falciparum infected red blood cells (iRBC) sequester in the microvasculature using the parasite cytoadherence system: a set of proteins exported to the host cell that modifies the mechanical attributes, surface appearance and adhesion properties of the infected erythrocyte. iRBC cytoadherence is the major factor for the onset of severe malaria and the resulting disease lethality. Important protein components of this system include the parasite adhesion receptor family PfEMP1, which locates on “knob”-like protrusions on the erythrocyte membrane, and KAHRP, which is critical for the formation of “knobs”. Despite the importance of this system to disease pathology, we currently lack a mechanistic understanding of its components. Here, we integrate solution NMR, SAXS and crystallographic data to construct the first molecular model of “knobs”. We had previously shown that the intracellular segment of PfEMP1 is an intrinsically disordered protein (1), as is the carboxy-terminal half of KAHRP. We have now identified three direct protein-protein interactions involving segments of erythrocyte spectrin, PfEMP1, KAHRP and a member of the parasite PHIST protein family. Together, these interactions provide a conceptually intuitive way for PfEMP1 and knob localization on the erythrocyte membrane, for natural formation of PfEMP1 clusters, and for a mechanical connection between extracellular adhesion and the cytoskeleton. We discuss our ongoing efforts to determine high-resolution structures of these proteins and complexes, which we hope will yield new potential therapeutics targets against severe malaria.

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Poster Presentation

482 TH

Structural and energetic details of the unfolding landscape of Δ+PHS staphylococcal nuclease from high-pressure NMR: effect of cavity creation.

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Because pressure sensitivity originates with a specific and unique property of the folded state, pressure unfolding contrasts with unfolding by temperature or chemical denaturants, which act globally and depend on exposed surface area in the unfolded state. Pressure perturbation coupled with NMR spectroscopy and novel computational approaches will enable unprecedented exploration of the complexities of protein energy landscapes. Unfolding by pressure implies that the molar volume of the unfolded states of proteins is smaller than that of their folded states. This decrease in volume has been proposed to arise from differential density of hydrating and bulk water, pressure dependent changes in bulk water structure, the loss of internal void volume, or some combination of the three. Here we demonstrate, using ten cavity-containing variants of the model protein Δ+PHS Staphylococcal nuclease, that pressure unfolds proteins owing to the presence of cavities in the folded state, thus solving a 100 year old conundrum, we also demonstrate how a combination of high pressure NMR spectroscopy and novel simulations constrained by NMR data can reveal structural and energetic details of the unfolding landscape of a protein with unprecedented detail (1, 2).

The time required to fold proteins usually increases significantly under conditions of high pressure. Taking advantage of this general property of proteins, we combined P-jump experiments with NMR spectroscopy to examine in detail the kinetics of the folding reaction of Δ+PHS staphylococcal nuclease and of some of its cavity-containing variants. The nearly 100 observables per protein that could be measured simultaneously, collectively describe the kinetics of folding as a function of pressure and denaturant concentration with exquisite site-specific resolution.

These studies illustrate the promise of pressure perturbation studies to determine how packing, conformational fluctuations and water penetration regulate essential solution properties of proteins.

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483 MO

NMR characterization of RKIP binding with Raf-1 peptides, locostatin and analogs

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The Raf-1 kinase inhibitory protein (RKIP) is part of the Phosphatidyl Ethanolamine Binding Protein (PEBP) family of proteins is widely distributed, from bacteria to mammals. In mammals, PEBPs have been described to modulate important cell mechanisms, such as the control of heterotrimeric G-proteins, the inhibition of Mitogen-actived protein-kinase . RKIP is the natural Raf-1 kinase inhibitor and is implicated in metastasis formation and Alzheimer's disease.

Since Raf-1 is very difficult to produce, we have use Raf-1 peptide fragments combined with ^{15}N HSQC and ^{31}P NMR to study the importance of the phosphorylation in the interaction with RKIP

Locostatin is the only known ligand of PEBP with an effect on its activity. Locostatin was found in a chemical genetics screen in a cell migration assay, and PEBP identified as the relevant cellular target.

Locostatin is a michael acceptor for which the nuclophilic reactant on the protein is unknown. We have used ^{13}C labelled locostatin and the combination of HCCH tocsy and ^{13}C HSQC to identify the different locostatin addition products.^{15}N labelled protein has been used to identify the locostatin and locostatin analogs binding sites.

484 TU

SIGNALING THROUGH DYNAMIC LINKERS AS REVEALED BY PKA

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PKA is a prototype of multi-domain signaling proteins functioning as allosteric conformational switches. Allosteric transitions have been the subject of extensive structural and dynamic investigations focusing mainly on folded domains. However, the current understanding of the allosteric role of partially unstructured linkers flanking globular domains is limited. Here, using the covariance analysis of NMR chemical shifts [1, 2], we show that a dynamic linker in the regulatory subunit (R) of PKA serves not only as a passive covalent thread, but also as an active allosteric element that controls activation of the kinase subunit (C) by tuning the inhibitory pre-equilibrium of a minimally populated intermediate (*apo* R). A*po* R samples both C-binding competent (inactive) and incompetent (active) conformations within a nearly degenerate free energy landscape and such degeneracy maximally amplifies the response to weak (~2RT), but conformation selective interactions elicited by the linker. Specifically, the R linker that in the R:C complex docks in the active site of C, in *apo* R preferentially interacts with the C-binding incompetent state of the adjacent cAMP-binding domain (CBD). These unanticipated findings imply that the formation of the inter-molecular R:C inhibitory interface occurs at the expense of destabilizing the intra-molecular linker/CBD interactions in R. A direct implication of this model, which was not predictable solely based on protein structure, is that the disruption of a linker/CBD salt bridge in the R:C complex unexpectedly leads to increased affinity of R for C. The linker includes therefore sites of R:C complex frustration and mutations that relieve frustration open new opportunities to design kinase inhibitors with enhanced potency.

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485 TH

STRUCTURAL STUDIES OF THE ANTIMICROBIAL PEPTIDE BREVININ-1BYa AND ITS ANALOGUES

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Cationic antimicrobial peptides (AMP's) are short, amphipathic peptides with a net negative charge that are expressed in all eukaryotic and most prokaryotic organisms. AMP's are of particular interest as a potential source of novel antibiotics due to their broad spectrum of activity.[1] AMP's typically work by interfering with the target membrane. The biggest limitation of these peptides is their cytotoxic activity.[2] Brevinin-1BYa is a 24 amino acid peptide, isolated from the skin secretions of the North American foothill yellow-legged frog Rana boylii, with promising antibacterial activity, including drug resistant strains. Brevinin-1BYa contains some interesting structural features including a conserved Pro^{14}, and a C-terminal disulphide bridge between Cys^{18} and Cys^{24}. A linear C-terminal analogue was developed by replacing the cysteine with serine residues and a dicarba analogue where the cysteine residues were replaced with modified allylglycine residues and the disulphide bridge with a double vinyl bond, and there antibacterial action was compared to the native peptide.[3]
The aim of this study is to investigate the structure, conformation and position of brevinin-1BYa and its analogues in membrane like. The solution structures of the AMP's were investigated by proton NMR spectroscopy and molecular modelling. This study revealed two alpha helical segments separated by a hinge region centred on the conserved Pro^{14} for brevinin-1BYa, and its analogues. The position of the peptides on membrane mimetic media was assessed using paramagnetic relaxation agents.
The effectiveness of AMP's against biofilm formation and preformed biofilms is also a current topic of study, which is aimed towards assessing the true potential of AMP's against the majority of bacterial induced, human infectious diseases.

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Poster Presentation

486 MO

INVESTIGATION OF THE ACTIVE SITE OF NI-CONTAINING SUPEROXIDE DISMUTASE

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Superoxide dismutases (SODs) are metallo-enzymes which catalyze the disproportionation of the superoxide anion (O_2^-) to hydrogen peroxide and molecular oxygen. SODs play an important role in the protection of cells against the toxic products produced during aerobic metabolism.[1] During the last few decades several independent classes of SODs have been identified, containing either dinuclear Cu/Zn or respectively mononuclear Fe, Mn or Ni cofactors.[2] NiSOD is structurally not related to the other known SODs. The coordination sphere is provided by the so called "Ni-hook" motif. Until now, the catalytic mechanism of O_2^- degradation by NiSOD has not been fully enlightened and is controversely discussed. [3] Resembling the Ni-hook structure, metallopeptide-based NiSOD biomimetics match the spectroscopic and functional properties of the native enzyme.[4] However, they also show several differences compared to the enzyme. One of those differences depends on the Leu4-Pro5-peptide bond which is a *cis*-confomer in the native enzyme, but a *trans*-conformer in the metallo-peptide.[5] The aim of our work presented here is to enlighten the structure and dynamics of nickel superoxide dismutase. Focusing on the enzyme's active site, appropriate peptide-based models are synthesized and studied. In both he models and the enzyme, the presence of two cysteines is crucial. Because of the unique role of cysteine in biochemistry, its influence on the properties of the active site is very interesting. In the next steps we will build up models in order to gain further insight into the spatial structure via NMR experiments (ROESY), complementary to results reported for NiSOD models in solid state.[6]

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487 TU

UNRAVELLING THE ALLOSTERIC EFFECTS OF SHP2'S SH2 DOMAINS IN THE JAK/STAT PATHWAY THROUGH NMR RELAXATION EXPERIMENTS

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SHP2 is tyrosine phosphatase involved in the attenuation of the cytokine-induced JAK/STAT signalling pathway. The protein binds through its two SH2 domains to phosphotyrosines located on the intracellular part of the implicated receptors. Although both SH2 domains bind peptides containing phosphorylated tyrosines, they seem to experience different dynamical effects in the activation process: The N-SH2 domain, which blocks directly the active site of the PTP domain through its D-E loop and connected β-strands, experiences a functional allosteric effect which induces it to release the active site when binding to a phosphotyrosine peptide. This dynamic behaviour is not observed for the neighbouring C-terminal SH2 (C-SH2), which links the N-SH2 to the phosphatase domain. Even more, this behaviour is clearly different from other SH2 family members like those involved in activation of kinases like Src and Fyn. To understand the dynamic differences between these SH2 domains our goal is to examine the changes in sidechain methyl dynamics measurements [1] caused by binding phosphorylated peptide related to intracellular part of EpoR and TpoR. Thus far we have fully assigned the bound and unbound forms of C-SH2 [2], and produced NMR ensembles of both forms. Currently we are studying the binding effects through a range of NMR relaxation experiments [3].

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DETERMINATION OF THE STRUCTURE AND DYNAMICS OF AMYLOID BETA (1-42) PEPTIDE IN DIFFERENT AGGREGATED STATES

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Alzheimer's disease is one of the most fatal neurodegenerative diseases. The deposition of Amyloid β fibrils has been suggested to play a central role in this disease: microscopic pictures of brain slices of Alzheimer's disease patients reveal Amyloid plaques, which consists mainly of the Amyloid β peptide. Alzheimer's disease is a protein misfolding disease, which means that it is caused by accumulation of abnormally folded proteins. If so, Amyloid β is converted from a soluble unstructured peptide into a insoluble aggregate, the so called Amyloid β fibril, which consists of highly ordered cross-beta sheets and therefore yield well resolve solid-state NMR spectra, where structural restraints can be derived. Amyloid β can display a variety of fibril forms or polymorphs. Here a condition was found where only one fibril form occurs, which is crucial for the elucidation of the structure. We present solid-state NMR data from the disease relevant Amyloid β (1-42) fibril. For the structural calculation with CYANA different restraints are used and first models can be shown. Additional data obtained with liquid state NMR using hydrogen-bonding constraints from quenched hydrogen deuterium-exchange NMR support the data from solid stat NMR.

Furthermore, the structure of the soluble peptide in a condition when it is in equilibrium with an aggregated state (micelles or oligomers) is studied. In respect to the method of the sample preparation and concentration one can get more monomeric species or aggregates, such as micelles. With higher concentrations STD (saturation transfer difference) is measurable, which shows that there exists an exchange between monomers and aggregates. In these conditions the structure and the dynamics of the peptide based on NOEs is elucidated.

489 MO

SOLUTION STRUCTURE OF THE HIV-1 VIF SOCS-BOX ELONGINBC COMPLEX

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Viral infectivity factor (Vif) is one of nine functional proteins encoded by human immunodeficiency virus. It neutralizes immune response by recruiting E3 ligase to ubiquitinate cellular APOBEC3G and APOBEC3F proteins, which are members of polynucleotide cytidine deaminase family and have anti-virus activity. The SOCS-box is a major functional domain in Vif that recruits ElonginB and ElonginC, two core proteins of E3 ligase complex, and includes a proline-rich motif whose function is still unclear. Here, we report the solution structure of the Vif SOCS-ElonginBC complex. In contrast to other members of the SOCS family, Vif SOCS-box contains only one α-helix domain followed by a β-sheet-like fold. It primarily binds to ElonginC by a hydrophobic interaction in the interface. The proline-rich motif on SOCS mediates another weak interaction with the ElonginB DVMK (residues 101-104) domain and induces folding of ElonginBC terminus, in order to perform further biological functions. The structure of the complex provides a detailed insight into the function of the Vif proline-rich motif, and reveals a new mechanism for Vif-ElonginBC interaction.

Poster Presentation

490 TU

RAMP – STRUCTURAL STUDIES OF A RECEPTOR ACTIVITY MODIFYING PROTEIN

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The family of Receptor Activity–Modifying Protein (RAMPs) consists of three single-transmembrane spanning proteins that facilitate receptor trafficking from the endoplasmic reticulum to the cell surface and alter the ligand specificity of the GPCR Class B receptors calcitonin receptors and calcitonin receptor-like receptors. RAMPs are required for receptors to act as a Calcitonin-gene-related peptide (CGRP) receptors (RAMP1), or adrenomedullin receptors (RAMP2 and 3) and thereby change the selectivity for hormones. In the absence of receptor RAMP1 and RAMP3 have been shown to form stable homodimers. The three RAMP family members share 56% sequence similarity and 30% sequence identity, including a potential dimerisation motif P-X-X-X-X-P within the transmembrane domain. With the goal of understanding the mechanism of dimerisation and the role of GPCR accessory proteins we have determined the monomeric and dimeric structures of the transmembrane domain of RAMP1 in detergent micelles by solution NMR, which provides unique possibilities for understanding the structure and dynamics of the RAMP proteins. Current studies are focussed on the dynamics and the biophysical properties of RAMP1, including the role of the P-X-X-X-X-P motif in the structure and dimerisation. These studies provide insights into how RAMP1 interacts with itself and its cognate receptors and hence help understand the signalling mechanisms of these GPCR accessory proteins.

491 TH

INTERACTION STUDIES OF HUMAN LAFORIN WITH OLIGOSACCHARIDES BY NMR, MST AND ITC

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Laforin is a unique human dual-specificity phosphatase as it contains an amino terminal carbohydrate binding module (CBM) and a carboxyl terminal phosphatase domain containing a HCXXGXXR(S/T) catalytic active site motif (1). Laforin mutations have been associated with Lafora disease, an early onset fatal progressive myoclonus epilepsy with autosomal recessive inheritance (2). The laforin CBM carbohydrate binding activity has been poorly characterized. We describe here our studies of the interaction of recombinant human Laforin (3) with a series of oligosaccharides using a variety of techniques. The Laforin oligosaccharide binding preferences were first screened by Microscale Thermophoresis (MST) using soluble oligosaccharides of increasing length, which has shown an increased preference of laforin for longer oligosaccharides (both linear and circular), with the highest affinity at pH 7.5. The affinity constant of Laforin to linear oligosaccharides decreased systematically from K_d = 2700 mM for maltotriose to K_d = 173 mM , while for the cyclodextrins K_d varied from 841 mM for a-CD to 114 mM for g-CD The Laforin affinity constants obtained for g-CD and maltoheptaose) by Isothermal Titration Calorimetry (ITC) titrations compare favorably with the above results. The thermodynamic parameters determined by ITC were also consistent with other protein-carbohydrate interactions. Finally we assessed ligand binding of g-CD and maltoheptose using STD (4) and CPMG NMR techniques (5). Both compounds were detected unambiguously on the two ligand-based NMR experiments. Although various resonance overlaps are observed for this kind of molecules, a group epitope mapping (GEM) from the STD-NMR experiment discloses the most likely binding profile for these sugars.

Acknowledgments

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Poster Presentation

492 MO

A NOVEL APPROACH TO THE BINDING OF THE TIA-1 PROTEIN TO RNA BY COMBINING SIA AND STD-NMR WITH SPR

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T-cell intracellular antigen-1 (TIA-1) is a key DNA/RNA binding protein that regulates mRNA translation by sequestering them into stress granules (SG) in response to stress conditions (1). It possesses three RNA recognition motifs (RRM) along with a glutamine-rich domain, with the central domains (RRM2 and RRM3) acting as RNA binding platforms. While RRM2 domain is primarily responsible for the RNA interaction, with high affinity for U-rich motifs, the RRM3 contribution to the RNA binding, as well as its targets sequences, are still unknown (2). Here we combine Nuclear Magnetic Resonance (SIA-NMR) and Surface Plasmon Resonance (SPR) techniques to elucidate the sequence specificity of TIA-1 RRM3 (3). With a novel approach using Saturation Transfer Difference NMR (STD-NMR) to study protein-nucleic acids interactions, we demonstrate and quantify RRM3 binds to those oligos enriched with cytosines. In addition, we show that in combination with RRM2, RRM3 significantly enhances the binding to RNA. Our findings provide a new insight into the role of RRM3 in regulating TIA-1 binding to C-rich stretches, that are abundant at the 5' TOPs (5' Terminal Oligopyrimidine Tracts) of mRNAs whose translation is repressed under stress situations (4).

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493 TU

SYNERGISTIC APPLICATION OF NMR SPECTROSCOPY AND X-RAY CRYSTALLOGRAPHY IN THE DRUG DISCOVERY OF THERAPEUTIC ANTIBODIES TARGETING INTERLEUKIN

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Antibodies form an emerging class of therapeutics that is present in the portfolio of drugs today. Whereas NMR contributions in our drug discovery pipeline of small molecules mainly focus on fragment-based screening or the validation and characterization of small molecules that bind to biologically relevant target proteins, contributions of NMR for antibody drug discovery have potential as shown by the present investigation. The interaction between one of our antibodies and the cytokine interleukin (IL) was investigated by NMR spectroscopy and X-ray crystallography. A sample of a complex of the Fab and IL was crystallized and different crystal forms were analyzed. Interestingly, two different complexes were observed. In the first, complex I, one Fab interacts with one molecule of IL (1:1 complex). In complex II, two Fabs interact with a dimeric IL using a binding epitope that comprises both sides of IL (2:2 complex). It was difficult to decide on the basis of X-ray analysis alone which complex represents the biologically relevant one. By NMR diffusion experiments of the complex, it could be shown that the 2:2 complex was predominantly present in solution. After resonance assignment of IL, the binding epitope of the Fab was mapped on IL and it was found to be in agreement with the structure of complex II. Subsequently, mutants of the Fab were designed on the basis of the crystal structure of complex II to weaken the interface with the second IL molecule. These Fab mutants indeed formed a significantly weaker dimeric complex with the cytokine as measured by NMR diffusion experiments.

Poster Presentation

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CONFORMATION AND DYNAMICS OF A 70 kDa MEMBRANE-PROTEIN–CHAPERONE COMPLEX

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The biosynthesis of integral outer membrane proteins (Omps) in Gram-negative bacteria relies on a chain of molecular chaperones that convey the hydrophobic Omp polypeptides in an unfolded form to the outer membrane. The trimeric 51 kDa protein Skp is one such transport chaperone[1,2], with a broad substrate range of more than 15 different Omps[3]. Skp binds its substrates with nanomolar affinity, but being periplasmic, it can not rely on cellular energy to release them. It is so far not understood in which conformation the substrates bind to Skp and how the periplasmic Skp fulfills its biological function of substrate holding and release.
Here, we address these questions by solution NMR spectroscopy. Based on complete sequence-specific resonance assignments, we use reduced spectral density mapping to characterize the backbone dynamics of the 70 kDa Skp–Omp. The Skp trimer provides a flexible architectural scaffold that rigidifies upon binding of the Omp substrate. The chaperone-bound Omps populate an equilibrium of rapidly interconverting backbone conformations, with exchange rate constants larger than 1 ms^{-1}. A combination of inter- and intramolecular PRE and NOE experiments was used to determine the spatial compaction of the Skp-bound substrate ensembles and to localize them within the chaperone cavity. Skp binds these Omp polypeptide ensembles with a global lifetime that is seven orders of magnitude longer than any of the local interaction lifetimes involved, showing that the high global affinity is added up by avidity from multiple local weak interactions. The unfolded Omp polypeptide is maintained stably in a high-entropy state, from which it can rapidly release and subsequently fold without external energy. Overall, our data reveal a novel conformational state for proteins, the “fluid globule” and the functional basis for substrate transport and fast release during Omp biosynthesis.

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495 MO

JUST DISORDERED LINKERS OR IMPORTANT INTERACTION SITES?
IDR in CBP/p300

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Single point mutations within intrinsically disordered domains of CBP (CREB binding protein) and its homologue p300 have been related with lung and cervical cancer development. Other critical diseases, such as acute myeloid carcinoma or the Rubinstein-Taybi syndrome are caused by mutations or chromosomal translocations affecting the corresponding genes. CBP/p300 are multi-domain transcriptional regulators involved in essential cellular functions such as growth, differentiation, apoptosis and DNA repair. Both co-activators, CBP and p300, have been intensely studied regarding their highly conserved structured domains. Interaction with multiple transcription factors is mediated by one or more of the several globular domains of CBP/p300, among them zinc finger motifs, the CREB-binding (KIX) domain, bromodomain and histone acetyl transferase (HAT) domain. However, little is known about the intrinsically disordered regions which link the structured domains. After analysing several related amino acid sequences of CBP/p300 with various bioinformatics tools focusing on conservation, functional divergence and linear motif scanning, we selected two promising disordered domains, ID3 and ID5, located in different regions of the proteins to study their dynamic behaviour in a comparative approach by solution NMR spectroscopy. While the acidic ID3 is a ~400 amino acid long disordered linker domain showing only a small number of conserved sequences of charged and hydrophobic amino acids, the C-terminal ID5 (~300 amino acids) carries positive net charge and harbours several short motifs highly conserved in both homologues, as well as some motifs conserved in CBP or p300 only. We used, ^{13}C-^{15}N labeled samples have been used for assignment of both domains, and, in the case of ID3, we employed ^{13}C direct detected experiments for better resolution of resonances. Additionally, several recombinant constructs of the ID3 and ID5 of CBP/p300 were employed in ^{15}N-relaxation studies to obtain information about the backbone dynamics of the conserved motifs in these domains. The identification of motifs structurally less flexible than their disordered surroundings provides information about putative functional interaction sites and may assist in the discovery of specific interaction partners and the characterization of interactions.

Poster Presentation

496 TU

LIQUID-STATE NMR INVESTIGATION OF MEMBRANE-ANCHORED FULL-LENGTH NS5A OF HEPATITIS C VIRUS

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Hepatitis C virus (HCV) infected over 170 million people worldwide and current medical treatments show limited efficiency. Recent high-throughput pharmacological inhibitor screening recognized HCV non-structural protein 5A (NS5A) as a promising therapeutic target for the treatment of HCV. NS5A consists of three domains which are essential for viral RNA replication, interferon resistance, and host cell apoptosis. A better understanding of the structural organization of this protein is required to develop a more effective therapy. However, until now structural work on NS5A has focused on individual domains: the N-terminal amphipathic helix, the well-folded domain 1, and the intrinsically disordered domains 2 and 3. Little is known about the synergetic action among the three domains due to the difficulty of expression and purification of full-length NS5A in a membrane-anchored form. Here we present our recent progress towards an atomic-level structural characterization of full-length NS5A in a membrane mimic environment by liquid-state NMR spectroscopy. The full-length protein has been successfully expressed and isotope-labelled in E. coli, and purified. An intensive detergent screen was performed to dissolve this protein. The commonly used detergents show a good solubility for the full-length protein, but interact unspecifically with peptide regions in the intrinsically disordered D2 and D3 domains. Neutral amphipols were found to be able to dissolve full-length NS5A without significant unspecific interaction. Despite the high molecular weight of full-length NS5A in detergent micelle of more than 80 kDa, we obtain high-quality NMR spectra that allowed sequence-specific resonance assignment. The visible correlation peaks correspond to domains D2 and D3 that remain highly disordered also in full-length membrane-anchored NS5A. Comparison of chemical shifts and 15N relaxation data between full-length NS5A and an isolated D2-D3 construct yields information on changes in the structural ensemble, and inter-domain interactions. Having a NMR-amenable model system of membrane-anchored full-length NS5A in hand, we will now be able to explore the interaction of NS5A with other proteins or drugs, in order to better understand its versatile function in viral replication and particle assembly.

497 TH

ULTRA-SENSITIVE FUNCTIONAL SWITCH MEDIATED BY POLY-PHOSPHORYLATION TO THE INTRINSICALLY DISORDERED PART IN CHROMATIN REMODELER FACT

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FACT (FAcilitate Chromatin Transcription) is a heterodimer of Spt16 and SSRP1 subunits, which remodels nucleosome structure to facilitate transcription in the context of chromatin. Spt16 roles as the histone binder, while SSRP1 binds to DNA. SSRP1 contains two intrinsically disordered (ID) regions and HMG domain. The ID parts are named as acidic and basic IDs according to their biased amino acid compositions. We found the AID (acidic ID) part is phosphorylated in cells, and the fully phosphorylated AID prohibited SSRP1 binding to nucleosome). The AID in SSRP1 has 10 phosphorylation sites, and the binding ability of SSRP1 reduced in a sigmoidal manner according to the extent of phosphorylation: it is an example of the ultra-sensitive switch).

In this work, we explored how the poly-phosphorylation to AID achieves the ultra-sensitivity in the SSRP1 binding to nucleosomal DNA, by using NMR, ITC and coarse-grained model analyses). NMR titration experiments showed that the phosphorylation to AID expanded its potential contact area with the following BID and HMG, which are the DNA binding parts. This was confirmed by the ITC experiments. The poly-phosphorylation to AID, therefore, increases the intramolecular encounter probability of the AID against the DNA binding surface of the BID-HMG segment; the contact interferes in the DNA binding. Numerical analyses using the 'poly-electrostatic model' demonstrated that the dynamic interactions between AID and BID enables the ultra-sensitivity in the DNA binding ability according to the phosphorylation extent. The ultra-sensitive switch by the poly-phosphorylation was analysed by the coarse-grained model, which gave further insights into the functional significance associated with transient contacts between the ID segments in SSRP1.

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Poster Presentation

498 MO

PROTEIN STRUCTURE DETERMINATION FROM A SET OF NON UNIFORMLY SAMPLED DIAGONAL-FREE 4D-NOESY SPECTRA

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Modern multidimensional NMR spectroscopy allows successful determination of double, or triple labeled protein 3D structures with molecular weights of up to 50 kDa. Typically determination of protein structure can be split into two main parts: sequence specific assignment of chemical shifts, and extraction of distances between proton pairs from NOESY spectra. Precision of the determined structures depends mainly on number of observed NOEs and their proper assignment. The main difficulty with assignment of NOEs is their ambiguity. Chemical shift values are derived from NMR measurements with certain level of uncertainty. A more severe problem arises due to frequent chemical shift degeneracy in proteins. It was shown that in larger systems less than 10% of NOE cross peaks can be assigned unambiguously. Currently, for protein structure determination, most commonly applied are standard three dimensional NOESY methods. Recent advances in NMR spectroscopy, especially introduction of non-uniform sampling, allows measurement of high dimensional NMR spectra in feasible experimental time, what significantly decreases problem of ambiguous assignment of NOE contacts. So far 4D NUS NOESY spectra were used solely to help with the protein structure determination from 3D techniques - herein we show that application of a set of 4D diagonal-free NUS NOESY experiments for determination of E32Q mutant human S100A1 protein structure leads to significant improvement in precision of determined structure. The fact that high dimensional NOESY spectra perform so well for medium size protein like S100A1 strongly suggest that they may prove crucial for determination of high quality structures in larger systems.

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499 TU

FIRST STEPS TOWARDS STRUCTURAL CHARACTERIZATION OF THE ABC TRANSPORTER BMRA FROM *BACILLUS SUBTILIS* BY SOLID-STATE NMR SPECTROSCOPY

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Here we report first steps towards structural studies of the bacterial ATP-binding cassette transporter BmrA, including protein overproduction, stable isotope labeling, reconstitution into a lipid environment and first two-dimensional solid-state NMR spectra. The spectra show good sensitivity, narrow lines and a dispersion typical for a well-folded protein.

ABC transporters are ubiquitous membrane proteins that couple the transport of a wide variety of substrates across biological membranes. Several members of this protein family are in involved in human diseases like adrenoleukodystrophy, cystic fibrosis or multidrug-resistance in cancer. For modulation of their activity in order to increase therapeutic efficiency a detailed structural understanding at an atomic level is necessary.

BmrA from *Bacillus subtilis* is a homologue of the human P-glycoprotein that is involved in multidrug resistance. The homodimeric drug exporter of 130 kDa was chosen as a model system because it can be overproduced in large quantities and allows stable isotope labeling. The aim is to investigate structural changes during substrate binding and translocation by solid-state NMR techniques.

^{13}C,^{15}N-BmrA could be reconstituted in a lipid environment. Given that structure and function of membrane proteins are highly dependent on the lipid composition we prepared for reconstitution a lipid extract from *Bacillus subtilis*. The lipid bilayer and BmrA without ligands form tube-like objects as observed before by electron microscopy (Chami *et al*. (2002). These are however no prerequisite for resolved NMR spectra, as further isotope labeled and reconstituted BmrA prepared in a drug-bound and in a post-hydrolytic transition state yield a comparable quality of spectra, but irregular structures as observed by EM.

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500 TH

STRUCTURAL STUDIES OF STREPTOCOCCAL M PROTEIN – WHAT CAN WE LEARN FROM NMR AND EPR?

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Streptococcus pyogenes is a major human pathogen causing about half a million deaths per year worldwide. Severe streptococcal immune sequelae such as acute rheumatic fever constitute a global health burden. M proteins, located on the bacterial surface and involved in binding to a wide range of host proteins, are a key determinant of streptococcal virulence. Very little is known about M protein structure and functional mechanisms, as the proteins are difficult targets for conventional structural biology and biochemical approaches. M proteins are generally believed to form elongated, parallel coiled-coil dimers. The N-terminal hypervariable regions (HVR, 50-100 residues) are of particular interest as they harbour several binding sites for host proteins including components of the complement system and collagen. No crystal structure of a full-length M protein has been solved. Due to the high molecular weight and anisotropy full-length M protein dimers are also not amenable to NMR.
We are using a combination of NMR and EPR to map the in-solution conformation of the HVR of the M3 protein. The native M3 protein does not contain cysteines, making it an ideal target for EPR using spin-labelled cysteine-containing mutants. A number of these mutants have been produced, with spin labels introduced in regions predicted to adopt or to lack coiled coil topology. PELDOR experiments in both X-band and HiPER with MTSL- or RX-labelled protein have resulted in distance information for a number of residues in homodimeric and heterodimeric samples and revealed an unexpected folded structure of the M3 HVR dimer. This informed the design of a short construct of the M3 protein (1-110C), which forms stable disulfide-linked dimers and is suitable for NMR. ^{1}H, ^{15}N-HSQC spectra indicate the HVR forms a folded structure containing disordered regions that is distinct from, and more complex than, a dimeric coiled coil. Long-range information derived from PELDOR and short-range restraints obtainable by NMR are a powerful combination that will allow structure determination of M proteins and analyses of their multifarious interactions.

501 MO

ATOMIC-LEVEL STRUCTURE ELUCIDATION OF AN ULTRAFAST FOLDING MINI-PROTEIN DENATURED STATE

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In this contribution, we present experimental data on the 6 M urea-unfolded state of the TC5b molecule, a small mini-protein sequence which exhibits a well-defined globular fold together with pronounced secondary and tertiary interactions present in its native state. Also, TC5b is known to fold on a submillisecond timescale which, together with its small size, has made it an attractive testing ground for computational folding studies and biomolecular dynamics simulations. By employing a combination of both multidimensional NMR spectroscopy together with heteronuclear relaxation experiments performed on the 6 M urea-denatured state of both TC5b and, in addition, a structurally optimized point mutant, we were able to highlight the importance of both native and non-native interactions for ultrafast and productive refolding of this mini-protein – a feature of the peptide's unfolded state whose significance for the direction of structure formation remained largely unclear until recently. Among other things, we detect unambiguously direct NOE cross-peaks between Trp 6 and several aliphatic amino acid side chains, i.e. Ile 4, Gln 5, Ile 7, Pro 12, and Arg 16, that exhibit both native and, more surprisingly, also non-native character employing relatively short mixing times. Moreover, we show experimentally that a mutationally induced enhancement of the nucleation site's hydrophobicity leads to the detection of not only additional non-random interactions but also, and more importantly, a concomitant acceleration of the refolding rate constant. To further rationalize these NOE-based results, the data is complemented with the determination of heteronuclear relaxation rate constants, additional het. NOE measurements and a subsequent reduced spectral density mapping, performed on the denatured states of TC5b and the point mutant thereby providing a distance-independent source of structural information.
In summary, our observations further emphasize the importance of pre-existing hydrophobic interactions involving sequence-remote side chains as a crucial prerequisite for fast folding kinetics and increased thermodynamic stability of the native state structure. It is believed that these results will productively contribute to the ongoing discussion of how only a few sequence determinants can direct the entire folding pathway of globular proteins starting from the very early stages of structure formation.

502 TU

INSIGHTS INTO THE STRUCTURE AND FUNCTION OF A LYTIC POLYSACCHARIDE MONOOXYGENASE BY NMR SPECTROSCOPY

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Lytic polysaccharide monooxygenases (LPMO) previously classified as carbohydrate binding module family 33 (CBM33) and glycoside hydrolase family 61 (GH61) and currently classified as Accessory Activities AA9 and AA10 [1], are likely to play central roles in future biorefining [2]. Still, the molecular basis of their unprecedented metal-dependent catalytic activity remains largely unknown. Here we have used NMR and ITC to study chitin binding protein 21 (CBP21), a chitin-active CBM33/AA10 [3]. NMR dynamic data showed that CBP21 is a compact and rigid molecule, the only exception being the catalytic site. NMR data further showed that H28 and H114 in the catalytic site bind a variety of divalent metal ions with a clear preference for Cu^{2+} (Kd = 55 nM; from ITC) and even better Cu^{1+} (Kd ≈1 nM; from an experimentally determined redox potential for CBP21-Cu^{2+} of 275 mV using a thermodynamic cycle). The higher affinity for Cu^{1+} was also reflected in a reduction in the pK_a values of the histidines by ~3.6 and ~2.2 pH units, respectively. Cyanide, a mimic of molecular oxygen, was found to bind to the metal ion only. These data support a model where copper is reduced on the enzyme by an externally provided electron, followed by oxygen binding and activation by internal electron transfer. Interactions of CBP21 with a crystalline substrate were mapped in a $^2H/^1H$ exchange experiment, which showed that substrate binding involves an extended planar binding surface, including the metal binding site. Such a planar catalytic surface seems well-suited to interact with crystalline substrates.

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503 TH

SHORT-RANGE AND LONG-RANGE INTERACTIONS OF THE INTRINSICALLY DISORDERED REGION IN THE TRANSCRIPTION FACTOR ENGRAILED 2

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Engrailed 2 is a transcription factor that possesses a well-folded homeodomain and a long, 200-residue, mostly unstructured N-terminal region. The role of the disordered region in the function of the protein is only partially known and, in particular, its importance for the modulation of DNA binding is still unclear.

Here we use nitrogen-15 relaxation and paramagnetic relaxation enhancements (PRE) to study the conformational space of the last 50 residues of the disordered N-terminal extension.

Nitogen-15 NMR relaxation measurements were carried out (auto- and cross-correlated relaxation) at four magnetic fields ranging from 9.4 to 23.5 T (400 MHz to 1 GHz). They reveal crucial structural and dynamical properties, in particular of the disordered N-terminal region, where a hydrophobic cluster constitutes a known interaction site with other transcription factors.

A series of mutants has been designed to introduce paramagnetic probes in several sites of the unstructured N-terminal region and at the surface of the well-folded homeodomain. Our PRE data reveal the presence of long-range interactions between the disordered region and the homeodomain, covering partially the DNA binding surface.

These long-range contacts suggest the possible extended role of the disordered region, which not only acts as an interaction site with other proteins, but may also modulate DNA binding in the absence of targeting proteins at its specific DNA target site.

Poster Presentation

504 MO

STRUCTURE OF THE BASEPLATE ANTENNA-COMPLEX OF *CHLOROBACULUM TEPIDUM* IN ITS FULL ORGANELLE NATIVE HETEROGENEOUS ENVIRONMENT

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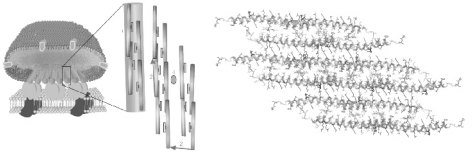
The photoreceptor complex of *Chlorobaculum tepidum is an* organelle antenna system, which is one of the most efficient for converting light into energy. This complex contains the protein CsmA forming an organized 2d crystal with the BChl *a* ligand, together with thousands of different carotenoids surrounded by a lipid monolayer. Very little is known about the precise three-dimensional organization of the different components of the system, since it is intrinsically very difficult to study such heterogeneous systems at high resolution. Here we use state-of-the art developed data analysis protocols to combine complementary information from solid state NMR providing atomic resolution local structural information with the global structural information from cryo-EM and with CD-spectroscopy to constrain the orientation of the BChl *a* pigments. The derived structure of the baseplate complex reveals rod-like structures composed by monomers of CsmA forming an amphipathic helix stacking head-to-tail and forming rods clustering the hydrophobic side of the helices in two-fold rotational symmetry around the BChl *a* ligand coordinated to the Histidine side-chain. The rod-like structures are associated co-aligned with side-by-side interaction through oppositely charged side-chains of the hydrophilic side of the helices. Our results reveal how the Bchl *a* ligand plays an integral role in the self-assembly of CsmA and our structure forms the basis for a hypothesis for the mechanism of energy transfer in the antenna system and possibly the design of artificial biological solar cells.



505 TU

STRUCTURAL STUDIES OF EIF4E-4EGI1-M7GDP COMPLEX

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EIF4E/eIF4G complex has an important role in eukariotic translation initiation. mRNA's transcribed from DNA are transported out of the nucleus for translation. mRNA's contain a modified ribonucleotide i.e. m7GDP referred to as mRNA cap. EIF4E is responsible for binding to the cap nucleotide, facilitating the translation initiation through interactions with eIF4G which is regulated by 4E-BPs and finally lead mRNA's to the ribosome. EIF4E's function is especially critical for long mRNA molecule's which have been evolved to encode many important hormone and cell proliferation factors. Because the same cell proliferation factors are also involved in cancer genesis it has been proposed that disruption of eIF4E activity might be therapeutic for patients.

4EGI-1 is a small molecule mimicking of eIF4E-4E-BP1 interaction. It has been found as the best hit by fluorescence screening of Chembridge Diver-Set E chemical library containing 16,000 of compounds. The activity of 4EGI1 has been further verified against human cancer cell cultures and in two different mouse models of human breast cancer.

Our aim is to purify eIF4E human variant and to co-crystallize it with 4EGI1. We want to locate the binding pocket and clarify the interaction mode of 4EGI1 to eIF4E. Preliminary studies by liquid state NMR have indicated the putative binding site. Unravelling of the exact binding mode will help us to design more efficient small molecule inhibitors of eIF4E-eIF4G assembly which can serve as anti tumour proliferation drugs. We are also collaborating with chemists in order to synthesize derivatives of 4EGI1 based on our findings and ideas of how to improve the drug activity. Furthermore we are planning to expand the cancer cell cultures and mouse model tests in other types of cancer as well.

Poster Presentation

506 TH

MAPPING THE CO-TRANSLATIONAL FOLDING ENERGY LANDSCAPE OF THE DDFLN5 IMMUNOGLOBULIN DOMAIN

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The process by which a nascent polypeptide acquires its native folded structure on emerging from the ribosomal exit tunnel, while avoiding the population of misfolded or aggregation-prone states, is of crucial biological significance. Within our group the ddFln5 immunoglobulin domain, from the *Dictyostelium discoideum* tandem repeat protein ABP-120, has been developed over several years as a model for protein folding as a ribosome-nascent chain complex, observed by NMR spectroscopy (1).

In this work, we have created an *in vitro* model of co-translational protein folding by preparing a series of C-terminal truncations of ddFln5, to mimic the progressive emergence of the nascent chain from the ribosome. Using NMR spectroscopy together with other biophysical methods we have characterised the emergence of folded structure from the unfolded state through a series of intermediate and increasingly native-like states. Using a combination of site-directed mutagenesis, EXSY and CPMG relaxation dispersion measurements these intermediates are related to the isomerisation of a highly conserved native state cis-proline residue, and to the formation of long-range contacts within the protein. The detailed structural and energetic description of the co-translational folding landscape that we create in this manner provides a high-resolution reference against which the effects of ribosome attachment on the folding of ribosome-associated nascent chains may potentially be discerned.

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507 MO

HIGHLY TUMOUR-SELECTIVE PROTEIN-FATTY ACID NANOPARTICLES: NMR STUDIES DESCRIBING THAT ITS REMARKABLE PROPERTIES ARE DUE TO CONFORMATIONAL MALLEABILITY AND WEAK LIGAND-BINDING AFFINITIES

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HAMLET (**H**uman **A**lpha-lactalbumin **M**ade **LE**thal to **T**umour cells) and its related partially unfolded protein–fatty acid complexes are novel biomolecular nanoparticles that possess relatively selective cytotoxic activities towards tumour cells, while leaving healthy differentiated cells intact. While progress in cell biology, transcriptomics, proteomics, imaging, *in vivo* studies, etc have provided clues to the biological mechanism(s) of cell death brought about by HAMLET and other related complexes[1], from a reductionists' / structural biologists' point of view, we have chosen to ask what would be the 'minimal cytotoxic unit' to give rise to this remarkable property[2]. Using diffusion, chemical shift perturbation, and temperature-dependent NMR measurements, It is now well known that one of the key characteristics is that the protein must be in the partially unfolded state to manifest this property, and furthermore that there is no requirement for the protein to recover to it native state[2]. These results show that it is possible to endow native proteins with additional, independent functions through the “alternatively folded states”.
Furthermore, our work has confirmed previous suggestions that the fatty acid moiety may be the ultimate cytotoxic agent, and that the protein moiety simply serves as carrier (or 'mule') by increasing its effective critical micelle concentration[3] (Nielsen SB et al, *J Mol Biol* 398: 351–361, 2010). We also show that the partially unfolded property of the protein as well as the nature of fatty acid binding is as much as important in determining the cytotoxicity – in other words, there is a delicate balance of structural malleability and related changes in binding affinities that determine the tumoricidal properties. Any efforts to design small-molecule mimics appear to require a better understanding of these structural aspects.

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Poster Presentation

508 TU

STRUCTURAL ANALYSES OF HIV-1 REVERSE TRANSCRIPTASE INDUCED BY NON-NUCLEOSIDE INHIBITORS, BASED ON NMR STUDIES AND MOLECULAR DYNAMICS SIMULATIONS

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Non-nucleoside reverse transcriptase (RT) inhibitors (NNRTIs) have become an inherent component in the treatment of HIV-1 infections. One of the most serious problems associated with NNRTIs is that the virus exhibits resistance to the drug through mutation. New inhibitors effective against these mutants and resistant to new mutations are needed in the treatment of HIV-1 infection. In the present study, the ^{1}H-^{13}C HSQC experiments of [methyl-^{13}C]methionine-labelled RT and computational approaches have been used to study the interaction between the NNRTI and RT and the dynamics of RT with and without a bound NNRTIs to help understand the mechanism of inhibition and NNRTI resistance. The HSQC spectra of RT with NNRTI compounds (nevirapine, efavirenz, rilpivirine, NA14 and NA15) were successfully obtained. In the presence of the nevirapine, both the M184 and M230 peaks were significantly perturbed upon binding of the inhibitor, indicating that conformational changes in the active site were induced by indirect interactions of nevirapine. In addition, the spectral changes of RT with efavirenz, NA14 and NA15 showed very similar mode of binding to nevirapine, while the rilpivirine was different. Importantly, the rilpivirine has an ability to bind and inhibit wild type RT and a number of clinically relevant NNRTI-resistant mutants. This ability derives from the strategic flexibility of the compound. Therefore, the focus for the development of next generation NNRTIs has to be the design of compounds with an improved resistance profile like rilpivirine. Furthermore, molecular dynamics simulations of the HIV-1 RT complexes to NNRTIs will be confirmed their interactions and mechanism. These experimental studies provide information for elucidation of the dynamic interactions of amino acid residues reside in the binding pocket, which will give a useful knowledge for further drug developments.

509 TH

INVESTIGATION OF THE INTRINSICALLY DISORDERED REGIONS OF THE RESPIRATORY SYNCYTIAL VIRUS P PROTEIN BY NMR

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The phosphoprotein P of human Respiratory syncytial virus (hRSV) is an essential co-factor of the polymerase during replication and transcription. As compared to other Mononegavirales P proteins, hRSV P is rather short and does not comprise domains with stable tertiary fold outside the central trypsin-resistant tetramerization domain, that most probably adopt a coiled-coil conformation (Llorente, 2006 & 2008). Although the regions at the N- and C-termini of the oligomerization domain are predicted to be mainly intrinsically disordered, alpha-helical propensity was suggested in both (Simabucco, 2011). Here we took advantage of NMR as a unique tool to investigate intrinsically disordered proteins and regions (IDRs) to analyze the secondary structure propensities of the N- and C-terminal regions of hRSV P, using backbone chemical shift analysis and 15N relaxation experiments. Data were measured with full-length P as well as several truncated forms, with deletions at the N- or C-termini. Our results provide experimental evidence that the latter are indeed IDRs. Nevertheless we also mapped regions at the N-terminus of the protein and downstream of the tetramerization domain, which clearly exhibit alpha-helical propensity. In contrast, the 10 residue long C-terminal stretch that was shown to bind to ribonucleoprotein complexes (Tran, 2007) appears to completely unfolded. The identified regions are likely additional sites of molecular recognition for other components of the hRSV polymerase complex and more particularly for the nucleoprotein, that could display different binding modes corresponding to the N°-chaperone or decapsidation functions of hRSV P.

510 MO

NMR- AND CD-BASED INTERACTION STUDIES SUGGEST A GENERAL ROLE FOR THE FATC DOMAIN AS MEMBRANE ANCHOR OF PHOSPHATIDYL-INOSITOL-3 KINASE-RELATED KINASES (PIKKS)

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Phosphatidylinositol-3 kinase-related kinases (PIKKs) regulate cellular processes such DNA repair, RNA surveillance, and cell growth and metabolism. All family members share a similar domain structure including besides a kinase domain a FAT and a C-terminal FATC domain. Various mutagenesis studies have shown that the FATC domain is important for the regulation of all PIKKs. Based on the structural characterization of the redox- and lipid-binding properties of the FATC domain of the ser/thr kinase target of rapamycin (TOR), it contains a redox-sensitive membrane anchor in its C-terminus (1, 2). Since the C-terminal regions of the FATC domains of all known PIKKs are rather hydrophobic and especially rich in aromatic residues, we analyzed if the ability to interact with lipids and membranes may be a general property. For some of the FATC domains we did this by using a newly established procedure (3). Here, we present NMR- and CD-data for the FATC domains of human SMG1, human ATM, human ATR, human TRRAP, and human DNA-PKcs that indicate that all can interact with different membrane-mimetics and only may have different preferences for membrane properties such as surface charge, curvature, and lipid packing (4). Except for the oxidized form of the TOR FATC that forms an -helix that is followed by a disulfide-bonded loop (1), the FATC domains of the other PIKKs are mostly unstructured in the isolated form and only significantly populate -helical secondary structure upon interacting with membrane-mimetics.

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511 TU

CPP-BASED CARRIERS FOR NUCLEIC ACID DELIVERY. STRUCTURAL INVESTIGATIONS BY SOLID-STATE NMR SPECTROSCOPY

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Small interfering RNA therapeutics has arisen in the early 2000's as promising remedy to fight many diseases including some that have escaped conventional methods (like hereditary diseases), and has also opened new horizons in cancer therapy. However, the intracellular delivery of these highly charged siRNA across the cellular barriers remains a hurdle to overcome. Carriers based on cell penetrating peptides are considered promising candidates, but the delivery of cargo by these peptides is complex, involving many steps and remains relatively inefficient when compared to the requirements for bio-medical applications.

In the current project we are studying in detail the interactions of designed peptides LAH4/LAH4L1 that exhibit potent DNA and siRNA transfection capabilities[1], with model membranes and nucleic acids. By dissecting the membrane translocation process into individual steps and investigating each step in thermodynamic and structural detail we are able to better evaluate the optimal characteristics for complex formation, membrane interactions and transport of those non-covalent nanoparticles. ssNMR, circular dichroism and isothermal titration calorimetry (ITC) are the methods of choice. On the one hand ITC reveals the thermodynamic parameters of pH- and concentration-dependent interactions of the peptide with lipid bilayers and short RNA sequences (as small interfering RNA) itself, and subsequently helped us to better design our NMR experiments.

On the other hand NMR measurements on peptide-in-model-membrane provides information about the peptide topology in lipid bilayers (^{15}N-labeled peptide in oriented samples) and the lipid acyl chain perturbation by the peptides. Furthermore, MAS solid-state NMR spectroscopy allows us to investigate the structural features of CPP - nucleic acid complexes. Intermolecular distances between the LAH4 peptides and nucleic acid residues were assessed by *Rotational Echo Double Resonance* (REDOR) solid-state NMR in order to confirm the mode of interaction earlier proposed in our team[2]. Some additional techniques were used to control the experimental conditions, and to assure their reproducibility, such as dynamic light scattering for tracking transfection complex size under various conditions; and agarose gel electrophoresis also for complex molecular ratio and size control.

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512 TH

SOLUTION STRUCTURE AND TRANSIENT DNA BINDING OF THE BACTERIAL ANTITOXIN MazE

Evelyne Schrank, Richard Fröhlich, Michal Respondek, Christoph Göbl, Lieven Buts, Remy Loris, Klaus Zangger*Institute of Chemistry, University of Graz*Chromosomal toxin-antitoxin (TA) systems play a crucial role in regulating the growth and possibly cell death of bacteria. The *Escherichia coli mazEF* system, consisting of the toxin MazF and its less stable antitoxin MazE were one of the first TA systems studied [1]. It is autoregulated by protein binding downstream of its promoter region.

Here we present an NMR investigation into the solution structure, dynamics and DNA binding of *E. coli* MazE. In solution MazE forms an N-terminal homodimeric AbrB-like motif like the crystal-structure of MazE in complex with a camel antibody [2]. The C-terminal region is highly flexible and is not involved in DNA binding. Using chemical shift mapping the strongest binding was observed for a palindromic region about 20 bases downstream of the *mazE* ORF. Weaker binding was observed for any double-stranded DNA tested from extended regions of the *mazEF* promoter.

The orientation of the DNA on MazE was studied using oligomers paramagnetically labeled with a proxyl tag bound to a PTO-modified nucleotide. Attachment of the spin label on both ends of the DNA yielded the same PREs, which is indicative of a hopping of the protein on the DNA, showing no preferred conformation.

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513 MO

NMR STUDY OF THE HEPATITIS C VIRUS E1 GLYCOPROTEIN TRANSMEMBRANE DOMAIN

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Oligomerization of hepatitis C viral envelope glycoproteins E1 and E2 is essential to virus fusion and assembly. Although their transmembrane domains (TMs) play an important role in the biogenesis of E1/E2 heterodimer, there is little structural information on this aspect of the entry mechanism. Our long-term goal is to investigate the interaction between E1-TM and E2-TM using structural biomolecular NMR methods. We have focused on expression, purification and characterization of the E1-TM domain. Membrane peptides are notoriously difficult to synthesize, requiring us to establish a MBP-fusion expression system which yields sufficient quantities of pure E1-TM, and to optimize peptide purification by HPLC, affording E1-TM suitable for NMR studies. The E1-TM structure was studied in two membrane-mimicking environments, SDS- and LPPG-micelles. Secondary chemical shifts, relaxation measurements and solvent exchange rates provided information on the secondary structure and global fold of E1-TM. In both micelles E1-TM adopts a kinked helical conformation, with helical stretches at residues 354-363 and 371-380 separated by a more flexible segment of residues 364-369. Replacement of key positively charged residue K370 with an alanine did not effect the secondary structure of E1-TM but did change the relative positioning within the micelle of the two helices. These results lay the foundation for structure determination of E1-TM and a molecular understanding of how E1-TM flexibility enhances its interaction with E2-TM during heterodimerization and membrane fusion.

Poster Presentation

514 TU

STRUCTURAL AND FUNCTIONAL INVESTIGATION OF THE PHOSPHOPANTETHEINYLATION REACTION IN NONRIBOSOMAL PEPTIDE SYNTHETASES FROM *BACILLUS*

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Carrier proteins play a crucial role in the metabolism of every organism. The topology of a four helix bundle is universal among the carrier proteins of fatty acid and polyketide synthases (FAS/PKS) as well as nonribosomal peptide synthetases (NRPS). Shuttling of substrates and intermediates (e.g. ketone bodies, growing fatty acid or peptide chains) by the carrier protein is facilitated by a phosphopantetheine cofactor attached to a conserved serine side chain making the phosphopantetheinylation a basic prerequisite for their proper function.
Two groups of phosphopantetheine transferases (PPTs) catalysing the transfer of the cofactor derived from coenzyme A are known. Group I PPTs consist of a single domain and are active as homotrimers. In PPTs from group II two similar domains are linked intramolecular and the enzyme is active in its monomeric state.
Previous work on a peptidyl carrier protein from *Bacillus* suggested that it undergoes a massive conformational change during its interaction with the group II PPT Sfp. Combining liquid state NMR and crystallization experiments we could show that a conformational change seems to play no role in the carrier protein/transferase interaction. Analysis of carrier protein mutants by isothermal calorimetry as well as a transfer assay revealed residues important for the productive interaction. Astonishingly the mode of interaction is conserved from the *Bacillus* NRPS to the human FAS carrier protein/transferase complex.

515 TH

STRUCTURAL INVESTIGATION INTO PEPTIDE INTERACTION AND REGULATION BY HEMIN USING NMR

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The heme – protein interactions have been an intense subject of interest especially in the last decade because of its vital role in diverse molecular and cellular processes. Heme as a prosthetic group part of several biologically relevant proteins, has been associated with important functions like chemical catalysis, electron transfer and role as an effector molecule. Nevertheless, no distinct classification and characterisation of heme-regulatory motifs is available. Solution NMR spectroscopy will be an excellent tool in determining different heme-binding modes and investigation into conformational changes of peptides upon heme-binding. A literature survey indicated cysteine, histidine and tyrosine as the three most reported iron-coordinating amino acids. Based on known heme-binding motifs, a combinatorial peptide library screening was performed by the Imhof lab to identify heme-binding preferences of short peptides (9-mers) using UV/Vis and mass spectrometry. NMR studies for the complex of cysteine-based peptides (especially CP motif) with Gallium protoporphyrin IX (GaPPIX) are being performed since complex studies with hemin in NMR might suffer from an increased spectral width and ferromagnetic effects leading to line broadening beyond signal detection limit. As a part of these investigations, we have reported the first structure of a CP motif containing 23 amino acid peptide in complex with GaPPIX. The 23-mer is a stretch of a full length DPP8 (Dipeptidyl peptidase 8) enzyme. The NMR results indicate the binding of the protoporphyrin IX to the centrally located cysteine residue while the proline ring acts as a spacer and prevents backbone:PPIX van-der-Waals clashes. The enzymatic assay on the DPP8 using hemin leads to its inhibition suggesting possible role of 23-mer in heme-binding and regulation. A second aspect of our research are voltage-gated potassium channels, responsible for the regulation of several ion conductance pathways. Their inactivation is one of the most critical events that shape the presynaptic action potential in CNS. According to results of our collaborators, hemin plays an important role in the regulation of the α-subunit of potassium channel Kv1.4 with important effects in downstream signalling. Intracellular free heme binds to the N-terminus of the channel leading to structural changes and impairment of the inactivation process. Functional studies and initial results of NMR based structure determination and heme docking will be presented.

Poster Presentation

516 MO

CLOCKING PROTEIN MOTIONS: ELUCIDATING THE DYNAMICS OF RECEPTOR-LIGAND COMPLEX FORMATION WITH TRANSIENT-EPR

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Protein functionalities are a direct consequence of protein motions. Protein structures, obtained with X-ray diffraction or NMR, reveal mostly static pictures of structure-function correlation. To truly connect structural changes of proteins' to function, we need to look at events happening in real time, i.e. the dynamics of the processes.
We have chosen mlCNBD as model protein to study the dynamics of receptor-ligand complex formation. mlCNBD is a cytosolic cyclic nucleotide-binding domain of a bacterial potassium channel (MloK1)[1]. mlCNBD binds to cyclic adenosine monophosphate (cAMP) and undergoes large conformational changes as evident from the NMR and X-ray structures of the *apo* and *holo* conformational states of the protein[2, 3]. Recent kinetic and NMR studies indicate that these structural transitions follow the "induced-fit" mechanism, i.e. these are a direct consequence of ligand binding[3,4]. However, the detailed mechanism of these structural rearrangements leading to receptor activation remains elusive.
We use transient Electron Paramagnetic Resonance (tr-EPR) spectroscopy in conjunction with Site Directed Spin Labelling (SDSL) to resolve the dynamics of mlCNBD-cAMP complex formation. We introduce single cysteine residues at different sites in the protein. The mutants are labelled with Methane Thio Sulphonate Spin Label (MTSSL). Binding of cAMP to the mutants is initiated either via a caged-cAMP approach or through a micro-mixer[5]. The time-resolved EPR data reveals the progression of structural change taking place at a particular site in millisecond time scale. Collating data over the whole protein will enable us to reconstruct the steps from the *apo* to the *holo* state of the protein. It will also provide the answer to the question whether the "induced-fit" mechanism follows a concerted (single step) or sequential (multi-step) path from the *apo* to the *holo* conformation.

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517 TU

NMR STRUCTURAL INVESTIGATION OF THE MEMBRANE PROTEINS INVOLVED IN THE DIMERIZATION OF THE MITOCHONDRIAL ATP-SYNTHASE

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ATP, the universal fuel molecule of any cell, is mainly produced by the F1Fo-ATP synthase. The yeast enzyme, like mammalian ones, is not only involved in ATP synthesis but also forms dimers essential for the organization of the mitochondrial membrane into cristae vesicles. Three subunits are involved in ATP synthase oligomerization: g, e and the N-terminal extremity of the subunit 4 (S4t). Deletion or partial mutation of these subunits lead to anomalous mitochondrial morphologies. No information has been obtained on the three-dimensional structure of these small hydrophobic proteins that create the membrane interface of ATP synthase oligomerization. A production of these proteins, in the presence or not of isotopically labelled amino acids, has been performed with the precipitate-based method using cell-free expression system. Solubilization of Nter4 has been achieved with zwitterionic detergent (DPC, LMPC) or with anionic detergents (SDS, LMPG). Circular dichroïsm spectra have indicated that the solubilized proteins contain mainly alpha helicoïdal structures. However, well dispersed $^{1}H^{15}N$-HSQC NMR spectra of ^{15}N-alanine labeled S4t were only obtained with SDS or LMPG. Assignment of resonances of $^{15}N^{13}C$ S4t has allowed us to solve its three-dimensional structure with the Rosetta program by using chemical shifts as restraints. S4t adopts in LMPG micelle a three helical fold, composed of a short helix at its N-terminus and a long helical hairpin. In a real membrane environment, this hairpin may be embedded in the membrane as two transmembrane segments, while the short helix may be located in the matrix.

Poster Presentation

518 TH

APPLYING A SEGMENTAL LABELLING APPROACH TO MULTI-DOMAIN HEAT SHOCK PROTEIN 90 FOR NMR STUDIES

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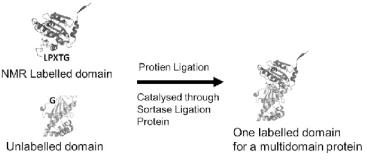
Studying large proteins (>30 kDa) by NMR is challenging. NMR spectra of large proteins suffer from broader line widths due to reduced tumbling times and increased spectral overlap. Several methods have been described to overcome these challenges such as specific isotopic labeling and relaxation-optimized pulse sequences. In order to reduce spectral overlap in a multi domain protein we have employed segmental isotope labeling, whereby one domain is isotopically enriched with NMR active nuclei while one or more remaining domains contain natural abundance nuclei. We have optimized protocols using expressed protein ligation with Sortase A.

The essential chaperone protein Heat Shock Protein 90 (Hsp90) is a three domain 90 kDa protein. Hsp90 is active as a homodimer in solution and is present from bacteria to higher order eukaryotes. It is present at very high concentrations, i.e. 1-2% of total cellular protein. Hsp90 has been extensively studied and structures of some conformational states are known. However, Hsp90 undergoes large conformational changes during its catalytic cycle where molecular details of these motions are poorly understood. Here, we employed segmental labelling on Hsp90 to study by NMR. We analyse NMR data comparing isolated and multi-domain constructs and study the interaction with client proteins of Hsp90 using segmentally isotope-labeled samples. We have observed differences in multi-domain Hsp90 compared to its individual domains in binding of nucleotides and client proteins. In our studies we have included a charged linker region.



519 MO

NMR CONFORMATIONAL DYNAMICS OF ARKADIA & ARKADIA-2 E3 UBIQUITIN LIGASES RING DOMAINS

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E3 ubiquitin ligases play a key role in the proteolytic degradation of proteins through the Ubiquitin-Proteasome pathway [Hershko A & Ciechanover A, Annu Rev Biochem 1998, 67, 425]. ARKADIA is the first example of an E3 ligase that positively regulates TGF–β family signaling through its C-terminal RING domain [Episkopou V et al. PLoS Biol 2007, 5, e67], while its homologue, ARKADIA-2, is implicated in BMP pathway.

The ARKADIA RINGs, were cloned and expressed in their Zn-loaded form and studied through NMR Spectroscopy [Kandias NG et al. *BBRC* **2009**, 378, 498]. The 3D NMR solution structure of ARKADIA-1 RING was determined and deposited in PDB (2KIZ). Additionally, NMR-driven titration studies were also performed to probe the interaction interface of ARKADIA-1 RING and the partner E2 (UbcH5B) enzyme and the RING-E2 complex was constructed through an NMR-driven docking [Chasapis CT et al. *Proteins* **2012**, 80, 1484].

Additionally, this study resulted to the identification of ARKADIAs RING functionally important residues, such as the conserved Trp972. Trp972 is considered as one of the key residues for E2 recognition and binding [Huang A, et al. J Mol Biol 2009, 385, 507]. According to recent experimental evidence, the mutation of the Trp972 to Arg abolishes the ability of ARKADIA to amplify TGF-b-Smad2/3 signaling responses in tissue culture transcription assays [Episkopou V, et al. Cancer Res. 2011, 71, 6438]. Various ARKADIA Trp mutants are now being studied through NMR in order to obtain an atomic-level insight about the structural base of ARKADIA-1 & -2 RING capability to selectively interact with the appropriate E2.

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POSTER PRESENTATION

520 TU

NMR INSIGHTS ON THE CONFORMATIONAL PLASTICITY OF THE EXTRACELLULAR DOMAIN OF A PROKARYOTIC nAChR HOMOLOGUE

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Pentameric ligand-gated ion channels from the Cys-loop family are of special importance for the rapid chemo-electrical transduction, but the mechanisms of ion permeation and gating of these membrane proteins remain elusive. Recently the X-ray structures of two prokaryotic homologues of the LGIC family most studied member, the nicotinic acetylcholine receptor (nAChR) have been determined. The first is the bacterial *Gloeobacter violaceus* pentameric LGIC homologue (GLIC) studied at 2.9 Å resolution in an apparently open conformation [Bocquet N et al *Nature* **2009** 457, 111] and the second is the bacterium *Erwinia chrysanthemi* (ELIC) pentamer, studied at 3.3 Å resolution defining a closed conformation of the channel [Hilf RJ et al *Nature* **2009** 457, 115]. Interestingly, the extracellular soluble domain of GLIC is found to be in monomeric state in solution.
The 200-residue extracellular domain of GLIC was cloned and expressed in high yields in *E. coli.* The ^{1}H-^{15}N HSQC exhibits signal dispersion typical for polypeptides with mainly beta structure. ^{13}C/^{15}N labeled GLIC is studied using heteronuclear multidimensional NMR spectroscopy and <40% of backbone nuclei were originally identified. Deuterated, triple labeled ^{13}C, ^{15}N and ^{2}H samples was used for the acquisition of triple-resonance NMR spectra and the so-far analysis of the data have allowed the identification of 50-60% of the backbone resonances. Selective labeling techniques were also used for the identification of ~80% of the backbone resonances. NMR data suggested that various GLIC segments are characterized by conformational exchange behavior. NMR data in higher temperature, H/D exchange experiments and ^{15}N relaxation measurements were used to determine the dynamics of the protein and the determinants of the GLIC ECD assembly and oligomerization [Chasapis CT et al. *Biochemistry,* **2009** 50, 9681].
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521 TH

A COMPARATIVE NMR STUDY OF FOUR VIRAL MACRO DOMAINS

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Macro domains are ancient and widely distributed throughout all eukaryotic organisms, bacteria, and archaea, indicating a ubiquitous and important basic biological function. Macro domains are also found in nonstructural proteins (nsPs) of several positive-strand RNA viruses, including hepatitis E virus, rubella virus and coronaviruses, as well as alphaviruses. The functions of the macro domain are poorly understood, but it has been suggested to be an ADP-ribose-binding module.
In this study we apply NMR spectroscopy to study the conformational properties and dynamics of four macro domains: (a) two from New World alphavirus (Mayaro & Venezuelan equine encephalitis virus), (b) one Old world alphavirus (Chikungunya virus) and (c) one from the Hepevirus genus (HEV-1).
The four macro domains are cloned and expressed with C-terminus poly(His)tag, in high yields in *E. Coli.*. All the protein constructs are soluble and using *E.coli* culture supplements prior to induction in typical (M9) minimal media the bacteria growth rates and protein yield were generally increased. Initial 1D ^{1}H & 2D ^{1}H-^{15}N HSQC NMR experiments suggest that all three macro domains are folded in solution. Acquisition and analysis of 2D/3D homo/heteronuclear NMR data are underway.
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POSTER PRESENTATION

522 MO

NMR STRUCTURAL INVESTIGATION AND RNA BINDING PROPERTIES OF LA PROTEIN DOMAINS FROM D. DISCOIDEUM

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Biosynthesis of RNA polymerase III transcripts requires binding of the La protein at their 3' end. La is an abundant nuclear RNA-binding protein which protects the nascent transcripts from 3' exonuclease degradation. It was originally discovered in patients with systemic lupus erythematosus and Sjogren's syndrome. It contains a conserved, predominantly helical structure which retains RNA, termed “La motif”. Interestingly, this motif is found not only in the bona fide La proteins. So far, the structure of full-length La protein is elusive. For a few species structural data of the conserved RNA binding motifs are available, providing important information on specific RNA-protein interactions with essential cellular function. Here, we report the high yield expression of two recombinant RNA binding domains (La motif and NRRM) from the La protein of Dictyostelium discoideum and their preliminary structural analysis through NMR spectroscopy. Both recombinant protein constructs were well-folded and allowed for an almost complete sequence-specific assignment of the 15N and 13C labeled domains. The NMR structure of LA N-terminal domains has also been determined.

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523 TU

CONFORMATIONAL DYNAMICS OF N-/C- TERMINAL DOMAINS OF ANTHRAX LETHAL FACTOR METALLOPROTEASE

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In the pathogenesis of Anthrax disease, the bacterium's lethal toxin (LeTx) is of critical importance, while its component called Anthrax Lethal Factor (LF), 90 kDa Zn-dependent highly specific metalloprotease, is particularly interesting [Pannifer AD et al Nature 2001, 414, 229.]. Its proteolytic activity is targeted in a highly specific way towards vital cellular signal transducers, the family of mitogen-activated protein kinase kinases (MAPKKs) . This leads to the disruption of their ability to interact with and phosphorylate downstream substrates, and consequently alter the corresponding signalling pathways, ultimately inducing apoptosis [Duesbery NS et al Science 1998, 280, 734]. What is more, this protein attracts the attention of modern research for its possible involvement in cancer, as it seems that MAPKKs to be over expressed in certain tumour cells [Davies H et al Nature, 417, 2002, 949], and related findings could be possibly form a novel, therapeutic, anti-cancer approach [Huang D et al. Cancer Res. 2008, 68, 81].

In order to elucidate how LF participates in the formation of (LeTx), as well as its catalytic site structural-functional activity towards its kinase substrates, we attempt the expression and the NMR study of the Domain I (233 a.a.) and Domain IV (225 a.a.) polypeptides. Structural analysis of these domains will be helpful in an effort to inhibit both these processes, as part of a possible therapeutic approach. In this regard, possible peptides that may antagonize MAPKKs in binding to the catalytic centre, thus preventing their pathological proteolysis, are also designed and expressed. Different experimental protocols are currently applied for the effective overexpression and labelling of these polypeptides, before the application of multi nuclear and multi dimensional NMR spectroscopy. Preliminary results suggest that the recombinant ^{15}N-labelled N-terminal polypeptide is soluble and folded in solution and suitable for NMR conformational analysis. On the contrary, the C-terminal polypeptide, while expressed in abundance, seems to be almost totally insoluble, thus making NMR study in solution impossible. Efforts are done to overcome this hurdle.

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524 TH

AN EFFICIENT STRATEGY FOR STUDYING WEAK INTERACTIONS OF POSTTRANSLATIONALLY MODIFIED PROTEINS

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[*]*Equal contribution*

Many cellular processes are regulated by posttranslational modifications that are recognized by effector domains in protein interaction partners. These interactions are often weak, thus allowing a highly dynamic and combinatorial regulatory network of protein-protein interactions. We report an efficient strategy that overcomes challenges in structural analysis of such weak transient interactions related to the dynamics and low affinities. The posttranslational modification is chemically introduced and covalently linked to the effector module by Expressed Protein Ligation. Covalent coupling of the two interacting moieties shifts the equilibrium to the bound state and stoichiometric interactions are formed even for low affinity interactions. The successful implementation of the method is demonstrated with the recognition of symmetrically dimethylated arginine by the Tudor domain of the Survival of Motor Neuron (SMN) protein.
The methodology has many advantages: 1) it allows the controlled and unambiguous introduction of specific modifications in the ligands by chemical synthesis, 2) it significantly stabilizes low affinity interactions, 3) stoichiometric interactions are obtained even for weakly interacting (peptide) ligands at protein concentrations suitable for structural and biophysical studies.

525 MO

SOLID-STATE NMR ASSIGNMENT OF A HIGH PH POLYMORPH OF A-SYNUCLEIN

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ETH Zürich

Parkinson's disease is a frequently occurring and devastating neurodegenerative pathology, which is tightly associated with the formation of Lewy bodies. Those deposits mainly consist of α-synuclein in fibrilar, β-sheet-rich assemblies. α-Synuclein can form numerous different polymorphs, which can be difficult to obtain in pure form. Here, we describe a solid-state NMR study to assign the chemical shift and to evaluate the secondary structure of this polymorph that was fibrilized at higher than physiological pH conditions. The fibrilar core of this species ranges from residue 40 to 95 with both the C- and N-terminus not showing any rigid and ordered parts. The chemical shifts differ considerably from previously assigned polymorphs (1-4). This assignment makes a first step towards obtaining a structure at atomic resolution and eventually getting insights into the rich polymorphism of α-synuclein.

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526 TU

PELDOR ON NEW TYPE OF SPIN LABELS FOR NUCLEIC ACIDS

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PELDOR (pulsed electron electron double resonance) is a magnetic resonance method for distance, orientation, and dynamic measurements of two or more paramagnetic centers in macromolecules like proteins, RNA, or DNA as well as polymers. Here we apply this method to analyze a series of different spin labels in DNA double helices. These spin labels have distinct different rotational freedom along the axis of the nitroxide.
The differences could be used to select specific labels for desired properties: only the distance or the distance and orientation between a pair of spin labels.

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Hydrogen-bonding controlled rigidity of an isoindoline-derived nitroxide spin label for nucleic acids D. B.Gophane, S. Th. Sigurdsson, Chem. Commun., (2013), 49, 999-1001.

527 TH

PRODUCTION OF SMALL TRANSMEMBRANE PROTEINS IN *E. COLI* VERSUS CELL-FREE SYSTEMS

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The human protein tetherin, also known as BST-2/CD317/HM1.24, is able to restrict the budding of enveloped viruses from the host cell [1]. The HIV-1 avoids this mechanism by using its own virus protein "U" (VpU) to antagonize and down regulate tetherin [2]. NMR studies require milligram amounts of protein. Here we compare two methods for production of various segments of the bitopic transmembrane (TM) proteins VpU and tetherin: Recombinant expression in *E. coli* cells and cell-free expression (CFE). We found that tetherin constructs containing the TM domain are cell toxic and impossible to express in *E. coli*. Full-length VpU as well as the TM domain of VpU could be expressed as ubiquitin fusion proteins in isotope labelled form, separated from the fusion partner and affinity tag by enzymatic cleavage and purified. However, protein yield was only moderate and the procedure is time-consuming. Cell-free expression of full-length VpU, the single TM domains of tetherin and VpU and of a tetherin fragment containing the TM and cytoplasmic domains delivered high amounts of protein. CFE works equally well in presence and absence of detergents in the medium resulting in solubilized and precipitated membrane protein, respectively. Isotope labelling, purification and initial NMR characterization of the produced proteins will be presented. We will discuss the pros and cons of the two production methods and compare the efforts required in terms of cost and time.

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Poster Presentation

528 MO

STRUCTURAL ANALYSIS AND MEMBRANE INTERACTION OF THE N-TERMINAL REGION OF DENGUE VIRUS NS4A PROTEIN

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Dengue virus (DENV) infection is a growing public health problem with more than one-third of the world population at risk. DENV is a mosquito-transmitted positive strand RNA virus that causes dengue fever, dengue hemorrhagic fever and dengue shock syndrome. Until now, no vaccine for DENV and no specific treatment for dengue fever are available. The non-structural proteins of DENV are believed to be crucial for replication of the viral RNA genome in association with modified cellular membranes that form a replication complex. The assembly of this replication complex is incompletely understood. The non-structural protein 4A (NS4A) of DENV is supposed to play a crucial role in anchoring components of the replication complex to intracellular membranes. We focus on the 48 amino acid long N-terminal fragment of NS4A which contains two conserved putative amphipathic helices (AHs) that seem to be critical for viral replication. AHs are known to be involved in membrane binding, membrane remodeling and protein-protein interactions. We studied the wild type NS4A peptide as well as a modified and functionally compromised version carrying two point mutations both without and in presence of membrane mimicking conditions. Circular dichroism (CD) spectra show that both peptides are unstructured in aqueous buffer. However, in presence of SDS micelles and liposomes of various lipid compositions they show a substantial degree of helical secondary structure. Qualitative surface plasmon resonance (SPR) data reveal a strong interaction of wild type NS4A peptide with various lipid membranes, while the mutant peptide interacts only weakly with immobilized lipid bilayers. A detailed secondary structure analysis of both peptides was conducted on the basis of backbone chemical shift NMR data. Both peptides, wild type and mutant NS4A, show significant changes of chemical shifts upon addition of detergent micelles. Secondary chemical shift data indicate formation of two helices in both peptides in SDS micelles. Surprisingly, position and length of the helices are very similar in the wild type and mutant peptides.

529 TU

SOLUTION ENSEMBLE OF OAA REVEALS BOUND-LIKE CONFORMATION IN THE ABSENCE OF LIGAND

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During the last decade several lectins were found to exhibit anti-HIV activity by binding with high affinity to the high mannose glycans on the viral protein gp120. One such lectin is the cyanobacterial *Oscillatoria Agardhii* Agglutinin (OAA). The crystal structures of both free and α3, α6-mannopentaose-bound states have been determined[1,2] and suggest that one of the two binding sites available on the surface of the protein may undergo conformational changes upon carbohydrate binding. NMR spectroscopy is a powerful technique for studying conformational ensembles in solution. Here we present the NMR solution structure of free OAA, based on NOE and RDC restraints. Interestingly, solution ensemble of free OAA contains the bound crystal conformation for both binding sites. This result shows that, in solution, OAA samples a "bound-like" conformation in the absence of ligand. In light of the two limiting scenarios invoked when describing molecular recognition, induced fit and conformational selection, our structural ensemble suggests that recognition of α3, α6-mannopentaose by OAA may proceed via conformational selection.

[1] L.M.I. Koharudin, W. Furrey, A.M. Gronenborn, The Journal of Biological Chemistry (2011), 286:1588-1597
[2] L.M.I. Koharudin, A.M. Gronenborn, Structure (2011), 19:1170-1181

Poster Presentation

530 TH

NMR STUDY OF *ARABIDOPSIS THALIANA* BolA2 : STRUCTURE, DYNAMICS AND INTERACTION WITH GLUTAREDOXINS

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BolA proteins are small ubiquitous proteins which participate in some cellular regulation and stress-response pathways. Because of their potential properties of DNA-binding, they may act as transcription factors. They are also able to form [2Fe-2S] cluster bridged heterodimers with CGFS-type monothiol glutaredoxins (Grx) involved in iron homeostasis.
We studied by NMR the 93-residue BolA2 of *Arabidopsis thaliana* and and its interaction with two glutaredoxins, the single domain GrxS14 and the multidomain GrxS17 from *A. thaliana*. Chemical shift mapping NMR experiments showed that the interaction area is similar in both cases. It includes His51 and several other conserved residues in its vicinity. Interestingly, the interface corresponds to the "helix-turn-helix" region involved in DNA-binding and not to the cluster binding region. Mapping in turn the region on GrxS14 interacting with BolA2 interestingly highlighted an interface described as a regulation site of glutaredoxins, dictinct from its cluster-binding area.
Finally, BolA proteins and Grx can form two very different heterodimers, an apo or a holo-heterodimer, depending on iron concentration. For both partners, the interfaces correspond to conserved regions either to a regulation area in the apo form, or to the region around the cluster ligand in the holo form. This result might be helpful to explain the mechanism of iron regulation in cells.

531 MO

USE OF ^{19}F-NMR FOR MEMBRANE-PROTEIN INTERACTION STUDY OF PORE-FORMING PROTEIN LISTERIOLYSIN-O

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Cholesterol-dependent cytolysins (CDCs) are a family of more than 20 different pore-forming proteins, expressed in various bacterial species. All of them are secreted as soluble 50-70 kDa monomers that bind to host membranes, oligomerize and form up to 35 nm pores. It has been shown that membranes must be rich in cholesterol that binding occurs, but the exact mechanism of cholesterol recognition is still unclear. The most known CDCs are pneumolysin, perfringolysin, streptolysin and listeriolysin-O (LLO), which is a virulence factor of Gram-positive pathogen bacteria *Listeria monocytogenes*. The bacteria causes listeriosis, a human disease that starts with the ingestion of contaminated foods and affects mainly immunocompromised persons, pregnant women, and newborns. Once within the host, bacteria is phagocytosed and in order to escape from acidic phagosome it uses numerous strategies that help it to survive and spread to other cells. One of them is the production of LLO, a protein with molecular weight of 56 kDa. It's unique among other CDCs, because its activity is pH dependent and reaches the maximum at pH 5,7. When the bacteria escapes from phagosome to pH neutral cytoplasm in host cell the protein aggregates and is therefore inactive. LLO consists of four domains (D1-D4) and is a homolog to other CDCs but its structure hasn't been determined yet. For cholesterol binding studies we have successfully expressed LLO with all seven tryptophans labelled with ^{19}F. Six of them are located in D4 where is also tryphophane-rich region that has been thought to be the cholesterol binding motif. The stability, structure and haemolytic activity of isotopically labelled LLO were undistinguishable from unlabelled protein and the ^{19}F-NMR spectra showed 7 clear signals. Single tryptophan deletion mutants are being prepared for their assignation. We have also prepared different membrane models with cholesterol for interaction studies, such as bicelles, liposomes, and nanodiscs. Its ability to successfully bind LLO was tested with haemolytic activity of LLO. We will record the spectra of ^{19}F-LLO bind to model membrane and in that way characterize conformational changes around triptophans during the binding to determine cholesterol recognition mechanism. This approach can be a usefull tool to study membrane-protein interactions with ^{19}F-NMR even when studied protein has higher molecular weight.

Poster Presentation

532 TU

MOLECULAR RECOGNITION OF ALTERNATIVE SPLICING FACTORS THAT MODULATE *FAS* ALTERNATIVE SPLICING

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The process of mRNA splicing is one of the most important aspects of gene regulation and leads to an expansion of the proteome by alternative splicing. Proteins with different, even antagonistic activities can be synthesised inside the cell by this method. One such example is FAS receptor in which the exon 6 could be skipped or included to make membrane bound or soluble FAS receptor respectively. Membrane bound FAS receptor promotes apoptosis whereas soluble FAS prevents it.
TIA-1 and SPF 45 are two proteins, which promotes exon 6 inclusion and skipping respectively by the alternative splicing of *FAS* gene. In addition to having domains for binding to mRNA, these proteins also interact with the proteins of the spliceosomal machinery by means of protein-protein interaction. These proteins, which govern these protein-protein and protein-mRNA interactions, are composed of a limited number of RNA binding domains. The RNA recognition motif (RRM) domain is the most abundant of these domains. Interestingly, atypical RRMs can act as protein-protein interaction domains in the splicing complexes. An important subfamily is the so-called U2AF homology motifs (UHMs), which harbour a conserved pocket for the binding of tryptophan in cognate UHM-ligand motif (ULM) peptides that are found in a number of alternative splicing factors.
Here by systematically varying amino acids in ULMs and structure activity relationship studies, we try to identify the residues that govern specificity of the ULMs for a given UHM by using SPF 45 as a modal system. Besides this, we identify residues surrounding the ULM binding pocket that govern selectivity of the ULMs it binds. In addition to this, we study RRM1 of TIA-1 protein by NMR, which is a potential UHM and the protein-protein interactions mediated by TIA-1. The knowledge gained from this study will help to understand the mechanism of molecular recognition of alternative splicing factors involved in *FAS* alternative splicing.

533 TH

SOLUTION STRUCTURE OF THE TETRAMERIZATION DOMAIN OF CIONA INTESTINALIS P53/P73-B AN ANCESTRAL ORTHOLOG OF THE VERTEBRATE P53 FAMILY OF TRANSCRIPTION FACTORS

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p53 is a tumor suppressor protein with a crucial role in cell cycle and cancer prevention. p73 and p63 are two additional ancestral members of the same protein family of transcription factors being involved in neurogenesis, epithelial stem cell maintenance and quality control of the female germ line. All three are products of gene-duplication events and subsequent specialization during vertebrate evolution starting from a single genomic copy. Predominantly dimeric until activation, the formation of tetramers, allowing co-operative binding to their DNA responsive elements, has been shown to be a common pre-requisite for their activity. The tetramerization domain (TD) is organized as a dimer of dimers with the primary dimers being formed via an intramolecular β-sheet and hydrophobic helix packing (H1). In the case of p63 and p73 this canonical motive is prolonged by an additional C-terminal helix (H2). In the active tetramer this is wrapping around the neighboring dimer like a clamp, stabilizing the overall architecture.
We are aiming to get further insights into the domain-domain interactions and the modes of activation of this very important protein family and are especially interested in the regulation of tetramer formation.
The urochordate Ciona intestinalis is a living fossil and believed be to one of the best representatives of a common vertebrate ancestor. It's much smaller genome codes for only two p53 like proteins which are being expressed in a dramatically lower number of isoforms. The supposedly much simpler network of interactions and regulations should make it easier to shed some light on the more fundamental and ancient functions.
Here we report the solution structure of the TD of Ciona intestinalis p53/p73-b (residues 374-419) determined by multidimensional liquid state NMR spectroscopy. As found in homo sapiens p53, p63 and p73 this domain forms a dimer of dimers with a D_2 symmetry. The second helix is conserved as a structural motiv although lacking significant sequence homology.
Furthermore we present some insides into the structural organization of the TD of Ci-p53/p73-a which aligns better to p63 or p73 than to p53, but does not form a second helix due to incompatible amino acids within the linker between H1 and H2.

534 MO

SOLUTION NMR OF INFLUENZA M2 IN ISOTROPIC BICELLES

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The protein M2 from influenza is a tetrameric membrane protein with several roles in the viral life cycle. The transmembrane helix (TMH) of M2 has proton channel activity that is required for unpackaging the viral genome. A membrane-interacting amphipathic helix (APH) that is C-terminal to the TMH is important for budding and scission; a second region that is predicted to be helical interacts with the matrix protein M1 for packaging of the viral ribonuclear protein complex. The APH of M2 (residues ~47-58) binds cholesterol and targets the protein to the edges of the host cell 'budzone', which is enriched in cholesterol.
Spectroscopic comparison of swM2 in micelles and bicelles reveals that changes in structure and dynamics that are localized to the linker connecting the TMH and APH, as well as the TMH kink at Gly34. In the APH there are chemical shift changes consistent with conformational rearrangement of the helix as well as large differences in the rates of chemical exchange with water in the TMH-APH linker region. Such changes are consistent with the differences observed between structures of M2 in lipid bilayers and detergent micelles, suggesting that the bicelle system is capable of recapitulating certain aspects of the membrane bilayer. Cholesterol-induced chemical shift changes are localized to the centere of the TMH and the APH, the latter of which contains the putative cholesterol-binding region.

535 TU

STRUCTURAL STUDY OF THE PARTIALLY DISORDERED FULL-LENGTH Δ SUBUNIT OF RNA POLYMERASE FROM *BACILLUS SUBTILIS*

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Studies of intrinsically disordered proteins (IDPs) are gaining significant importance in the biochemical research, as these systems are highly abundant in eukaryotic proteome and their malfunction has been related to serious human diseases. Nuclear magnetic resonance spectroscopy (NMR) is the only biophysical technique which is capable of providing information about both local conformation and long-range contacts in IDPs at atomic resolution. The fast conformational averaging of the disordered polypeptide chain accompanied by the repetitions in the primary structure results in significant signal overlap in the NMR spectra which may prevent from detailed characterization of their conformation and dynamics effecting thus the level of understanding of their function in the organism. Thanks to the technical and methodological advances accomplished in the field of NMR spectroscopy in the last decade, the studies of unstructured or partially disordered proteins have become accessible for the systematic research.
We have investigated the partially disordered δ-subunit of RNA polymerase (RNAP) which is unique for Gram-positive bacteria such as *Bacillus subtilis*. Recent results indicated that the presence of δ subunit increases the transcription specificity and the efficiency of RNA synthesis. On the other hand, the absence of delta subunit is proposed to decrease a virulence of some pathogens. The full-length partially disordered δ-subunit was investigated in this study. The three-dimensional structure of the well folded N-terminal domain was determined based on the set of proton-proton distance extracted from the analysis of the NOESY. Completely different approach had to be applied for the characterization of the disordered C-terminal domain of the RNAP δ-subunit whose primary structure consists of repetitive motifs of acidic amino acids. The acquisition of the five-dimensional NMR experiments provided high resolution of the signals in the spectra which afforded the unambiguous assignment of both backbone and side-chain resonances. The analysis of the observed chemical shifts revealed the trends to form transient extended structure along the length of the disordered domain. To study the transient long-range contacts between the individual parts of the C-terminal domain and between the well-ordered and disordered domains of the RNAP δ-subunit, the paramagnetic relaxation enhancements of the 1H-15N cross-peaks were measured using the cysteine attached MTSL spin label in single-cysteine mutants of the δ-subunit. The strongest contacts were found between the stretch of positively charged lysine residues and stretches of negatively charged residues. The increased propensity for transient ordering in the lysine rich region was also confirmed by the decreased flexibility determined from measurement of heteronuclear steady-state nuclear Overhauser enhancements.

Poster Presentation

536 TH

STRUCTURES AND INTERACTIONS OF PROTEINS INVOLVED IN NEURONAL REGENERATION

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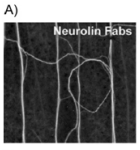
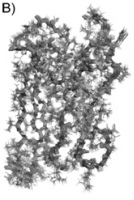
In contrast to mammals fish have the amazing capacity of regenerating their central nervous system after injuries. Upon optic nerve lesion, new axons grow out from retinal ganglia towards the brain, find their target areas, and re-establish vision.
A number of proteins are involved in this process. Among these are reggie/flotillin proteins that play a key role during the organization of membrane microdomains, and knock out of which in fish or mice leads to severe impairment of neuronal development. The protein neurolin, a member of the immune globulin superfamily, has a specific function during axonal pathfinding that can be selectively inhibited by antibodies (Fig. A).
We have determined the solution structure of neurolin's second immune globulin domain (Fig. B) and characterized the interaction with the pathfinding error-inducing monoclonal antibody N518.
Furthermore, we investigate structures and interactions of prokaryotic reggie/flotillin proteins. By combining NMR spectroscopy and fluorescence microscopy, we have obtained detailed information on the function of these proteins during formation of membrane microdomains and recruiting of proteins into these microdomains. These results will be transferable to membrane microdomains present in eukaryotic organisms and have implications for the role of reggie/flotillin proteins during neuronal development.





537 MO

INVESTIGATION OF THE ROLE OF A LINKER REGION IN THE AUTOTRANSPORTER NALP

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Autotransporters are outer membrane proteins from gram-negative bacteria, which are important determinants of pathogenicity. These proteins contain a membrane bound translocator domain that is folded in the form of a β-barrel, and a functional passenger domain that is transported to the outer surface of the bacterial cell. The two domains are connected by a short linker peptide constituted of about 30 amino acids. The linker peptide is of particular interest, since its deletion in autotransporters eliminates the transport function. Additionally, the crystal structure of the translocator domain of NalP, the linker region has been shown to adopt an α-helical conformation located in the central pore of the barrel [1]. In the work presented here, NMR spectroscopy is used in combination with other biophysical tools to investigate the role played by this linker in the folding and transport process of the autotransporter NalP from Neisseria meningitidis. Circular dichroism (CD) spectra, NMR chemical shifts and nuclear Overhauser effect spectroscopy reveal significant structural differences in the linker peptide dissolved in aqueous medium, sodium dodecyl sulfate micelles and trifluoroethanol. Additionally, the presence of the linker region is found to impact the folding of the translocator domain in vitro, as evidenced by CD spectroscopy and transverse relaxation optimized (TROSY) NMR of constructs of the transporter domain in detergent micelle solutions (Figure 1). The data obtained suggests a role for the linker in the function of autotransporters.

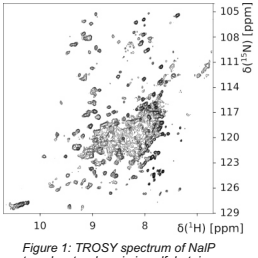

Figure 1: TROSY spectrum of NalP translocator domain in sulfobetaine 3-12 detergent micelles

[1] Oomen et al., EMBO J. 2004, 23: 1257

Poster Presentation

538 TU

ANALYSIS OF VEGETABLE OIL QUALITY TO PRODUCE BIODIESEL USING MULTIVARIATE METHODS WITH DATA OBTAINED FROM LOW-FIELD NMR

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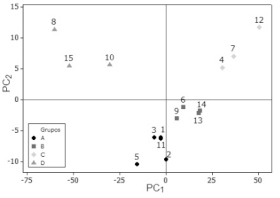

Fig 1. Graph of scores resulting from the PCA analysis of data obtained by CPMG.

In order to carry out the selection from the wide range of oilseeds to produce biodiesel, it is essential to understand the quality of vegetable oils. The method of low-field ^{1}H NMR can be applied in these analyses. The studies are based on the existing correlation between the T_2 values of oils, usually obtained through CPMG experiments, and the oil properties such as viscosity, iodine index and cetane index. In this work, fifteen oil samples were analyzed through CPMG experiments and the obtained results were used for principal component analysis (PCA). The resulting scores graph is shown in Figure 1. The results were significant, so the oilseeds could be separated according to their quality. Group A is made up of matrices presenting high oleic acid content: peanut, canola, dende and oilseed turnip, as well as babassu (*Orbignya speciosa)*, which is rich in lauric acid. These fatty acids provide biodiesel with adequate viscosities, low iodine indices and high cetane indices. The oleaginous seeds in group B (sunflower, corn, soybean and jatropha) have lower fraction of oleic acid compared to group A, but they are made of higher concentrations of linoleic acid. Therefore, group B biodiesels will be less viscous and will have higher iodine indices and lower cetane indices than those derived from group A. Cutieira (*Joannesia princeps*), linseed and candle nut seed (*Aleurites moluccana*) make up group C: the matrices that are rich in polyunsaturated fatty acids. They are less viscous, but have high iodine indices and low cetane indices. Group D is made up of the most viscous oleaginous seeds: Castor bean (Ricinus communis), munguba seed (*Pachira aquatica*) and tungue seeds (*Aleurites fordii*). Thus, the low-field ^{1}H NMR technique, combined with multivariate methods, stands out when choosing matrices to produce biodiesel, because it is a fast and non-destructive technique, that yields results strongly correlated with many physical properties of relevance for the potential of the matrices.

Acknowledgement
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539 TH

NMR STUDY ON DYNAMICS OF FSA^-, NTf_2^- and $BETA^-$, WHICH ARE REPRESENTATIVE ANIONS OF IONIC LIQUIDS, AND INCORPORATED 1,3-DIMETHYLIMIDAZOLIUM CATION

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The ^{1}H- and ^{19}F-T_1, T_2 values of 1,3-dimethylimidazolium salts ($[C_1mim]^+X^-$) (X: bis(fluorosulphonyl)-amide (FSA), bis(trifluoromethylsulphonyl)amide (NTf_2) and bis(pentafluoroethylsulphonyl)amide (BETA)) were measured as a function of temperature in the wide range from 203 K to 393 K. As ^{1}H and ^{19}F are included in the cation and anion respectively, the dynamics of cation and anion can be investigated independently. In this study, we mainly focus on the degree of cooperative dynamics of the anion and cation and also the anion dependence on the dynamics. In the cooling process, the ^{1}H and ^{19}F-T_1, T_2 values of $[C_1mim]FSA$ and $[C_1mim]NTf_2$ changed discontinuously at 303 K and 283 K due to crystallization, respectively. The both minimum points on the ^{1}H and ^{19}F-T_1 values of $[C_1mim]FSA$ appear at 273 K after crystallization. The ^{1}H-T_1 minimum point of $[C_1mim]NTf_2$ appears at 213 K after crystallization in the cooling process. However, the minimum point cannot be recognized clearly in the ^{19}F-T_1 values of $[C_1mim]NTf_2$ because the ^{19}F-T_1 values did not change in the temperature range from 273 K to 243 K. The ^{1}H-T_1 and T_2 values of $[C_1mim]BETA$ discontinuously changed at 223 K and 273 K, respectively, in the cooling process. The ^{19}F-T_1,T_2 values of $[C_1mim]BETA$ changed discontinuously at 273 K and then continuously decrease with lowering temperature. The first discontinuous change of the T_2 values may be caused by partial crystallization. The second discontinuous change of the T_1 values at 223 K may be caused by crystallization and/or phase transition. The details of phase behaviors observed by the ^{1}H and ^{19}F relaxation time measurement will be demonstrated and discussed using a poster.

Poster Presentation

540 MO

NMR STUDY ON MOLECULAR DYNAMICS AND PHASE TRANSITIONS OF 1-ALKYL- 3-METHYLIMIDAZOLIUM BIS(TRIFLUOROMETHYLSULPHONYL)AMIDE

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The molecular dynamics and phase transition of ionic liquids 1-alkyl-3-methylimidazolium bis(trifluoromethylsulphonyl)amide [$C_n$mim]NTf_2 with n=1, 2, and 4 were investigated using the ^{1}H- are ^{19}F-T_1, T_2 values measured as a function of temperature in the wide temperature range. ^{1}H and ^{19}F are included in cation and anion, respectively. Therefore, the separate information about dynamics of cation and anion can be obtained from ^{1}H-T_1, T_2 and ^{19}F-T_1, T_2, individually. The ^{1}H- and ^{19}F- T_1, T_2 values of [C_1mim]NTf_2 discontinuously changed at 283 K by crystallization in the cooling process. The melting point was recognized at 303 K by discontinuous changing of both ^{1}H- and ^{19}F-T_1, T_2 in the heating process. The minimum point of ^{1}H-T_1 was clearly recognized at 213 K. However, the minimum point of ^{19}F-T_1 cannot be recognized clearly because ^{19}F-T_1 values changed a little in the temperature range from 273 K to 243 K. The ^{1}H-T_1, T_2 values of [C_2mim]NTf_2 discontinuously changed at 233 K by crystallization in the cooling process. However, the T_1 minimum value could not be observed in the measurement temperature range from 393 K to 193 K. The ^{19}F-T_2 values of [C_2mim]NTf_2 discontinuously changed at 233 K in the cooling process. In the cooling process, however, the ^{19}F-T_1 values continuously changed in the measurement temperature range and the T_1 minimum point was observed at 203K. The ^{1}H- and ^{19}F-T_1, T_2 values of [C_4mim]NTf_2 continuously changed in the cooling process in the observed temperature range from 393 K to 273 K. However, the ^{1}H-T_2 values discontinuously changed by crystallization at 213 K in the heating process. The crystallization speed was extremely slow, and it took one hour for only about 0.5 ml sample to crystallize. The details of phase behaviors of these samples will be shown and discussed using a poster.

541 TU

NMR STUDY ON CATION DYNAMICS OF 1-ALKYL-3-METHYLIMIDAZOLIUM BROMIDE

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1-Alkyl-3-methylimidazolium ions are the representative cations constituting ionic liquids. Our group reported cation dynamics of 1-alkyl 3-methylimidazolium bromide [$C_n$mim]Br (n = 2, 3 and 4) based on T_1 and T_2 measurements.[1)] In this study, we measured [$C_n$mim]Br with longer alkyl chains (namely n=5, 6, 7and 8) as a function of temperature in the wide temperature range from 173 K to 403 K in order to comprehensively investigate
relationship between alkyl chain length and phase transition caused by the ion dynamics. The ^{1}H-T_1,T_2values of [C_5mim]Br continuously changed in the cooling process. In the heating process, however, [C_5mim]Br discontinuously changed at about 253 K by crystallization. The crystallization speed was very slow like that of [C_4mim]Br which had been investigated in the previous work[1)]. It took approximately an hour for the sample of 0.5 ml to crystallize. The melting point was 343 K.
However, [C_6mim]Br, [C_7mim]Br and [C_8mim]Br did not crystallize in the both cooling and heating processes. The quantitative behaviors of the ^{1}H-T_1,T_2 values as a function of temperature were the same as that of [C_3mim]Br[1)]. The T_1-minimum points of all these samples appeared at around 313 K. The T_1 values at the minimum point increased with elongation of chain length. Moreover, we measured the ^{13}C spectra and the ^{13}C-T_1 values as a function of temperature for the samples in order to obtain information on the cation dynamics in the liquid state. We will demonstrate the details of the phase transition behavior and the cation dynamics depending on the alkyl chain length.

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Poster Presentation

542 TH

NMR SPECTROSCOPY PROBES MICRO-SOLVATION IN "NUMBERS"

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How many solvent molecules and in what way do they interact directly with biomolecules? This is one of the most challenging questions regarding deep understanding of biomolecular functionalism and solvation. Biomolecular solvation, especially hydration, still remains enticing for numerous research groups with the view to biomolecules' behaviour and structure understanding. As far as protein hydration is concerned, many studies exploit model peptides (NALMA etc.) as a vehicle to understand protein-solvent interactions, providing significant information about protein-water interactions.1
In this work, we present a novel, "global" NMR spectroscopic study, employing the longitudinal relaxation times (T_1) of several neutral dipeptides' $^{13}C_\alpha$ and $^{13}C_\beta$ nuclei at **three** different magnetic fields, succeeding in the accurate quantification of the directly strongly interacting H_2Os with them, namely 8 ± 1 H_2Os. It is the first time a spectroscopic method detects the number of directly interacting water molecules with oligopeptides (e.g. dipeptides), which is further supported by both molecular dynamics simulations and density functional theory calculations, advanced analysis of which allowed the identification of the direct interactions as well as the topology between the zwitterionic L-alanyl-L-alanine dipeptide/water system. Beyond the quantification of dipeptide/water molecules direct interactions, this "**global**" NMR technique could be useful for the determination-elucidation of small to moderate bio-organic molecular groups' direct interactions with various polar solvent molecules. In other words, the T_1 values of any small (larger than the solvent molecule) to moderate sized bio-organic molecular group's protonated ^{13}C nuclei in at least two different magnetic fields could lead, through the minimization of a **unique equation**,2 to the quantification of the directly interacting water, dimethyl sulfoxide, ionic liquids, etc molecules.

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543 MO

PROBING PROTEIN DYNAMICS BY FIELD-CYCLING NUCLEAR MAGNETIC RESONANCE (NMR) RELAXATION TECHNIQUE

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Field dependent relaxation has been known in NMR field for decades. However, its application in dynamics investigation in macromolecular systems is restricted by hardware development until a shuttle mechanism applied on a high field magnet was built by A. Redfield in 2003. Using this concept of shuttling, we decided to push this application line to protein systems. High speed and high stability for a shuttling device is essential for macromolecular applications, especially for protein systems. To fill these essential requirements, we have designed a compact shuttling device, named "field cycler", composed by a servo motor and a rail system mounted on 600MHz spectrometer. This field cycler shuttles the sample stably from the center of the magnet at 14.1T to the top of fringe field at 0.01T in 100ms which permit us to determine NMR dynamics parameters up to the order of 10^{-1}s in a routine basis. This opens up many experiments which could not be done previously, thus extends the capability of NMR on determining macromolecular dynamics. We will demonstrate the first field-dependent NMR relaxation analysis on protein backbone dynamics in ubiquitin.

POSTER PRESENTATION

544 TU

MOLECULAR DYNAMICS IN PBA/PEO MIKTOARM STAR COPOLYMERS

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The designing of the new type of mesoscopic materials, which can bridge the gap between the linear polymers and colloids is a challenge for modern science. The most promising class of such a type of systems constitute the miktoarm (or heteroarm) star copolymers, containing certain number of various types of polymer arms attached to a centrally located core [1]. The ability of the miktoarm star copolymers to self-assemble into organized microdomain structures, occurring for the systems having incompatible polymeric arms, leads to phase separation in a similar way as for block copolymers. The goal of the study is to determine the influence of poly(butyl acrylate) and poly(ethylene oxide)) PBA/PEO arms composition on molecular dynamics in PBA/PEO miktoarm star copolymers with the use of Nuclear Magnetic Resonance (NMR) and Broadband Dielectric Spectroscopy (BDS) methods The structural characteristics of the systems under study have already been reported [2,3]. The spin-lattice relaxation (T_1) dispersion data obtained for the studied miktoarm copolymers were analyzed in terms of a susceptibility $\chi''(\omega)$. The time-temperature superposition principle was applied to obtain a master curve describing the molecular mobility over a wide frequency range. The analysis of the experimental NMR (temperature and frequency dependence of T_1) and BDS data reveals the existence of both local and segmental motions in the studied systems at temperatures below melting point [4]. The temperature dependence of motional rates of segmental motions in the studied systems, obtained from NMR and BDS investigations, was analyzed using Vogel-Fulcher-Tamman (VFT) relation.

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545 TH

THE SEGMENTAL AND GLOBAL DYNAMICS IN LAMELLAR MICROPHASE-SEPARATED POLY(STYRENE-B-ISOPRENE) DIBLOCK COPOLYMER STUDIED BY ^{1}H NMR AND DIELECTRIC SPECTROSCOPY

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Block copolymers are composed of two or more chemically distinct polymer blocks covalently bound together. They exemplify an interesting class of polymer materials which are increasingly drawing the attention of both researchers and engineers. The self-organization processes in block copolymers lead to the formation of various different nanoscale morphologies. Due to complex phase behaviour block copolymers, belong to a very promising material group which might be implemented in microelectronics and nanolithography. They also find applications as surfactants, adhesives, and compatibilizers of polymer blends [1].

Since, the first copolymer systems were synthesized, many research groups attempt to explain how immiscible blocks of two different types influence one another in terms of chain mobility. One of the most powerful methods to study polymer dynamics are broadband dielectric spectroscopy (BDS) and nuclear magnetic resonance dispersion (NMRD) In this study, the susceptibility representation of the NMR relaxation data [2] was applied to the analysis of the molecular dynamics in the lamellar microphase separated polystyrene b–polyisoprene diblock copolymer. As a reference samples the polyisoprene and polystyrene homopolymers were examined. This approach in combination with the frequency-temperature superposition (FTS) allows one to compare directly the NMR and DS data in an extended frequency range providing a unique comprehensive picture of various relaxation processes present in the system studied. The findings of these investigations include structural relaxations of the polyisoprene (PI) and the polystyrene (PS) blocks, a normal mode relaxation of the PI block, and an extra low frequency interfacial relaxation. Special attention has been devoted to influence of the copolymer morphology on the segmental and global dynamics in PI.

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Poster Presentation

546 MO

THE INTERPLAY OF MD AND QM CALCULATIONS TO PREDICT SINGLET STATE RELAXATION

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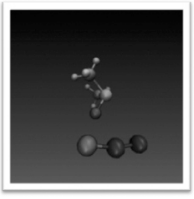

Under certain conditions systems of two spin-1/2 support singlet states that are immune to the intra-pair dipolar relaxation superoperator therefore often showing characteristic decay constants T_S which exceed T_1 by an order of magnitude or more. These enhanced lifetimes are of particular interest as polarization (and indeed hyperpolarization) can be stored for longer time intervals with important application in MRI, for example. Because of the extended time regime, many other "minor" relaxation mechanism interplay to relax these states. An exact knowledge of these mechanisms can indicate the conditions to maximize and extend even further singlet lifetimes. However, even relatively small molecules in solution impose a number of challenges in the task of modeling T_1 and T_S from molecular dynamics simulation (MD) and quantum mechanics calculations QM. Computational cost is one major bottleneck. In addition, in order to accurately model long T_S, attention is required to the weak and rather poorly explored relaxation mechanisms such as spin-rotation.

In this work we use novel approaches to address chemical shift anisotropy, spin-rotation and inter-molecular dipole-dipole relaxation mechanisms within the joint framework of MD and QM calculations. We take particular care of molecular flexibility. The CSA contribution, for example, is computed using a *timescale-separated* spin Hamiltonian, paying particular attention to internal molecular degrees of freedom. In the case of spin-rotation, the explicit time dependent spin Hamiltonian is considered. We demonstrate a good quantitative agreement between experiments and calculations *without adjustable parameters*.

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ON-RESONANCE SATURATION EFFECTS IN MT RESEARCH OF SOLUTIONS CONTAINING SPIO NANOPARTICLES.

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To test the efficiency of the on-resonance saturation magnetization transfer (MT) method for the contrast enchancement of MR imaging of molecular-cellular structures containing superparamagnetic iron oxide nanoparticles (SPIO), the authors have carried out experimental research of on-resonance saturation MT effect in model samples of aqueous solutions and agar gels with different mass concentration and the addition of SPIO nanoparticles with different average effective size and concentration.

For numerical estimating of the magnetization transfer effect we have used magnetization transfer ratio – $MTR = (M_0 - M_{sat}) / M_0$, where M_0 represents the signal intensity when no saturation pulses are applied and M_{sat} the corresponding intensity with MT saturation on.

Data obtained in samples of 6% gel of agar-agar containing nanoparticles with different concentrations demonstrate that optimal time of exchange decreases with increasing of nanoparticles concentration. In addition direct saturation depends on concentration of the iron oxide nanoparticles caused by the decreasing of spin-spin relaxation time T_2 of water protons. Maximal value of *MTR* for optimal time of exchange linearly increases with increasing of SPIO concentration. We have found that *MTR* depends on many factors such as a concentration and a size of SPIO nanoparticles, the time of exchange, the sample structure (gel, fluid, suspension and e.c.), the magnitude of polarizing magnetic field. We have concluded from our research that the exchange between a free water and restricted biomolecular protons become stronger with the increasing of SPIO concentration. But we must control the time of exchange because the increasing of SPIO concentration shorten the T_2 relaxation time of free water protons, and therefore will bring to decreasing of MT effect.

This work was supported by Russian High Education Ministry (grant No.14.B37.21.0568).

POSTER PRESENTATION

548 TH

MOLECULAR DYNAMICS OF METHANOL CONFINED IN CAGES OF FAUJASITES. DEUTERON NMR INVESTIGATION

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Nuclear magnetic resonance (NMR) provides means to investigate molecular dynamics at every state of matter. Molecules confined in nanoscale cages of zeolites represent a particularly interesting system. Features of molecular dynamics characteristic for the gas phase, liquid-like layers and immobilized molecules were observed in the temperature range from 300K down to 20K. Narrow lines were observed at high temperature indicating basically isotropic reorientation. Spin-lattice relaxation rates provide evidence for a transition from translational diffusion to isotropic reorientation as the main mechanism of relaxation for molecules confined in nanoscale zeolite cages for D_2, CD_4 and CD_3OD. Other molecules like D_2O, ND_3, and $(CD_3)_2CO$ are strongly bonded, both mutually and to zeolite framework, and exhibit a much more restricted diffusion.

Deuteron spin-lattice relaxation was measured for methanol-d_4 molecules confined in zeolite NaX and NaY cages. Experimental evidence was given for the formation of trimers, their existence was so far proposed only by theory. The conclusion was based on observation of different relaxation rates for methyl and hydroxyl deuterons undergoing a common dynamics. A change in the slope of the temperature dependence of both relaxation rates indicates a transition from the relaxation dominated by translational motion to prevailing contribution of reorientation at 222K. Trimers undergoing isotropic reorientation disintegrate and separate methanol molecules become localized on adsorption centers at 169.5K and 153.8K for NaX and NaY, respectively as indicated by extreme broadening of deuteron NMR spectra. The transition temperature, higher for NaX, indicates the dominating role of the hydrogen bonding to framework oxygen. NMR spectra at low temperature are consistent with the model in which molecules are bonded at two positions: horizontal (methanol oxygen bonded to sodium cation) and vertical (hydrogen bonding of hydroxyl deuteron of methanol to zeolite framework oxygen).

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A GENERAL APPROACH FOR CHARACTERIZING RNA DYNAMICS AT ATOMIC-RESOLUTION USING NMR RESIDUAL DIPOLAR COUPLINGS: THE BASIS FOR INTER-HELICAL MOTIONS REVEALED

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NMR Residual Dipolar Couplings (RDCs) have recently been used to investigate dynamics of biological macromolecules. The use of several independent alignment media has allowed to determine solely on the basis of RDCs and at atomic resolution, the dynamics of proteins occurring on timescales ranging from picoseconds to milliseconds. However the application of such strategies to characterize RNA dynamics remains essentially limited by the inability to experimentally modulate molecular alignment and therefore to obtain a sufficient amount of independent information.

To address this problem, we extended the recently proposed elongation strategy, by using variable elongation as a general tool for modulating the alignment. If this approach allows for a significant increase of the potential number of measurable RDC datasets it requires a new theoretical framework to extract structural and dynamic information even in the presence of strong couplings between internal motions and overall alignment. We proposed here a protocol that combined the use of long molecular dynamic simulations, *in silico* elongations and structure-based predictions of the alignment to overcome this problem.

We successfully employed this approach to construct and validate an atomic resolution ensemble of HIV-1 TAR RNA using four RDC data sets and a broad conformational pool obtained from an 8.2s molecular dynamics simulation. If this ensemble allows to probe inter-helical reorientation, it also reveals local motions in and around the bulge involving changes in stacking and hydrogen bonding interactions, which are undetectable by traditional spin relaxation. By revealing new insights in both fast and slow dynamics of TAR, this ensemble allows us to propose a mechanism that describes how local dynamic rearrangement of the bulge and its surroundings can trigger the overall reorientations of the two constitutive helices of HIV-1 TAR.

By making a significant step towards quantitative analyses, this new and generally applicable approach broadens the scope of using RDCs in characterizing the dynamics of nucleic acids.

Poster Presentation

550 TU

FUNDAMENTAL AND PRACTICAL APPLICATIONS OF NMRD PROFILES IN THE STUDY OF PARTICULATE MRI CONTRAST AGENTS

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Biological Physics Department

Magnetic nanoparticles can be used to enhance the contrast in Magnetic Resonance Imaging. This is related to their ability to shorten water proton relaxation times. The evolution of the relaxation rates with magnetic field (the NMRD profile) is bearing important information about these systems. Of course it allows a direct estimation of the efficiency of the magnetic particles at multiple magnetic fields, but this is not their only advantage. Iron oxide (maghemite) superparamagnetic particles1 are intensively used in preclinical imaging as well as in cellular imaging. Thanks to the development of relaxation models, the NMRD profile provides - through an appropriate fitting of the curve - an estimation of the characteristics of the system, as particle size and magnetization. Moreover, as the relaxation of magnetic particles is very sensitive to the clustering, NMRD profiles can also be used to check the stability in time of a colloidal suspension.
New paramagnetic particles (based on rare-earth ions) are currently developed and tested in animal models2. These must be divided into two groups. The gadolinium containing particles (oxide, hydroxide and fluoride) on the one hand, that could be used as T1 contrast agents at clinical imaging fields. On the other hand, one finds particles containing dysprosium, holmium, erbium and terbium that could be used as T2 or T2* agents at high fields3,4. Besides the magnetic susceptibility and the size of the particles, the imaging magnetic field has a tremendous influence on the relaxation efficiency of paramagnetic particles even for T2, which is not the case for iron oxide particles. The theoretical modelling of the relaxation induced by these compounds helps determining a priori which particles will be suited for a given experiment. In this context, NMRD profiles are essential to test the relaxation models. But they can also help monitoring the release, by the paramagnetic particles, of rare-earth ions, which could result in a serious toxicity.
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551 TH

SINGLE-SCAN T_1-T_2 RELAXATION CORRELATION MEASUREMENT

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Power of traditional NMR spectroscopy relies on the versatile chemical information reflected by chemical shifts and couplings. Multidimensional experiments multiply the chemical resolution and information content, but they also multiply the experimental time, restricting the investigations of fast processes. Frydman *et al* introduced single-scan multidimensional NMR as an ingenious solution for this issue [1].

Typically, NMR spectrum of a fluid absorbed in a material contains little information; it may even consist of a single broad peak. However, the relaxation time distribution may reveal various environments of the fluid molecules, and consequently provide detailed information about the structure of the material. As in traditional NMR spectroscopy, resolution and information content of the relaxation time experiment can be increased by a multidimensional approach such as T_1-T_2 correlation experiment [2]. A 2D Laplace inversion is used to extract 2D relaxation time spectrum from the experimental data. Original T_1-T_2 correlation experiment is a 3D experiment, and the long experiment time restricts its applicability in the study of fast processes.
Inspired by Frydman's single scan multidimensional NMR approach, we introduce a novel method for single scan measurement of T_1-T_2 relaxation correlation. The method shortens the measurement by orders of magnitude; typical experiment time is about one second while it is from tens of minutes to hours in the conventional experiment with equal resolution. We show that the 2D relaxation correlation spectra are in agreement with the relaxation time distributions measured by the conventional method. The concept of the single-scan T_1-T_2 correlation experiment is applicable to a broad range of other multidimensional experiments, as we will show in future.
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Poster Presentation

552 MO

LOW-FIELD INVESTIGATIONS OF ENZYMATICALLY DEGRADED ARTICULAR CARTILAGE AND ITS CONSTITUENTS

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The proton relaxation times in healthy cartilage tissue show a strong dependence on magnetic field strength which is particularly pronounced in the low-field range between the fields of typical clinical scanners and the Earth magnetic field. Superposed onto this continuous function are the so-called quadrupolar dips, significantly reduced relaxation times in the region between about 10 and 70 mT which indicate cross-relaxation of protons with the partially immobilized nitrogen nuclei in amino acids in collagen and glycosaminoglycans (GAGs). Varying the composition, water content or structural integrity of cartilage affects both the general frequency dependence of T_1 and the shape of the quadrupolar dips, providing a possible diagnostic access to arthropathies such as osteoarthritis.
In this work, we investigate the effect of enzymes to identify a connection between the quadrupolar dips and the different components in cartilage in order to address the problem which of the components is predominantly affected during osteoarthritis [1]. While trypsin is known to separate GAGs from the proteoglycan backbone, collagenase attacks the collagen structure exclusively. Nitrogen nuclei in both substances are shown to contribute to the quadrupolar dips in a similar way. Experiments for both constituents as well as fresh and enzyme-treated bovine articular cartilage were carried out and the relaxivity in the region of the quadrupolar dips were quantified. The observed strong dependence on water concentration is interpreted by a fast-exchange model and is discussed in conjunction with low-field one-dimensional relaxation imaging results with high spatial resolution [2].

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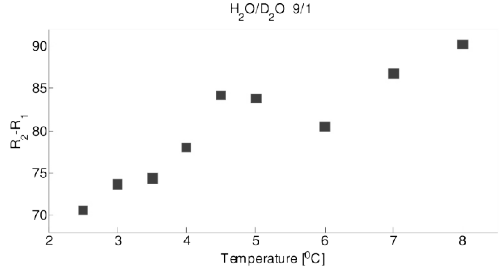
553 TU

UNUSUAL RELAXATION OF OXYGEN-17 IN WATER AT TEMPERATURE OF MAXIMUM DENSITY

Eli Bendet-Taicher, Alexej Jerschow

New York University

The study of spin-lattice and spin-spin relaxation times of ^{17}O in water in a large range of temperatures was previously measured. Relaxation processes in water generally exhibit non-Arrhenius temperature dependence, and for a small range of temperatures around the temperature of maximum density (TMD), the difference between the transverse and the longitudinal relaxation rates of ^{17}O as a function of temperature shows an anomalous behavior, expressed by local maximum at TMD. It is shown that he same anomalous behavior shifts to the different temperature of maximum density for different proton and deuterium concentrations and different salt concentrations. This phenomenon can also be seen in proton relaxation of water and can shed more light on the physical picture of structural and diffusive anomalies of water, and the anomalous mobilities of the hydronium and hydroxyl ions and proton transfer.



Poster Presentation

554 TH

DISCRIMINATION OF DIFFERENCES IN MEMBRANE CHOLESTEROL CONTENT USING ^{129}XE HYPER-CEST

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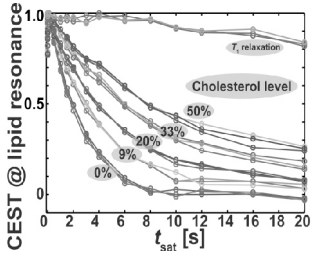
Leibniz-Institut für Molekulare Pharmakologie

In NMR spectroscopy and magnetic resonance imaging (MRI) chemical exchange saturation transfer (CEST) is used to enhance signals from dilute spin pools. The combination of hyperpolarization and CEST (Hyper-CEST) by means of hyperpolarized xenon (^{129}Xe) that is temporarily bound to cryptophane-A (CrA) enhances the signal of thermally polarized ^{129}Xe up to a factor 108. The high specificity of ^{129}Xe to its molecular environment in terms of its chemical shift range is increased upon binding to CrA. Taking advantage of Hyper-CEST's high specificity and high sensitivity we sensed differences in membrane fluidity of diluted liposomes made up of POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) – containing different fractions of cholesterol (10:1, 10:2.5, 10:5, 10:10 molar ratios) at 37 °C in 1 ml 10 mM hepes buffer containing 5 µM CrA. When the cholesterol level in the liposomal bi-layer was higher and thus the membrane fluidity lower, the ^{129}Xe depolarization process was slower when applying saturation pulses of variable lengths at the resonance frequency of the Xe host embedded in the lipid bi-layer. The detected (multi-)exponential depolarization processes were analyzed using an algorithm based on inverse Laplace transform. The high signal stability of our setup (< 1% variation) allowed us to distinguish differences in cholesterol level of less than 5 %. The algorithm was also integrated into MRI where a pixel-wise analysis led to a detailed depolarization-time map in which solutions containing POPC and DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine) liposomes could be distinguished spatially (0.39 mm² resolution). Thus the Hyper-CEST method might be a valuable tool for biomedical applications to obtain information about differences in membrane fluidity including non-invasive selective mapping of membrane distributions.



555 MO

COMBINING FIELD-CYCLING RELAXOMETRY AND STIMULATED-ECHO EXPERIMENTS: A POWERFUL-APPROACH TO STUDY ION DYNAMICS IN SOLID-STATE ELECTROLYTES

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Modern solid-state electrolytes feature complex ion dynamics, which occur on a large variety of time scales. We demonstrate that a combination of field-cycling and stimulated-echo experiments allows us to continuously follow ion dynamics over 10 orders of magnitude in time [1]. Field-cycling relaxometry provides access to the dispersion of the spin-lattice relaxation rate. We use this method for the first time to determine the frequency-dependent spectral density of ionic motion. The time window of our field-cycling experiments, 10^{-9}-10^{-5} s, complements in an ideal way that of stimulated-echo experiments, which allow us to measure correlation functions of ion dynamics in a range of 10^{-5}-10^{1} s [2-4]. We show that both methods yield a coherent picture of ion motion. In particular, they probe the elementary ionic jumps underlying the macroscopic charge transport, providing valuable information about local activation barriers controlling ionic migration.

The existence of strongly non-exponential correlation functions is a key feature of ion dynamics in solid-state electrolytes. To determine the microscopic origin of this phenomenon and, thus, to achieve a fundamental understanding of the transport mechanism, we develop stimulated-echo methods for measurements of three-time and four-time correlation functions of ion dynamics [2-4]. These higher-order correlation functions indicate that the non-exponential behaviour is due to pronounced dynamical heterogeneities, i.e., the ionic jumps exhibit a broad rate distribution, while their directions are uncorrelated on the time scale of the repopulation of ionic sites.

^{6}Li, ^{7}Li, and ^{109}Ag NMR studies are presented. The studied materials include crystals, glasses, and ceramics. It turns out that the broad time window of our combined approach is particularly useful when inhomogeneous structures lead to ion dynamics on vastly different time scales.

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Poster Presentation

556 TU

MULTIPLE TIME SCALE DYNAMICS AND MEMORY EFFECTS IN PROTEIN DYNAMICS: EVIDENCE FROM MD SIMULATIONS

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The investigation of protein dynamics has been a very active field of research in the NMR community, mainly motivated by its possible implication in biological function. However, despite the development of extremely effective NMR spectroscopic probes, the interpretation of the data still represents a central issue. Indeed, on the one hand, internal mobility in such **complex environments** as proteins are not likely adequately represented by too simple models. On the other hand, models used for the interpretation of data should be based on firm physical grounds. Progress in this direction can be made from molecular dynamics simulations, which have proven a very useful complementary tool indeed to access information at the microscopic level.

One of the aspects often overlooked in the interpretation of protein NMR relaxation is the presence of memory effects in the dynamics. The presence of non-Markovian stochasticity introduced by the complexity of the environment inside the protein, leading to a **multiplicity of time scales** was suggested by MD simulations. We showed how a **fractional Brownian dynamics** approach could account for these in the case of backbone or side-chain bond vectors. A simple model based on a two-parameter Mittag-Leffler function was shown to efficiently reproduce MD internal correlation functions.

More recently, we have undertaken a similar kind of analysis for the **chemical shift correlation functions**, and relationships between short-time and long-time behaviors are investigated. We show preliminary investigations aiming at tracing memory effects at the most fundamental level. To this aim, force correlation functions are analysed, based on Kubo's **generalized Langevin equation**, in order to observe the potential signature of a non-Markovian behaviour.

Several implications of these approaches in in the analysis of actual experimental data will be discussed.

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557 TH

INVESTIGATION OF CHLOROMETHANE COMPLEXES OF CRYPTOPHANE-A BY NMR SPIN-ECHO EXPERIMENTS

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Cryptophane molecules possess a three dimensional cavity which can encapsulate small organic compounds[1]. Because of this feature, they may have potential applications in many areas (biology, pharmacy…). But, in order to be used, the encapsulation process, leading to the formation of these host-guest complexes, has to be understood. This can be achieved with the help of Nuclear Magnetic Resonance (NMR) spectroscopy by determining the physico-chemical properties of these systems.

In our case, host-guest complexes between cryptophane-A as host and chloroform or dichloromethane as guest were investigated by spin-echo experiments: i) PGSTE diffusion experiments and ii) transverse relaxation dispersion.

A first series of diffusion experiments allowed us to measure the self-diffusion coefficient for cryptophane and dichloromethane, but also for other molecules present in the solution such as water. The results obtained have shown that dichloromethane enters the cavity while water does not, thus proving the hydrophobicity of the host cavity.

Because the encapsulation of a guest changes the probability distribution of the cryptophane conformers[2], a second series of experiments was performed. These experiments, called CPMG-relaxation dispersion[3], aim at the determination of the transverse relaxation time T_2 as a function of the repetition rate of the π pulses in a CPMG train. In the presence of chemical reactions, occurring on the time scale of micro- to milliseconds, the measured transverse relaxation rate varies with the repetition rate. This methodology was employed on our systems. The ^{13}C transverse relaxation dispersion curves obtained were fitted by assuming a fast conformational exchange. A tentative interpretation of the physico-chemical parameters deduced from the fitting is proposed.

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Poster Presentation

558 MO

SUB-t_c MOTIONS IN PROTEINS REVEALED BY HIGH-RESOLUTION NMR RELAXOMETRY

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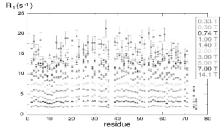
Centre National de la Recherche Scientifique and Ecole Normale Supérieure, Département de Chimie, Paris

The function of a protein is governed not only by its tri-dimensional structure but also by its internal dynamics. NMR relaxation is a powerful technique to characterize such dynamics. Traditional high-field measurements of relaxation rates provide high-resolution information but limited sampling of fast motions over narrow frequency ranges of the spectral density of motions. On the other hand, conventional relaxometry allows one to explore motions over a broad range of frequencies, albeit at the sacrifice of high resolution. High-resolution relaxometry, as introduced by A. G. Redfield, can, in principle, combine the best of both worlds but the ability to record relaxation in proteins at low magnetic fields has been limited so far by signal losses during sample motions. Here, we report the design and performance of a new shuttle system installed on a 600 MHz spectrometer, where the polarization and detection take place at high field and relaxation at various points of the stray field. We have measured longitudinal nitrogen-15 relaxation rates in ubiquitin over nearly two orders of magnitude of magnetic field from 0.33 to 14.1 T. In order to analyse low field relaxation rates and correct for the effects of relaxation during shuttling, we have developed a protocol called Iterative Correction and Analysis of Relaxation Under Shuttling (ICARUS).



We show than some key regions of ubiquitin, in particular the β1-β2 turns, are more dynamic on a nanosecond timescale than previously thought. This study demonstrates that high-resolution relaxometry with fast sample shuttling offers unprecedented information about local motions in proteins on timescales that are faster than their overall tumbling.

559 TU

HYDRATION PROPERTIES OF FUNCTIONAL GROUPS OF ORGANIC MOLECULES IN AQUEOUS SOLUTIONS

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A clear understanding of interaction of organic molecules functional groups with different environment allows us to understand the formation of the spatial structure of proteins in complicated biological solution and live tissues. It can be supposed the formation of biological nanostructures due to mutual influence of hydrophobic and hydrophilic interactions in complex solutions.

The hydration parameters of the different functional groups and the hydrocarbon skeleton can be calculated from the solvent nuclei (^{1}H, ^{2}H, ^{17}O) NMR-relaxation and results of quantum chemical calculations. The water molecules in the investigated solutions with organic molecules can be divided into several groups: hydration shells of carboxylic, amino, methylene groups and pure solvent. Relaxation rate of the solvent nuclei should be calculated as a sum of contributions of each substructure. In the case of fast exchange of the water molecules between all substructures, the spin-lattice relaxation of the solvent nuclei in the investigated system is given by simple formula.

The microstructure of the investigated water clusters around different functional groups of the organic molecules and values of relative concentrations were determined by quantum-chemical calculations (Hartree-Fock and DFT theory levels). The coordination numbers of functional groups, obtained by calculated methods, is in a very good agreement with those obtained experimentally.

As result we have investigated the temperatures and concentration features of the hydration properties of different functional groups: methylene, carboxylic, methyl and amino groups.

Poster Presentation

560 TH

ENTANGLEMENT IN QUASI-EQUILIBRIUM STATE

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It was recently shown that entanglement in quantum systems being in a non-equilibrium state can appear at much higher temperatures than in an equilibrium state. However, any system is subject to the natural relaxation process establishing equilibrium. The work deals with the numerical study of entanglement dynamics in a dipolar coupled spin-1/2 system under the transition from a non-equilibrium state to an equilibrium state. The spin system is characterized by a two-temperature density matrix, and the process of the establishment of equilibrium is in the equalization of these temperatures. The method of the non-equilibrium statistical operator is used to describe the evolution of the system. The process of establishing an equilibrium state in the homonuclear spin systems at low temperature was first considered. It was shown that the time dependences of the inverse temperatures of the spin subsystems are given by a solution of non-linear equations in contrast to the linear equations in the well-known high temperature approximation. It was first studied the entanglement dynamics during the equilibrium state establishment and found that, during establishing equilibrium, the concurrence changes non-monotonically with time and temperatures. Entanglement fades long before equilibrium is established in the system. It was shown that the entanglement dynamics depends strongly on the ratio of the Zeeman energy to the dipolar energy. At a high ratio, the concurrence in the system decreases quickly for time about 100 μs while, at a low ratio, establishment of equilibrium and fading entanglement take prolonged time up to 1 ms.

561 MO

9-BROMOTRIPTYCENE IN $CDCL_3$ SOLUTION – A NUCLEAR SPIN RELAXATION AND DFT STUDY

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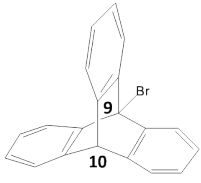
^{13}C NMR parameters of carbons connected with bromine are of great interest as their values are expected to be influenced by the relativistic effects of the substituent being an atom of the third-row element. Some of these parameters, such as ^{13}C NMR chemical shifts, can be easily measured, whereas others, e.g. ^{13}C-^{79}Br scalar spin-spin coupling constant or electric field gradient at the bromine nucleus are accessible only indirectly, via interpretation of the nuclear spin relaxation data. Essentially, the values of NMR parameters can be calculated theoretically using quantum chemistry methods. The reliability of theoretical results, however, strongly depends on the level of the method used and, when relativistic effects are involved, frequently remains unknown. Thus, it is still desirable to perform due tests.



9-bromotriptycene

The structure of 9-bromotriptycene molecule possesses co-linear C-H and C-Br bonds. This advantageous feature has allowed us to determine the reorientation correlation time, common for both bonds, from the dipolar relaxation rate of ^{13}C nucleus of C-10 atom. Simultaneously, interpretation of the relaxation rates of ^{13}C of C-9 occurring mainly by the scalar relaxation of the second kind (SC2 mechanism) has enabled determination of ^{1}J(C-Br) and EFG(Br) values. The values of these parameters as well as the magnetic shielding constants of carbon nuclei in the investigated molecule have been calculated using more or less standard [DFT B3LYP/6-311++G(2d,p) PCM] theoretical method. The comparison of the obtained experimental and theoretical data has provided a possibility of verification of the effectiveness of the theoretical method used in this work.

POSTER PRESENTATION

562 TU

EFFECT OF PARTIALLY HYDROPHOBIC ADDITIVES ON TEMPERATURE-SENSITIVE POLYMERS

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Institute of Macromolecular Chemistry AS CR v.v.i

The ability of the stimuli-responsive polymers to change their behaviour according to external conditions, and the possibility to moderate this effect make such systems interesting for the various potential applications. The thermal response of PVME and EO-PO-EO copolymers was compared in the presence of different partially hydrophobic ketone-, ether- and alcohol-based additives. According to proton and carbon NMR spectra, relaxations, and PFG NMR self-diffusion measurements, partially hydrophobic additives facilitate the conformation states decrease with the increasing hydrophobicity and bulkiness of the additive. The interaction of the ketone-based additives is indirectly proved by the decrease of their rotational and translational mobility in the presence of copolymer Pluronic L64 and PVME homopolymer. For Pluronic L64, it was shown that the temperature at which the PO chain starts to change its conformation under dehydration decreases by 6 K for each additional methyl group in the alcohol molecule (i.e. with increasing its hydrophobicity) and the analogous conformation states are attained at temperatures about 10 K lower compared with the use of ketonic analogues of the alcohols under the same conditions. According to diffusion measurements, the molar fraction of the alcohol hydrogen-bonded to L64 increases with its hydrophobicity and, in an apparent conflict with thermodynamics, with increasing temperature at which also higher NOE can be observed. Strong hydrogen bond interaction, which is in mutual cooperation with hydrophobic interaction, does not preclude the exchange between bound and free states of the alcohol, however. On the other hand, for PVME, presence of ether-based additives leads to the time dependence of the reversibility mechanism due to the precipitation of additive molecules on polymer globules. On the other hand, ketone-based additives lead to the formation of small cooperative domains caused by strong polymer-water-additive interaction.

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563 TH

STUDY ON NMR TEMPERATURE CHARACTER OF CRUDE OIL

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23MHz low field NMR instrument was used to analyze the ^{1}H transverse relaxation of crude oil at different temperature. Four kinds of crude oil were measured, which were extracted from one kind of shale and three kinds of sandstones respectively. The NMR system (produced by Shanghai Niumag Corporation) was equipped with a temperature control device allowing for ±0.1 temperature regulation. The measurement temperature changed from 35 to 105 with the increment of 10 .

Fig 1 shows that T_{2g} of crude oil increases as the temperature increasing because of the viscosity reduction. And the signal intensity should be decrease according to the NMR theory. But the signal intensity of shale oil increases oppositely as shown in Fig 2. It is indicated that we can't detect the whole signal produced by shale oil at a normal room temperature because a part of it decays so fast to catch which means we need to use high temperature when we measure fresh shale rocks. And the proper temperature should be more than 75 when the signal doesn't increase obviously.

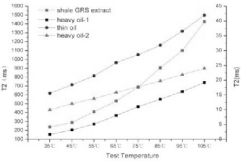

Fig.1. T_{2g} (geometry mean) of crude oil as the temperature increasing

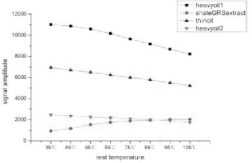

Fig.2. Oil signal intensity of crude oil as the temperature increasing

Poster Presentation

564 MO

MAGNETIC PROPERTIES AND NMR RELAXATION OF DIFFERENT-SIZED IRON OXIDE NANOPARTICLES

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Iron oxide nanoparticles are of great interest in nanomedicine. They are used in Magnetic Resonance Imaging (MRI) as negative contrast agent. In this work, we explored magnetic properties and Nuclear Magnetic Resonance (NMR) relaxation of different-sized magnetite nanoparticles (Fe_3O_4).
First, by using Vibrating Sample Magnetometry (VSM), we performed Zero-Field-Cooling (ZFC) and Field-Cooling (FC) curves. Samples were cooled to very low temperature under zero magnetic field (for the ZFC) or under 10 mT (for the FC). At 2 K, the magnetic field was fixed at 10 mT for both curves and magnetization measurements were made for increasing temperatures. The average blocking temperature of the iron oxide crystals was determined as the maximum of the ZFC.
Then, we carried out, at different temperatures, magnetization measurements in function of the magnetic field. Best Langevin fits to data yielded the average nanoparticles radius and a standard deviation estimation of the lognormal size distribution. Based on the average blocking temperature and the average radius, we calculated the uniaxial magnetic anisotropy constant K_A.
Finally, we performed relaxometric measurements using low resolution relaxometry and Fast Field Cycling (FFC) relaxometry. T_1 and T_2 were determined at two Larmor frequencies, 28,8 MHz and 38,7 MHz corresponding respectively to 0,68 T and 0,91 T. All the relaxometric measurements were made at 37°C. The longitudinal relaxation rate R_1 was obtained by a saturation-recovery sequence (SR) and the transverse relaxation rate R_2 by a Carr Purcell Meiboom Gill sequence (CPMG). Solutions with different concentrations were prepared for each sample. The relaxivities r_1 and r_2 were calculated as the straight line slope linking R_1 and R_2 relaxation rates to the concentration. The obtained results are compared to the prediction of well established relaxation theories.

565 TU

^{2}H NMR STUDY OF MAGNETIZATION RELAXATION BEHAVIOR OF SELECTIVELY LABELED MESOGENS IN MONODOMAIN LIQUID CRYSTAL ELASTOMERS

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Liquid crystal elastomers (LCEs) are materials with combining properties of elastomer elasticity and orientational ordering of liquid crystal molecules [1]. One of the most exciting features of these materials is their macroscopic shape change as a result of the order-disorder transition of the mesogens. This unique feature has resulted in a rising interest in LCEs, since it renders them exploitable to many different fields of applications.
With increasing temperature, monodomain LCEs exhibit a mechanical deformation, i.e., a thermomechanical contraction, related to disordering and thermal motion of the mesogenic molecules, attached to the polymer backbone. Apart from the studies of temperature dependences of the quadrupole-perturbed ^{2}H-NMR spectra, related to orientational reorganization of the LCE network, spin-spin and spin-lattice relaxation has never been studied in detail. Owing to the recent availability of selectively-deuterated systems, we performed a systematic study of the T_1 and T_2 temperature behavior of the M6 mesogen, ^{2}H-labeled in the phenyl ring and at the α- position, respectively, as well as of the phenyl ring labeled M6 mesogen dopant, which was introduced into the elastomeric matrix by soaking in a M6-doped toluene/cyclohexane solution.

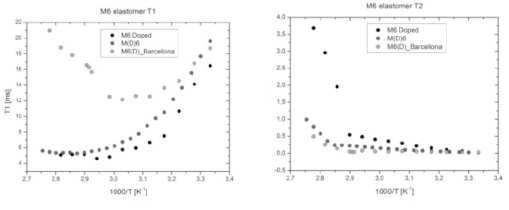

Figure 1: T_1 (left panel) and T_2 (right panel) of selectively deuterated LCEs

Poster Presentation

566 TH

EVALUATING THE CONTRIBUTIONS TO ^{1}H LONGITUDINAL RELAXATION ENHANCEMENT THROUGH ^{13}C DETECTED EXPERIMENTS FOR THE INVESTIGATION OF INTRINSICALLY DISORDERED PROTEINS

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Intrinsically disordered proteins (IDPs) have recently attracted the attention of the scientific community thanks to their peculiar properties that confer them functional advantages compared to well folded proteins. Thanks to its ability to characterize both structured and unstructured proteins, NMR thus becomes a unique tool to access atomic resolution information. However the peculiar properties of IDPs, such as lack of a stable 3D structure, the high flexibility, the largely exposed backbones, do have an impact on NMR observables. Therefore NMR experiments should be optimized to address the peculiar features of IDPs. Heteronuclei, characterized by a large chemical shift dispersion, are indeed mandatory to study IDPs. Thanks to recent improvements in instrumental sensitivity, ^{13}C detected NMR experiments have been developed and provide a useful tool to study IDPs.
We present here several variants of the 2D CON experiments to determine ^{1}H inversion recovery profiles under different initial conditions in order to evaluate which are the most efficient longitudinal relaxation enhancement mechanisms and if they can provide some information on the local solvent accessibility or local compactness. The experiments are tested on two well studied proteins, a structured one (ubiquitin) and an intrinsically disordered one (-synuclein).

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567 MO

FLEXIBLE PARTS OF THE PRION PROTEIN FRAGMENT HET-S(218-289): SPECTRAL ASSIGNMENT AND DYNAMICS ANALYSIS

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The HET-s protein is produced by the filamentus fungus Podospora anserina. This protein propagates as a self-replicating amyloid and is involved in programmed cell death [1]. It has been shown by solid-state NMR that the prion part of the HET-s protein [HET-s(218-289)] contains rigid, well-ordered parts. The structure of HET-s(218-289) has been solved on the basis of intra- and intermolecular distance restraints with four β-strands forming two windings of a β-solenoid [2]. On the other hand, well-resolved ^{13}C, ^{15}N and ^{1}H resonances have also been observed in INEPT-based experiments [3]. These flexible residues are probably located in the N and C termini and in the loop connecting the rigid β-sheets β2b and β3a.
We performed an HNCA experiment based on INEPT transfers [4] to assign the flexible parts of HET-s(218-289) ^{2}H, ^{13}C, ^{15}N with back-exchanged amide protons. We found convenient in terms of signal-to-noise ratio to use an HRMAS probe with coils for creating pulse field gradients for solvent suppression. MAS averages susceptibility differences due to the presence of the fibrils. We assigned about 90% of the residues present in the N terminus (residues 218 to 225), in the C terminus (residues 283 to 289) and in the loop connecting the rigid β-sheets β2b and β3a (residues 247 to 261). We are trying to gain more insight about the dynamics of HET-s(218-289) with the help of site-resolved relaxation studies. The correlation time of the motion within the protein can be calculated using T_1 relaxation values together with order parameters obtained via measurement of dipolar couplings.

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568 TU

THE EFFECT OF THE MANUFACTURE METHODOLOGY ON THE DOSIMETRIC CHARACTERISTICS OF NORMOXIC N-VINYLPYRROLIDONE BASED POLYMER GELS AS ASSESSED BY qMRI METHODS

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Purpose
The significance of polymer gel dosimeters is their ability of 3D assessment of the irradiation dose. Precision and accuracy are crucial factors for their application in clinical practice. A major limitation regarding their dosimetric performance is their sensitivity in oxygen contamination. In the current study, the effect of manufacture methodology for two modified batches of normoxic N-vinylpyrrolidone-based polymer gels fabricated in anoxic (VIPAR^{N2V}) and in normal atmospheric (VIPET^{2V}) conditions was investigated.
Materials and Methods
200mL VIPAR^{N2V} were manufactured inside an Argon glove box, containing 5% w/w gelatin, 8% v/v N-vinylpyrrolidone, 4% w/w bisacrylamide and 5mM THPC and 200 mL VIPET^{2V} were manufactured inside a laminar flow hood with the same recipe. One day post-preparation, the phantoms were irradiated at different areas covering a dose range from 0.5 to 60Gy. The irradiated phantoms were scanned at a clinical MRI system one day post-irradiation, utilizing a specially designed Multi Echo Spin Echo pulse sequence (Phase Alternating Phase Shift) technique. The R2 and dose color parametric maps were produced for the assessment of the dosimetric characteristics of VIPAR^{N2V} and VIPET^{2V} gels.
Results
Both VIPAR^{N2V} and VIPET^{2V} gels exhibited a linear R2-dose response in the range of 1 up to 30 Gy. However, differences in the sensitivity and R2 values were observed between the polymer gel batches. Dose resolution values of VIPET^{2V} were increased compared to VIPAR^{N2V} gels. The comparison of the dose profiles of both polymer gels one day post-irradiation revealed that they presented a stable dose distribution.
Discussion
The VIPAR^{N2V} gels were improved as compared to VIPET^{2V} dose sensitivity and dose resolution. This result revealed the effect manufacturing procedure in the dosimetric characteristics of each polymer gel. Regarding the spatial integrity, both polymer gels exhibited a spatially stable dose distribution.

569 TH

FITTING REGRESSION ALGORITHMS IN qMRI POLYMER GEL DOSIMETRY

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Department of Medical Physics, University of Crete, Greece

Purpose
The unique characteristic of polymer gel dosimeters is their capability of 3D dose verification in radiotherapy. In the current study, three fitting regression algorithms were utilized for the estimation of the dosimetric characteristic of N-vinylpyrrolidone based polymer gels.
Materials and methods
200mL VIPAR^{N2V} were manufactured inside an Argon glove box, containing 5% w/w gelatin, 8% v/v N-vinylpyrrolidone, 4% w/w bisacrylamide and 5mM THPC. One day post-preparation, the phantoms were irradiated at different areas covering a dose range from 0.5 to 60Gy. The irradiated phantoms were scanned at a clinical MRI system one day, one week, two weeks and one month post-irradiation, utilizing a specially designed Multi Echo Spin Echo pulse sequence (Phase Alternating Phase Shift) technique. The calculated T2 color parametric maps were produced by the utilization of three different fitting regression algorithms. Finally, the dosimetric characteristics of VIPAR^{N2V} gels were assessed and compared with the three different fitting methods.
Results
All the algorithms were sensitive for the same dose range. Minor deviations were observed in dose response at low doses and greater at doses above 20Gy. The dose sensitivity, the offset ($R2_0$) and the dose resolution values were estimated for each algorithm. The dosimetric characteristics differed amongst them. The dose response remained stable for the period of one month post-irradiation independently of the used algorithm.
Discussions
The dose sensitivities were improved; the offset and the dose resolution values were increased in relation to the amount of the noise reduction for the used algorithm. On the other hand, their corresponding errors were also increased, respectively. Moreover, the temporal stability of the dosimetric characteristics was not affected by the selected algorithm.

Poster Presentation

570 MO

FISHNET COIL: NEW TRANSVERSE RESONATOR FOR NMR/MRI EXPERIMENTS

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The reciprocity theorem relates the sensitivity in NMR experiments with the efficiency of the coil, which is working as radio-frequency (RF) detector [1]. Therefore, the higher is the magnetic field per unit current produced by the coil, the higher is the sensitivity observed during the experiment.
The most efficient coil is the solenoid. It provides the highest RF magnetic field per unit current and the best spatial field homogeneity. However, the RF magnetic field is oriented parallel with respect to the axis of the coil. This orientation presents some practical drawbacks when working with longitudinal magnets for accessing the sample and for achieving large sample volumes.
During the last decade, different types of transverse resonators have been developed. They produce a transverse RF magnetic field with respect to their longitudinal axis [1,2]- however, they have lower efficiency than solenoids. Our purpose is to present a new RF transverse resonator which has an efficiency closer to the solenoid performance, than the above-mentioned coils. We report nutation experiments performed with the new design at high field (11T) and low magnetic field (1T). We compare the nutation curves with commercial probes and equivalent saddle coils in order to show the performance of the new resonator and we showed an up to 30% better sensitivity. These results allow us to present new configurations and applications where this design could increase the sensitivity considerably.

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571 TU

ON THE POTENTIAL OF RADICAL-BASED, FAST-DISSOLUTION DNP FOR PROTEIN NMR STUDIES

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Hyperpolarization of water protons is proposed as a way to improve spectra quality in biomolecular Nuclear Magnetic Resonance spectroscopy (NMR). High-field dynamic nuclear polarization methods were developed in the past few years to facilitate NMR structural biology applications. Most promising among these methods is the use of dissolution DNP, to improve NMR sensitivity in liquids studies. A main obstacle in this aim is the fast relaxation that hyperpolarized biomolecules would undergo upon transferring from the cryogenic hyperpolarizer, to the NMR spectrometer where their observation would take place.
In order to cope with this limitation the present study explores the use of hyperpolarized water, as a mean to enhance the sensitivity of nuclei in biomolecules.
Upon dissolution within the NMR spectrometer tube, the biomolecule's amide groups could undergo rapid exchange of their protons with the hyperpolarized species coming from the water. This exchange allows for the fast polarization transfer between the water protons to the amide protons and the backbone 15N nuclei, and to significant signal enhancement.

Poster Presentation

572 TH

IMAGING OF 1H LONG LIVED STATES ORIGINATED FROM PARA-HYDROGEN INDUCED POLARIZATION IN CS SYMMETRICAL MOLECULES

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Parahydrogen Induced Polarization (PHIP) creates a hyperpolarized spin state by hydrogenation of a double or triple bond with parahydrogen[1]. A drawback of all the hyperpolarization techniques is the limited lifetime, since it is usually relaxing to the thermal equilibrium with the spin-lattice relaxation time T_1. A longer lifetime can be achieved by storing the hyperpolarization as a singlet spin state. A requirement for the singlet state preservation is the reducing of the chemical shift (Cs) difference usually achieved by moving the sample to a low field or applying dedicated pulse sequences[2]. Recently, it was shown for the Cs-symmetric molecule dimethyl maleate that the hyperpolarized proton singlet state can be stored at 7 T for 4 min with no need of rf pulses[3,4]. To yield measurable magnetization the sample was transported to the resonance magnetic field at 0.1 T allowing for singlet-triplet conversion via level anti-crossing. Here, the singlet-triplet conversion is performed inside the observation field by an RF pulse sequence and combine with imaging.

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573 MO

EXPLOITING LEVEL ANTI-CROSSINGS FOR EFFICIENT AND SELECTIVE TRANSFER OF NUCLEAR SPIN HYPERPOLARIZATION

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Nuclear spin hyperpolarization can be coherently transferred to other nuclei in field-cycling NMR experiments. At low magnetic fields, where nuclear spins are strongly coupled, hyperpolarization is redistributed in the spin-coupled network. Polarization transfer is most efficient at fields where level anti-crossings (LACs) occur for the nuclear spin-states. A further condition is that field switching to the LAC positions is non-adiabatic in order to convert the starting population differences into spin coherences that cause time-dependent mixing of states. The power of this method has been demonstrated by studying transfer of photo-Chemically Induced Dynamic Nuclear Polarization (photo-CIDNP) in amino acids. We have investigated the magnetic field dependence and kinetics of coherent CIDNP transfer and directly assessed nuclear spin LACs by studying polarization transfer at specific field positions.

We also performed a study of Para-Hydrogen Induced Polarization (PHIP) transfer at low external magnetic fields. Although it was known that PHIP is efficiently transferred at low field the transfer mechanism, *i.e.*, coherent spin mixing or cross-relaxation, remained unknown. To this end polarization we for the first time measured and modelled theoretically the transfer kinetics, which contained pronounced quantum beats indicating coherent transfer mechanism. Spin coherences have been excited by passing through a LAC of the nuclear spin energy levels. By exploiting LACs it is also possible to achieve selectivity of the transfer. Our work gives evidence that coherent PHIP transfer mechanism is dominant at low magnetic field.

The proposed approach based on LACs is not limited to CIDNP and PHIP but is advantageous for enhancing NMR signals by spin order transfer from any type of hyper-polarized nuclei.

This work has been supported by the Russian Foundation for Basic Research (projects 12-03-31042, 12-03-33082, 12-03-31775, 13-03-00437), the Alexander von Humboldt Foundation, EU-COST Action TD1103 and the program of Russian Government P220 (agreement No.11.G34.31.0045).

Poster Presentation

574 TU

PHIP IN THE WATER-PHASE HYDROGENATION OVER IMMOBILIZED IRIDIUM CATALYSTS

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It is known that the method of nuclear magnetic resonance (NMR) has low sensitivity, which is associated with small population differences of spin energy levels. Parahydrogen induced polarization (PHIP) is one of the methods which can significantly increase the population differences, and consequently increase the sensitivity of NMR techniques. Commonly, PHIP produced in homogeneous hydrogenations using solution of transition metal complexes as catalysts. The advantages of these catalytic systems are high extent of pairwise addition and high rate of hydrogen transfer to a substrate molecule in comparison to the nuclear relaxation and orto-para conversion rates. These properties allow achieving significant enhancement of NMR signals. It should be noted, however, that the homogeneous catalysts are difficult to separate from the reaction mixture, which sets restrictions for PHIP application in studies of biological objects, for instance. The catalytic systems based on immobilized transition metal complexes are promising for generation of PHIP, because they combines the advantages of both homogeneous and heterogeneous catalysts. Here, hydrogenation of 3-butene-2-ol and 2-propenamide in water-d_2 solution over silica immobilized complexes was investigated in terms of production of hyperpolarized substances. The immobilized catalysts was prepared starting from iridium complexes $[Ir(COD)Cl]_2$ (1) and $[Ir(COD)(Py)_2][PF_6]$ (2), which were immobilized on phosphine-modified silica gel (PPh_2/SiO_2). Both PASADENA and ALTDENA PHIP was observed. In the case of 2-propenamide hydrogenation over (1)PPh_2/SiO_2, a two orders of magnitude enhancement of NMR signal was detected, while for 3-butene-2-ol hydrogenation the PHIP is not observed despite the fact that hydrogenation reaction took place. The reasons of such interesting finding are discussed.

This work was supported by the grants from RFBR (11-03-00248-a, 12-03-00403-a, 12-03-31386-mol_a), RAS (5.1.1), SB RAS (60, 61, 57, 122), the program of support of leading scientific schools (NSh-2429.2012.3), the program of the Russian Government to support leading scientists (11.G34.31.0045).

575 TH

DYNAMIC NUCLEAR POLARIZATION USING FREQUENCY MODULATION AT 3.34 TESLA

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Dynamic nuclear polarization (DNP) is a method that can transfer polarization from unpaired electrons to their neighbouring nuclear spins, resulting in dramatically enhanced NMR signals. This polarization transfer depends on microwave (MW) irradiation of samples that contains radicals. In most cases continuous wave irradiation is applied. It was recently shown by Thurber et al. (JMR 2010) that modulating the field during DNP on solid samples can increase the DNP enhancements by a factor of <2.4.

In this work we examine the DNP enhancement efficiency under conditions of MW frequency modulation, using our 3.34 T static DNP system (Feintuch et al., JMR 2011). This allows for more flexibility in terms of the modulation parameters, and results in less heat deposition on the sample with respect to magnetic field modulation.

The efficiency of triangle shaped MW modulated fields was explored on the ^{1}H signal enhancement in frozen solutions containing TEMPOL radicals. This was explored for different radical concentration (5-40 mM) and sample temperatures (10-50 K), and as a function of the modulation frequency, amplitude and base frequency, and under the most favourable conditions resulted in as much as 3 times higher enhancements with respect to constant frequency DNP.

The optimal range of the modulation parameters will be described, and the key features will be explained based on simulating a small model systems exhibiting either solid effect DNP or cross effect DNP (Hovav et al.: JMR 2010, JCP 2011, JMR 2012; Shimon et al., PCCP 2012).

576 MO

ENHANCING NMR SENSITIVITY THROUGH ENGINEERED SINGLETS AND HYPERPOLARISATION

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Recent progress in the understanding of singlet relaxation mechanisms enables one to engineer molecules exhibiting very long-lived nuclear singlet order in room-temperature liquids. These systems may serve as vehicles for storing and transporting hyperpolarized nuclear spin order. We have developed models for the most important singlet relaxation mechanisms and used molecular dynamics and quantum chemistry calculations to guide the design of molecular systems with long singlet relaxation times for 13C pairs. We have achieved singlet lifetimes exceeding 30 min in a room temperature solution at a low field strength of 2 mT. However, stabilizing singlet states in high magnetic field is even more challenging. Here, the differences in chemical shift anisotropy between the two spins forming the singlet become an important source of relaxation.
We have designed and synthesized molecules that significantly reduce the difference in chemical shift anisotropy leading to singlet relaxation times of approximately 10 min at 7 Tesla, which is about 30 times longer than T_1 under the same conditions. By means of *J*-synchronized echoes, singlet order can be converted into triplet order and vice versa at will. The combination of singlet NMR with dissolution dynamic nuclear polarisation leads to detectable amounts of spin order at times exceeding the spin-lattice relaxation time by nearly two orders of magnitude.

577 TU

TOWARDS NANOMOLAR DETECTION BY NMR THROUGH SABRE HYPERPOLARIZATION

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NMR spectroscopy has proven to be an extremely important analytical tool in the field of (bio)chemistry and medicine. However, it suffers from an inherent low sensitivity due to the small population difference between nuclear spin states at thermal equilibrium. Hyperpolarization techniques have been developed to overcome this inherent insensitivity by creating non-Boltzmann spin state distributions. Signal Amplification By Reversible Exchange (SABRE)[1], uses a nuclear spin isomer of molecular hydrogen as source of polarization. This *para*-hydrogen interacts reversibly with a substrate molecule through a metallo-organic complex, resulting in greatly enhanced signals of unmodified substrate molecules in solution.

So far, SABRE has been used at relatively high concentrations, mostly millimolars of substrate. Hereby we present a method to extend the applicability of SABRE to dilute samples, making the detection of analytes in the low micromolar to nanomolar concentration range possible in a single scan experiment. Detecting these concentrations with NMR spectroscopy is exceptional, and promising for the application of NMR in trace analysis.

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Poster Presentation

578 TH

PARAHYDROGEN INDUCED POLARIZATION STUDY OF UNSATURATED COMPOUNDS HYDROGENATION OVER SUPPORTED METAL CATALYSTS

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Parahydrogen-induced polarization (PHIP) is a very informative method for the investigation of mechanisms of hydrogenation reactions. It also can be used as a source of a significant enhancement of NMR signals which is very important for magnetic resonance applications. This method is based on the pairwise addition of two hydrogen atoms from the same parahydrogen molecule to the multiple bond of substrate molecule.
Hydrogenation of α,β-unsaturated carbonyl compounds over supported metal catalysts is an industrially important process, which has a great significance for producing pharmaceuticals, perfumes and flavouring materials. The hydrogenation of C=O bond in these compounds is complicated due to thermodynamically favored hydrogenation of C=C bond. So, it is highly important to investigate the mechanism of this reaction.
In this work it was shown that PHIP effect can be successfully observed in hydrogenation of acrolein and crotonaldehyde C=C bond over different supported monometallic and bimetallic Pd-, Pt- and Rh-based catalysts.
For the first time the PHIP effect was observed in heterogeneous hydrogenation of unsaturated compounds over bulk metals (Pd, Pt, Rh) and oxides (CaO, Cr_2O_3, CeO_2, PtO_2, PdO, Rh_2O_3) instead of supported metal catalysts.
It was shown that the using of single-atom Au supported on carbon nanotubes as a hydrogenation catalyst provides significantly higher (no less than 10%) percentage of pairwise hydrogen addition compared to supported metal nanoparticles. It was found that the reaction orders with respect to H_2 for propene hydrogenation over Pt/Al_2O_3 are equal to 0.1 and 0.7 for the non-pairwise and the pairwise hydrogen addition, respectively. Therefore, different types of active sites on catalyst surface are responsible for the pairwise and the non-pairwise hydrogen addition.

This work was supported by the RFBR (11-03-00248-a, 12-03-00403-a), RAS (5.1.1), SB RAS (60, 61, 57, 122), (NSh-2429.2012.3), the Council on Grants of the President of the Russian Federation (MK-4391.2013.3) and program of the Russian Government to support leading scientists (11.G34.31.0045).

579 MO

A CRYOGENIC GENERATOR FOR THE CONTINUOUS FLOW PRODUCTION OF HYPERPOLARIZED PARAHYDROGEN GAS

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Dissolution DNP[1] combines the substantial polarization achievable in low temperature solid state DNP with detection at room temperature in the liquid state. It is rapidly becoming the technique of choice to enhance the sensitivity of NMR and MRI experiments in particular in the study of metabolic processes *in vivo*[2]. It has however the disadvantage of being a single shot technique and it is inherently impossible to infuse DNP hyperpolarized substrate into the subject of interest over a prolonged period of time.
Other well-known methods to produce high level of polarization in liquid state samples are PHIP[3] and SABRE[4], which is the non-hydrogenative version of PHIP. Both methods use the high nuclear polarization of parahydrogen to polarize other nuclei in the sample and are in principle capable of producing hyperpolarized molecules “on the fly”, thereby enabling infusion studies of metabolic processes. In this contribution, a novel design is presented for a cryogenic parahydrogen generator. The instrument was specifically developed for the high yield, continuous flow production of parahydrogen gas, enabling hyperpolarized NMR and MRI experiments under conditions of prolonged infusion of hyperpolarized material. The calculated parahydrogen yield, based on the operating temperature of the instrument, is verified by Raman spectroscopy. Early hyperpolarized NMR results using the SABRE method are shown.

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Poster Presentation

580 TU

A STRUCTURALLY LOW INVASIVE LABEL FOR PHIP IN BIOMOLECULES

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Nuclear Magnetic Resonance (NMR) spectroscopy and Magnetic Resonance Imaging (MRI) are essential methods widely applied in medicine and drug research. Currently, functional imaging or molecular imaging is improved to get deeper insight into processes on a molecular scale.[] Nonetheless, magnetic resonance applications are limited by the inherent low sensitivity which is mainly caused by the low population difference of the nuclear spin levels. During the last decades efforts were made to increase this population difference, employing Para-Hydrogen-Induced Polarization (PHIP).[,] The PHIP method bases on polarization transfer *via* a hydrogenation reaction with para enriched hydrogen.
The challenge is to find structurally low invasive building blocks to generate biomolecules for applications of PHIP[, ,] The unsaturated side chain of L-propargylglycine (Pra) was used in a systematic study of synthetic oligopeptides *via* PHIP. For the first time PHIP-initiated NMR signal enhancement was observed in peptides bearing various functional side chains.[]This enables new approaches to the investigation of biomolecules and may be of crucial importance in the development of new signal-enhanced MRI methods.

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581 TH

ADIABATIC CROSS POLARIZATION VIA DIPOLAR ORDER AS A B_1-INSESITIVE AND LOW-B_1 ALTERNATIVE TO HARTMANN-HAHN CROSS POLARIZATION IN DISSOLUTION DNP

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The gain in polarization using dissolution dynamic nuclear polarization (dDNP) is accompanied by a significant lengthening of the minimum repetition rate. Typical repetition rates are in the order of one hour in conventional polarizers using direct ^{13}C DNP. Besides the development of multi-sample polarizers, it has been shown that the repetition time can be reduced by the combination of dDNP with Hartmann-Hahn cross polarization (CP) [1,2]. The main technical challenge here is the demand for B_1 fields sufficiently high to be efficient and sufficiently homogeneous enough to match the Hartmann-Hahn condition over the full sample. Lee et al. [3] have shown the possibility of partial adiabatic polarization transfer from ^1H to ^{13}C Zeeman order via heteronuclear dipolar order in static powders using very low-B_1 fields for adiabatic de-/remagnetization in the laboratory frame (AD/RLF) at room temperature.
Here, we present an investigation and the realization of the AD/RLF polarization transfer applied under dDNP conditions. We describe the implementation and optimization of the AD/RLF pulses using an automatic frequency-sweeping mode in the FPGA-based OPENCORE spectrometer [4]. We show that the optimum polarization transfer occurs at B_1 field strengths in the range of 1.5 kHz (^1H) and 8 kHz (^{13}C) for TEMPO-doped [^{13}C]urea samples and that the transfer efficiency is insensitive to B_1 variations around the optimum. The required B_1 fields are well within the range of the performance of typical dDNP probes. To further optimize the transfer efficiency, the spin-lattice relaxation times of the dipolar order are measured as a function of the radical concentration. Furthermore, the dipolar rotating-frame relaxation times and the mixing times of dipolar and Zeeman order in the rotating frame are presented as a function of B_1 strength supplying valuable information for the optimization of the AD/RLF – CP sequence.

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Poster Presentation

582 MO

DNP AT 9.4T AND 4.3K: PROSPECTS OF DISSOLUTION DNP AT HIGH FIELD

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Dynamic Nuclear Polarization (DNP) at low temperatures in combination with rapid dissolution to the liquid state is a hyperpolarization technique of choice for low gamma nuclear spins such as ^{13}C. Extensive levels of polarization (typ. $P(^{13}C) = 30 - 40\%$) can be attained by direct ^{13}C polarization with narrow ESR line polarizing agents (for example trityl or BDPA, etc.) at $T = 1$ K and $B_0 = 3.35$ T, however at the expense of lengthy DNP build-up of several hours. We have recently demonstrated how DNP could be boosted by utilizing the broad ESR line polarizing agent TEMPOL in combination with low temperature cross polarization (CP) from protons to ^{13}C. Increasing the magnetic field to $B_0 = 6.7$ T provided substantial improvement over results obtained at $B_0 = 3.35$ T. Polarization values as large as $P(^{13}C) = 70\%$ at $T = 1.2$ K and $P(^{13}C) = 15\%$ at $T = 4.2$ K were reported. In view of investigating DNP at even higher field, we have designed and built a static CP-DNP probe operating at $T = 4.3$ K and $B_0 = 9.4$ T in conjunction with a conventional 400 MHz/263 GHz Bruker DNP machine equipped with a gyrotron (see abstract "Probe for Static DNP at 263 GHz and Liquid Helium Temperature" by Armin Purea *et al.*). $P(^1H) = 22\%$ was reached within a short build-up time $t_{DNP} = 39.5$ s, and readily transferred to ^{13}C by CP in a 1-^{13}C sodium acetate sample containing 55mM of TEMPOL as polarizing agent. $P(^{13}C) > 18\%$ was attained, which confirmed that DNP further increases with increasing field. DNP results with irradiation over of the whole ESR spectrum will be presented, as well as DNP polarization levels and build-up rates as a function of radical concentration. The possibility of further improvements, for example by utilizing bi-radical polarization agents such as TOTAPOL or btbk, will be discussed.

Even though CP-DNP at higher fields $B_0 \geq 9.4$ T delivers high polarization levels, the cost of microwave sources at 263GHz is a severe drawback. The prospect of having cost-effective solid-state microwave sources available in the future makes high field DNP an attractive proposition.

583 TU

VERY HIGH CONCENTRATION SENSITIVITY PULSE EPR

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For many biological applications involving pulse EPR such as PELDOR and ENDOR, concentration sensitivity is often the most critical parameter. In this poster we show experimentally that by operating at high frequencies, with high powers and high sample volumes it is/will be possible to increase concentration sensitivity by two to three orders of magnitude relative to standard measurements made at X-band using optimised cavities.

In particular, we describe a W-band pulse EPR system operating at power outputs of 1kW, in non-resonant induction mode with a large instantaneous bandwidth. We demonstrate that this system offers increases in sensitivity of the order of 30 for PELDOR measurements relative to X-band using similar sample volumes. In this system the sample is multi-wavelengths long, where we stress the importance of sample annealing to create a high quality glass.

We also experimentally show that further very significant increases in sensitivity are possible for PELDOR (and other EPR applications) using a variety of methods including:

I Further increasing the sample volume – making use of simple shimming techniques to correct for B_0 inhomogeneity

ii) Making use of composite pulses (or optimal control) to increase sensitivity for both pump and detection pulses in PELDOR sequences

iii) The use of 3-pulse Stitch DEER, which can offer significant increases in sensitivity for this particular system

iv) The use of higher power levels. New broad-band amplifiers offer increased power levels over extended frequency ranges – offering the potential of PELDOR involving metal centres

We believe three orders of magnitude increase in instrumental sensitivity is possible, relative to standard X-band measurements, with further major gains (at any frequency), if it is possible to deuterate the protein – for PELDOR measurements involving long distances.

POSTER PRESENTATION

584 TH

FINDING ACTIVE LIGANDS TO kRAS USING SOLUTION STATE NMR

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Pharmacology, [+]Protein Chemistry and [¯]Cell Regulation, Genentech Inc. 1 DNA Way, South San Francisco 94080 CA, .USA

RAS has been the classical oncology target for many years and yet to date; no small molecule drug has been described that targets this protein directly. RAS mutations are drivers for a number of cancers (1) and kRAS (2) is mutated in the majority of pancreatic cancers. We have used a fragment based lead discovery (FBLD) workflow (3) to identify and biophysically characterize binders to kRAS from a library of diverse fragments. Solution state ligand-detected NMR methods were used to identify fragments that bind to the protein. The binders were validated using isotope-labeled protein and amide proton and nitrogen chemical shift perturbations. Ligands were identified that bind to a pocket on a functionally relevant face of kRAS. This region is involved in the interaction of the GTPase activating protein (GAP), the GTP exchange factor (SOS) and the effector (PI3K). The ligand-based screen of 3300 compounds produced 786 primary hits; a cascade of follow-up measurements validated 83. Of those, 25 compounds showed similar chemical shift perturbations indicating one preferred binding site. High-resolution RAS/ligand complex structures were determined by crystallography. Guided by the complex structures, we have identified a small molecule ligand that shows activity in a biochemical assay, underlining the functional relevance of the binding site (4).

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585 MO

31P NMR STUDY ON GTP HYDROLYSIS IN TAXOL-STABILIZED MICROTUBULES

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Rapid exchange and hydrolysis of the tubulin-bound guanine nucleotides are important steps in controlling the dynamics of microtubules [1]. However, the instability microtubules and a low concentration of the nucleotides have been the obstacle to direct observation of the status of the microtubule-bound GTPs [2]. In this study, we report the first 31P NMR signals acquired from the microtubule-bound GTP by employing a solid-state NMR technique. A distinctive 31P CPMAS NMR spectrum (S/N ~ 7.5) was acquired from a lyophilized microtubules which was freshly prepared with taxol. Hydrolysis of the GTPs in the microtubules is presumably halted by lyophilisation, and the 31P NMR signals from a low concentrations (1~2 μmoles) are enhanced by 1H 31P cross-polarization.
Analysis of the 31P NMR spectrum clearly indicates the presence of gamma-phosphates as much as the alfa- and beta-forms. In addition, the resonance from the gamma-phosphates was shifted by 4-ppm to a higher frequency compared with a control sample, while others stayed almost at the same shifts. Our results suggest that the dominant form of guanine nucleotides in the taxol-stabilized microtubules is GTP whose gamma-phosphates may be bound directly to the metal Mg(2+) ions.

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Poster Presentation

586 TU

MOLECULAR STRUCTURE AND SPECTROSCOPIC ANALYSIS OF HOMOVANILIC ACID AND ITS SODIUM SALT – NMR, FT-IR AND DFT STUDIES

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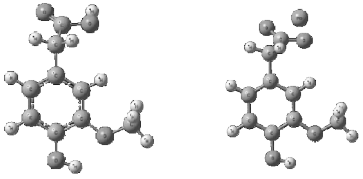
Division of Chemistry, Bialystok University of Technology, Zamenhofa 29

Homovanilic acid has been investigated by various researches because of its biological activities. The estimation of the electronic charge distribution in metal complexes and salts allows to predict what kind of deformation of the electronic system of ligand would undergo during complexation. It also permits to make more precise interpretation of mechanism by which metals affect the biochemical poperties of ligands. In this paper the influence of sodium cation on the electronic system of homovanilic acid was studied. Optimized geometrical structures of studied compounds were calculated by B3LYP/6-311++G** method. Mulliken and NPA (Natural Population Analysis) atomic charges were analyzed. The theoretical NMR and IR spectra were obtained. ^{1}H and ^{13}C NMR as well as FT-IR spectra of studied compounds were also recoreded and analyzed. The calculated parameters are compared with experimental characteristics of these molecules.



Acknowledgements: The project was funded by the National Science Center granted by decision number DEC-2011/01/B/NZ9/06830.

587 TH

A COMPARISON STUDY ON MOLECULAR STRUCTURE OF PHENYLACETIC- AND 3-HYDROXYPHENYLYCETIC ACIDS USING NMR, FT-IR SPECTROSCOPY AND THEORETICAL CALCULATIONS

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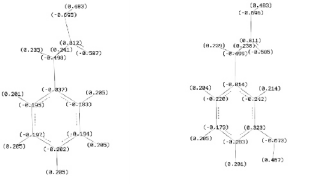
Phenylacetic and 3-hydroxyphenylacetic acids are biologically active compounds. The changes in physical, chemical and biological properties of them decided about their effect on the biological systems. In this paper molecular structure of 3-hydroxyphenylacetic acid in comparison to phenylacetic acid is studied using NMR, FT-IR spectroscopy as well as density functional hybrid method (DFT) calculations .



The ^{1}H and ^{13}C NMR spectra of DMSO saturated solution were recorded with the NMR AC 200 F, Bruker unit at room temperature. TMS was used as an internal reference. The B3LYP/ 6-311++G** method was used to calculate optimized geometrical structures of studied compounds. The Mulliken, APT and NPA (Fig.) atomic charges, dipole moments and energies were calculated as well as the chemical shifts in NMR spectra and the wavenumbers and intensities of IR spectra. Theoretical parameters were compared to experimental characteristic of studied compounds.

Acknowledgments

The project was funded by the National Science Center granted by decision number DEC-2011/01/B/NZ9/06830.

Poster Presentation

588 MO

NMR INVESTIGATION OF STRONG INTRAMOLECULAR HYDROGEN BONDS IN 5-NITROSOPYRIMIDINES

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Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nam. 2, 16610, Prague, Czech Republic

Pyrimidine derivatives are naturally occurring as precursors for nucleic acid (DNA, RNA) synthesis. Furthermore, there is a large class of pharmacologically important pyrimidine derivatives that act for example as compounds with antiviral or antibacterial activities, antidepressants and inhibitors of cyclin-dependent kinase as potential drug candidates for cancer therapy.
In this work, a series of 5-nitrosopyrimidines with *para*-substituted phenylamino substituent in position 6 and monosubstituted amino group (cykloalkylamino, alkylamino, amino and acylamino) in position 4 was prepared. The nitroso group in the position 5 can exist in two rotamers and form intramolecular hydrogen bonds with the NH group in the position 4 or 6. The resulting hydrogen bonds are very strong and usually both forms were observed in ^{1}H and ^{13}C NMR spectra acquired at room temperature. Even at 150 °C we did not observe fast interconversion of the two forms. The ratio of the two possible rotamers could be finely tuned in the whole range (35 – 91% of the 6-NH hydrogen bond) by the selection of the substituents in the positions 4 and 6. For example, electron-donating substituents in the *para*-position of the phenyl ring increased the amount of the form with the nitroso group heading towards the 6-NH group. This effect correlated well with Hammett constants. Furthermore, a strong influence of the substitution on chemical reactivity and UV-VIS absorption spectra was observed. The ratio of the two possible rotamers was successfully predicted by DFT calculations. We have also calculated chemical shifts of both forms and the calculated data agreed well with the experimental ones. Additionally, we calculated the barriers of interconversion between the two nitroso group rotamers and the exceptional stability of the intramolecular hydrogen bonds observed by NMR was confirmed.
This work was supported by Grant Agency of the Czech Republic No. 13-24880S.

589 TU

INTER-LIGAND NUCLEAR OVERHAUSER EFFECT (ILOE) NMR

Tomas Jacso, Ulf Borjesson, Victoria Ullah, Thomas Olsson, Tomas Akerud

Astra Zeneca

Fragment Based Lead Generation (FBLG) has emerged as a potent tool to complement High Throughput Screening (HTS) in the search of drug candidates in the pharmaceutical industry. Identifying and linking vicinity binding fragments can result in highly potent binders. To guide this process accurate spatial information is required. Solution state NMR is able to give such information through NOE experiments. Here we use the Inter-Ligand Nuclear Overhauser Effect (ILOE) to retrieve distance information between protons in close proximity of two fragments in their protein bound state.
The distance restraints between the two molecules are then used to design a linked high-affinity binder. Using libraries of small fragments to probe proximity binding to a known hot-spot warhead molecule, with the aim of creating high-affinity binders, would greatly aid the lead generation process. The proximity binders probes the vicinity of the binding pocket, in respect to the warhead molecule, identifying the appropriate chemistry for the expansion.

POSTER PRESENTATION

590 TH

ANALYSIS OF COMPLEX MIXTURES USING SPECTRAL ALIASING. APPLICATION TO THE GLUCOSE OXIME FORMATION

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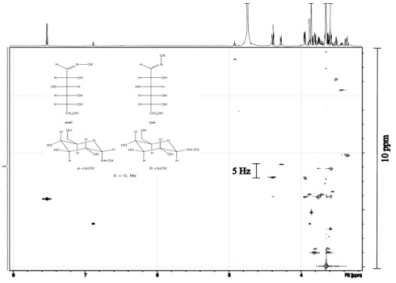
30, quai Ernest Ansermet 1211. Geneva Switzerland

Carbohydrates are important biomolecules that mediate many biological processes and exist in different conformations that make them difficult to characterize. The chemical reactions of carbohydrates give rise to several derivatives with biological relevance, *e. g*. oximes and hydrazones which have found widespread applications, such as biomolecules labelling, analyzing protein−protein interactions and in vivo cell imaging. Spectral aliasing is a technique that improves the resolution in the indirect dimension (F1) dimension of 2D spectra and can be accessed with a simple reduction of the spectral window to the desired value.



The 1H, 13C -HSQC with 10 ppm in the carbon dimension was used for achieving a complete assignment of 1H and 13C of the components of the reaction mixture without any further purification. This makes spectral aliasing an useful tool for the analysis of complex mixtures of compounds.

591 MO

DETECTION OF LOW NATURALLY ABUNDANT (ENANTIO)-ISOTOPOMERS BY 2D-NMR IN LIQUIDS AND CHIRAL ORIENTED SOLVENTS: THE INTERESTING CASE OF ^{2}H-^{13}C PAIRS

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[b] *Unité de Catalyse et de Chimie du Solide, UMR-CNRS 8181, Université de Lille 1, ENSCL, Cité Scientifique, Bât. C7, F-59652, Villeneuve d'Ascq cedex, France.*

The recent technological and methodological developments in NMR spectroscopy have dramatically decreased its detection limits, thus expanding its application areas (1).
In this work, we report the first experimental detections of ^{2}H-^{13}C isotopomers in natural abundance (1.7×10^{-4}%) both in liquids and chiral oriented systems by combining cryoprobe, high magnetic field (21 T) and optimised heteronuclear 2D experiments (2,3).
The 2D pulse sequence entitled "NASDAC" derives from the ^{2}H-^{13}C HETCOR sequence with ^{13}C detection (2). Compared to HMQC and HSQC methods, this scheme exhibits a higher sensitivity, especially for short recycling delays. As NASDAC experiments require the detection of one molecule among 580,000, the coherence pathway selection is achieved by combining both pulsed field gradients and phase cycling. A variant of this experiment was designed to refocus the anisotropic ^{2}H chemical shifts in F_1. In liquids, a systematic isotopic shielding of ^{13}C resonances was measured by comparing the F_2 projection of NASDAC spectrum with the ^{13}C-{^{1}H} 1D spectrum. This stems from the influence of (^{1}H/^{2}H) substitution on ^{13}C chemical shifts. For the first time, we have access to (^{1}H/^{2}H) isotope effects without any deuterium enrichment (4). Interestingly, we show that ^{2}H-^{13}C enantio-isotopomers can be distinguished using 2D NMR in chiral polypeptide alignment media, thus providing a new tool for the chiral analysis. This method could open fruitful prospects in various analytical fields such as molecular source authentication, metabolism studies or the fight against counterfeiting.

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POSTER PRESENTATION

592 TU

SCULPTING BIOACTIVE COMPOUNDS THROUGH ENZYME-CATALYZED REGIOSELECTIVE ACYLATION OF NATURAL PRODUCTS: RAPID PRODUCT SCREENING AND BIOLOGICAL EVALUATION

Alexandra Chatzikonstantinou[1], Eleni Kyriakou[1], Nisar Sayyad[1], Athena Papadopoulou[2], Ioannis P. Gerothanassis[1], Haralambos Stamatis[2], Andreas G. Tzakos[1*]

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Natural products cover a very interesting chemical space of biological relevance due to their vast chemical diversity, and fine-tuning for optimal interactions with biological macromolecules through evolutionary selection[1]. However, their inherent scaffold intricacy and the associated complexity in their synthetic chemistry have challenged their effective integration in the drug discovery pipeline. To overcome this, we exploit chemoenzymatic tools to regioselectively enable rapid installation of chemical functional groups to the natural product core[2,3]. Transformation efficacy and regioselectivity can be readily monitored *in situ* through rapid and simple NMR spectra of the crude reaction products without any prior isolation or fractionation[2,3]. The functionalized natural products have been then accessed for their bioactivity in traditionally thought "undruggable" targets[4]. Different examples of natural product based molecular hybridization[5] as also natural product scaffold sculpting and associated biological evaluation in *in vitro* cancer cell lines will be presented.

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593TH

DIRECT NMR IDENTIFICATION AND QUANTIFICATION OF GEOMETRIC ISOMERS OF CONJUGATED LINOLEIC ACID IN MILK LIPIDS WITHOUT ISOLATION OR DERIVATISATION STEPS

Theodore Skarlasa, Constantinos G. Tsiafoulisb, Ouranios Tzamaloukasc, Despoina Miltiadouc, Ioannis P. Gerothanassis

Department of Chemistry, University of Ioannina, Ioannina GR-45 110, Greece
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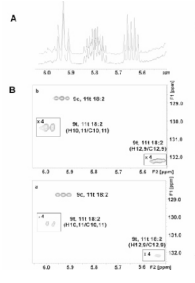
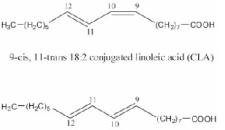
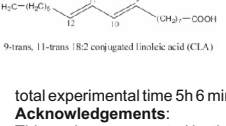
We report the first successful direct and unequivocal identification and quantification of four minor geometric 9-cis, 11-trans 18:2, 9-trans, 11-cis 18:2, 9-cis, 11-cis 18:2 and 9-trans, 11-trans 18:2 conjugated linoleic acid (CLA) isomers in milk with the combined use of 1D 1H, 2D 1H-1H TOCSY and 2D 1H-13 C HSQC NMR. The significant sensitivity and resolution barriers have been successfully overcome under selective suppression of the major NMR resonances, with over 10 4 greater equilibrium magnetization of the -(CH2)n- 1H spins compared to that of the 1H spins of the conjugated bonds of the CLA isomers, and reduced 13C spectral width in the 1H- 13C HSQC experiment. The method does not require any isolation or derivatisation steps.







Selected regions of 500 MHz 1D 1H (A) (298 K, ns = 256, AQ = 4.3 sec, total experimental time 40 min) and 2D 1H-13C HSQC (B) spectra of a raw milk sample without (a) and (b) with spiking 40 μl of 2.31 mM solution of the 9-trans, 11-trans 18:2 CLA isomer. (298 K, 40 repetitions of 256 increments, total experimental time 5h 6 min)

Acknowledgements:
This work was supported by the Cyprus Research Promotion Foundation and Charalambides-Christies Ltd dairy industry.

Poster Presentation

594 MO

ENZYMATIC AND CHEMICAL SYNTHESIS OF A NOVEL ANTIOXIDANT-ANTICANCER HYBRID WITH POTENT ANTIPROLIFERATIVE ACTIVITY

Nisar Sayyad[1], Elena Geromichalou[1], Alexandra Chatzikonstantinou[1], Eleni Kyriakou[1], , Efstathia Giannopoulou[2], Haralabos P. Kalofonos[2], Haris Stamatis[3], Ioannis P. Gerothanassis, Andreas G. Tzakos[1*]

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Recent science evidenced the interlinkage of oxidative stress and cancer. Due to the inherent complexity of cancer and its accompanying effect of oxidative stress, novel molecules, containing combinatorial functionalities should be targeted[1]. Herein, we synthesized gemcitabine-5`-O-lipoate derived from a regioselective coupling of the chemotherapy drug gemcitabine, a first-line agent for cancer therapy and α-lipoic acid, a potent antioxidant. Gemcitabine-5`-O-lipoate was obtained in 4 chemical steps. To avoid the tedious and laborious chemical steps we also utilized biocatalysis and the optimum conditions for the regioselective and one-pot synthesis of gemcitabine-5`-O-lipoate where established by exploiting different solvents (organic solvents and ionic liquids) and enzyme immobilization (acrylic resin and carbon nanotubes). Cytotoxic activity of co-administrating gemcitabine and α-lipoic acid was proven to be synergistic against non-small cell lung cancer cells whereas antagonistic for bladder cancer cells. In contrast, the Gemcitabine-5`-O-lipoate hybrid was found to be superior to the parent compounds against both non-small cell lung cancer and bladder cancer cells.

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Enzymatic hybridization of α-lipoic acid with bioactive compounds in ionic solvents. Papadopoulou AA, Katsoura MH, Chatzikonstantinou A, Kyriakou E, Polydera AC, Tzakos AG, Stamatis H. Bioresour Technol. 2013 Feb 27;136C:41-48.

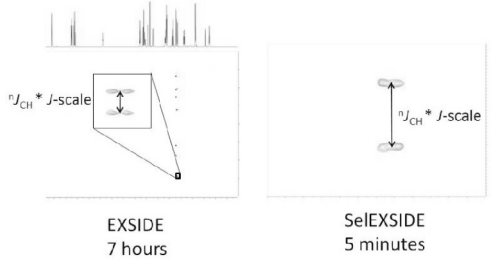
595 TU

NEWER! FASTER! BETTER? WAYS TO MEASURE $^{N}J_{CH}$ IN SMALL MOLECULES - SELEXSIDE

Craig Butts, Godiraone Tatolo, Bert Heise, Ikenna Ndukwe

University of Bristol

Multiple-bond ^{13}C-^{1}H scalar coupling constants ($^{n}J_{CH}$) are substantially under-utilised in studies of small molecule structure and dynamics – primarily because the methods available for their measurement can be less than reliable, sensitive or accurate. We present a ^{13}C-band selective-EXSIDE (SelEXSIDE) as a very rapid, easily interpreted approach to measuring individual $^{n}J_{CH}$ values with high accuracy in only a few minutes. Ease-of-use, reliability, accuracy and sensitivity of SelEXSIDE will be discussed and contrasted with alternative methods.



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Poster Presentation

596 TH

^{3}C NMR Spectroscopy for the differentiation of enantiomers in complex systems using chiral solvating agents

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InstituteServei de Ressonància Magnètica Nuclear, Universitat Autònoma de Barcelona, Bellaterra, Catalonia, Spain

Chiral molecules are present in nature (natural chiral compounds) and in the world of synthetic compounds (synthetic chiral compounds). Most of the endogenous metabolites are chiral compounds and so are many drugs and reactants. The different character of two enantiomeric molecules is manifested when they are within a chiral environment, what happens to be intrinsic in nature. There is an important interest in distinguishing enantiomers in different areas, not only in the pharmaceutical industry (drugs and their degradation products) but also in areas working with natural chiral compounds such as metabonomics.

NMR spectroscopy used in combination with chiral solvating agents (CSAs) is a simple, rapid and robust method to differentiate enantiomers without requiring derivatization or purification of the sample. These advantages make that methodology suitable for the differentiation of enantiomers within complex mixtures as we have recently proved in the field of metabonomics, named Chiral Metabonomics.[1] ^{1}H is by further the most used nuclei using such methodology. However, when dealing with complex systems such as complex biological mixtures (e.g. biofluids) or pure chiral compounds with complex ^{1}H spectra, typical situations of overlapping and partial resolution of signals impede the enantiodifferentiation study. We evaluate 1D ^{13}C NMR spectroscopy as an alternative since ^{13}C is also present in most organic molecules and has two main advantages: (i) a much higher dispersion and (ii) an easily obtention of a well resolved decoupled spectrum. On the contrary, the sensitivity of ^{13}C is much lower than that of the ^{1}H nuclei. Parameters such as sensitivity, resolution, quantification and signal overlapping are assessed.

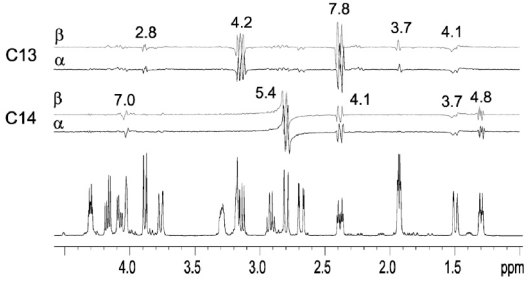
597 MO

ON THE INTERFERENCE OF J(HH) MODULATION IN HSQMBC-IPAP AND HMBC-IPAP EXPERIMENTS

Josep Saurí and Teodor Parella

Universitat Autònoma de Barcelona, Servei de RMN (SeRMN)

The effects of phase signal modulation due to homonuclear proton-proton coupling constants in HSQMBC-IPAP and HMBC-IPAP experiments are experimentally evaluated.
We shown that accurate values of small proton-carbon coupling constants, ^{n}J(CH), can be extracted even for phase-distorted cross-peaks obtained from a selHSQMBC experiment applied simultaneously on two mutually J-coupled protons. On the other hand, an assessment of the reliability of ^{n}J(CH) measurement from distorted cross-peaks obtained in broadband IPAP versions of equivalent HMBC and HSQMBC experiments is also presented. Finally, we show that HMBC-COSY experiments could be an excellent complement to HMBC for the measurement of small ^{n}J(CH) values.



Poster Presentation

598 TU

COMBINED NMR-UV/VIS AND NMR-FTIR STUDIES OF HYDROGEN BONDED COMPLEXES IN APROTIC SOLVENTS: HYDROGEN BOND CORRELATIONS AND PROTON TRANSFER PATHWAY

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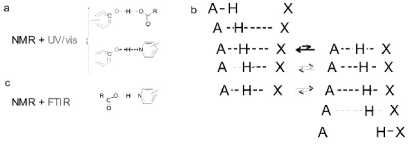
A quite recently developed method for the in-situ measurement of UV-vis absorption spectra inside an NMR probe (Tolstoy et al., Angew. Chem. Int. Ed., 2009, 48, 5745) is employed to study phenol carboxylate and phenol pyridine complexes (Figure a) in aprotic solvents. It is shown that the ^{13}C1 chemical shifts and UV-vis absorption frequencies of the phenolic residues reflect time averaged and time resolved proton positions, respectively, and quantitative hydrogen bond correlations as well as a qualitative scheme for a detailed proton transfer pathway involving equilibria between tautomeric structures interconverting fast on the NMR time scale are proposed (Figure b; Koeppe et al., J. Am. Chem. Soc. 2011, 133, 7897). Further studies of the impact of solvent and temperature conditions on the geometries of the hydrogen bond in phenol carboxylate complexes reveal that medium effects can qualitatively be explained in terms of stabilization of localized charges by polar media (Koeppe et al., J. Am. Chem. Soc. 2013, accepted). Chloroacetic acid pyridine complexes (Figure c) are studied by NMR and FTIR spectroscopy. ^{12}C-^{13}C isotope effects are exploited for the spectral assignment of the C=O stretching mode. Notably, for systems with shortest hydrogen bonds, dual bands (50 cm^{-1} splitting) are observed which are – again - indicative of the coexistence of tautomeric structures (Figure b).



599 TH

SYMMETRIC [N-X-N]$^+$ HALOGEN BONDS ARE PREFERRED IN SOLUTION

Anna-Carin Carlsson, Jürgen Gräfenstein, Alavi Karim, Jesse Laurila, Ulrika Brath, Roland Kleinmaier, Martin Uhrbom, and Máté Erdélyi

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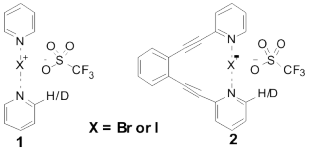
Halogen bonding (XB) is a close to linear, noncovalent interaction between an electropositive halogen (X) and an electron donating species. Due to its strong resemblance with hydrogen bonding (HB), XB is now regarded as a potential complementary tool to HB in the control of molecular recognition and crystal engineering. Whereas the symmetry of NHN and OHO H-bonds has been extensively investigated, the understanding of the behaviour of the analogous [NXN]$^+$ halogen bonds in solution is still in its infancy.
By applying the NMR technique isotopic perturbation of equilibrium (IPE) using ^{13}C NMR detection to two bispyridine-based [N−X−N]$^+$ model systems, one freely adjustable (1) and one conformationally restricted (2), we have successfully determined the symmetries of [N−I−N]$^+$ and [N−Br−N]$^+$ halogen bonds in CD_2Cl_2 solution. IPE NMR is a sensitive method based on isotopic substitution. The observation of isotope shifts makes it possible to distinguish a static symmetric complex ([N···X$^+$···N]) with a single well energy potential from an asymmetric complex comprised of two rapidly equilibrating tautomers ([N$^+$–X···N]$^+$ [N···X–N$^+$]) with a double well energy potential. Preference for a symmetric arrangement was observed for both [N−X−N]$^+$ model systems studied. Diffusion ^{1}H and ^{19}F NMR experiments for determination of the degree of ion pairing of the [N−X−N]$^+$ cation and the triflate revealed a strong coordination. The fact that the symmetric arrangement of the [N−X−N]$^+$ cation was not disturbed despite a strongly attached counterion, indicates a high energetic gain upon the formation of symmetric [N−X−N]$^+$ halogen bonds. Computational studies on the DFT level confirmed the preferred symmetric arrangement. Even in the more polar solvent CD_3CN, the [N−X−N]$^+$ complexes remained static symmetric.



Poster Presentation

600 MO

CONFORMATIONAL STUDY OF BIS-NITROGEN CONTAINING OLIGOMERS FOR THE DESIGN OF NANOTUBES

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Foldamers are oligomers able to adopt a well-defined secondary structure (1). Numerous pseudopeptidic motives have been described and most of them adopt a helix conformation (2-4). Some of these helical foldamers, such as oligourea (2) present an extra nitrogen atom in the backbone. Bis-nitrogen containing pseudo-peptides are particularly interesting since it was assume that the additional nitrogen atom could be an additional acceptor site for a hydrogen bond.
In this context we present oligo-2,1-[a/aza]-mers containing aza aminoacids in which the a carbon is replaced by a nitrogen. The N-N pattern has already been described as a turn inducer and it has been shown that the induced hydrazino-turn is stabilized by an two hydrogen bonds (5). Various sequences have been synthesized with difference length. Linear as well as cyclic oligomers have been studied and their conformations have been characterized by X-ray diffraction, infrared spectroscopy and NMR. ^{1}H, and ^{13}C complete resonances have been assigned in chloroform. ROESY spectra showed characteristic NOE correlations which allow us to solve the structure. Finally, the association of cyclic bis-nitrogen containing oligomers has been studied and nanotubes have been observed.

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601 TU

STRUCTURE ELUCIDATION AND VALIDATION OF SMALL MOLECULES: THE LSD AND CASA SOFTWARE

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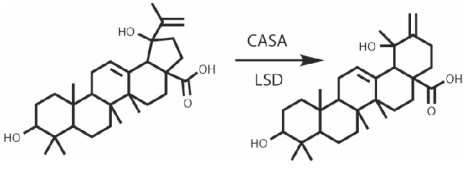
The validation of a molecular organic structure on the basis of 1D and 2D HSQC, COSY and HMBC NMR spectra is proposed as an alternative to the methods that are mainly based on chemical shift prediction. The CASA software was written for this purpose. The tentative matching between the structure and the NMR data of a triterpene unexpectedly lead to the hypothesis of an incorrect structure. The LSD software was used to find an alternative structure that improved the 2D NMR data interpretation and the carbon-13 chemical shift matching between experimental values and those produced by the nmrshiftdb2 prediction tool.



The LSD software proposes the structures of small organic molecules that fit with structural constraints from 1D and 2D NMR spectroscopy. Its initial design introduced limits that needed to be eliminated in order to extend its scope and help its users choose the most likely structure among those proposed. The LSD software code has been improved, so that it recognizes a wider set of atom types to build molecules. More flexibility has been given in the interpretation of 2D NMR data, including the automatic detection of very long-range correlations. A program named pyLSD was written to deal with problems in which atom types are ambiguously defined. It also provides a carbon-13 NMR chemical shift-based solution ranking algorithm. PyLSD was able to propose the correct structure of hexacyclinol, a natural product whose structure determination has been highly controversal. The solution was ranked first within a list of ten structures that were produced by pyLSD from literature NMR data.

Poster Presentation

602 TH

USING NMR TO UNDERSTAND THE DISSOLUTION OF PHARMACEUTICAL TABLETS

Leslie P. Hughes, Steven Coombes, Andrew R. Phillips, Stephen A.C. Wren

Astra Zeneca

Dissolution is the process by which drugs are released from tablets into aqueous solution and so available for absorption into the body. Measuring the *in-vitro* dissolution of drugs and other soluble tablet components, e.g. soluble fillers and carrier matrices, is important for a greater understanding of the mechanisms that control the performance of the tablets. Dissolution testing is pivotal to demonstrating product quality and manufacturing control as well as underpinning new formulation development.
Soluble fillers and carrier matrices control the dissolution performance of immediate release products and enabling formulations, e.g. polymers in solid dispersions, and yet the vast majority of these are invisible to conventional pharmacopeial testing methods using ultra-violet (UV) detection.
Here we report how the innovative application of NMR techniques combined with a flow-cell have given insight into the relationship between the dissolution rates of an excipient and three active ingredients in the commercially available combination product: Beechams All-in-One Tablets™. One of the excipients, lactose, is invisible to UV and the UV absorption spectra of the three active ingredients are so severely overlapped that the solution concentration of each could not easily be determined.
Conventional thinking suggests that NMR is unsuitable for dissolution testing given its low sensitivity, compared to UV, and the preference for deuterated solvents. We have challenged this by using a medium field NMR spectrometer fitted with a cryo-probe and the implementation of solvent suppression techniques on-flow. This means that "lossy" protic dissolution media such as pH 6.8 phosphate buffer can be used, and dissolution carried out under normal pharmacopieal conditions.
A flow-cell, developed at the U.o.Manchester, fits easily into a conventional 5 mm cryo-probe. Using flow-rates of 2 ml/min the peak line shape and signal:noise ratio were sufficiently good to enable the determination of the solution concentration of each of the individual active ingredients and the excipient lactose with a temporal resolution of 80 s.

603 MO

NATURAL POLYPHENOL INTERACTION WITH BCL-2 AND BCL-XL VIA IN VITRO STUDIES, NMR SPECTROSCOPY AND DOCKING CALCULATIONS

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Apoptosis, the programmed cell death, when is deregulated participates in the pathogenesis of several diseases including cancer thereby allowing the expansion of tumor cell clones that eventually develop resistance to apoptotic cell death. Indeed, the latter is one of the six hallmark of cancer.[1] The current knowledge on the process of apoptosis suggests that it is regulated by a balancing interplay between pro- and anti-apoptotic members of the Bcl-2 family of regulatory proteins. Currently, designing small molecules that mimic the BH3 domain of the pro-apoptotic Bcl-2 family proteins is a currently challenging topic aiming at developing agents that inhibit anti-apoptotic Bcl-2 proteins (BH3 mimetic) driving cancer cells to apoptosis.[2] Quercetin is a natural polyphenol that has drawn much attention because it exerts anticancer effects, while sparing normal cells and has been reported to intervene with the anti-apoptotic proteins of the Bcl-2 family.[3] Though, the exact mode of apoptotic-driven action of quercetin remained elusive. In the present study a multidisciplinary approach has been employed in an effort to reveal its mode of action, including cytotoxicity cell assays, biochemical approaches, ^{1}H-^{15}N HSQC NMR spectroscopy and docking calculations.

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Acknowledgments This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: Heracleitus II.

Poster Presentation

604 TU

WHAT IS THE LOWEST CONCENTRATION OF A MINOR COMPONENT THAT CAN BE DETECTED BY ^{1}H DQ MAS EXPERIMENTS IN PHARMACEUTICAL SOLIDS?

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1) Analytical & Quality Evaluation Research Laboratories, Daiichi Sankyo Co., Ltd., 2) Department of Physics, University of Warwick

Salt formation is the most common and effective method of improving the aqueous solubility and dissolution rate of acidic and basic pharmaceutical products.[1] However, during various processing steps a salt can become unstable chemically and, physically, it may transform to a free acid/base or another polymorph or solvate. The solid state transformation from the salt to free form will alter the properties of the API and this is extremely undesired. In order to monitor the quality of drugs, an effective and highly sensitive quantitation method is required. Solid state NMR spectroscopy has become a standard tool for analytical research in pharmaceutics.[2] While the workhorse method is ^{13}C CP MAS, the potential of applying ^{1}H solid-state NMR is being increasingly recognised. Specifically, the ^{1}H double-quantum (DQ) solid-state NMR experiment (DQ MAS and DQ CRAMPS) is a powerful probe of dipolar-coupled protons, with DQ peaks being observed for close (typically less than 3.5 Å) through-space H-H proximities.[3,4] Thus, a two-dimensional ^{1}H DQ spectrum represents a "fingerprint" for a specific three-dimensional packing arrangement adopted by an organic molecule.[5] A drawback of the ^{1}H DQ CRAMPS method is the presence of spectral noise due to the homonuclear decoupling, i.e., a lower detection limit could be expected in a ^{1}H DQ MAS experiment at high magnetic field. We have recorded ^{1}H DQ MAS spectra at 850 MHz for a cimetidine hydrochloride salt/free form mixture (20%, 10%, 5%, 1% and 0.5% free form) samples and determined the limit of detection for the free form component as 1%. This result is better than published limits of detection by both powder X-ray diffraction and ^{13}C CP MAS NMR for minor polymorph components. This results is important for the pharmaceutical industry, e.g., offering the potential to enable polymorph conversion to be detected much earlier in e.g. stability testing.

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605 TH

MECHANISTIC INVESTIGATION OF THE 1,4-ADDITION REACTION OF ORGANOZINC REAGENTS CATALYSED BY CHIRAL PHOSPHORAMIDITE-COPPER COMPLEXES

Carina Koch, Felicitas von Rekowski, Ruth M. Gschwind

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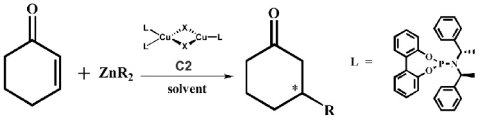
Due to high enantioselectivity, nearly quantitative yields and relatively low cost, the 1,4-addition reaction - catalysed by a chiral phosphoramidite-copper complex - is a widely used method for the formation of new C-C-bonds.[1-4]

Recently our group was able to elucidate the temperature-dependent complex structures in solution. Using a catalytic system consisting of chiral phosphoramidite ligands and copper(I) salts, we were able to identify complex **C2** as the precatalytic complex of this reaction. This new structural motif shows a trigonal/tetrahedral coordination on copper, and thus offers a free coordination side for the transmetalation reagent.[5-7]



After this elucidation we focused on the transmetalation step, which is postulated as first step in the proposed mechanism after the addition of an organozinc reagent to the precatalytic system.[7] In our studies we are using variable temperature NMR spectroscopy (170-230 K) and a combination of 1D and 2D ^{1}H^{31}P-HMBC spectra. After a screening of different organometallic reagents (MeLi, ZnR_2; R=Et, Me, Ph) we are able to present the first direct experimental proof of such a transmetalated species. However the structure of the found transmetalated species is not completely elucidated until now.

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Poster Presentation

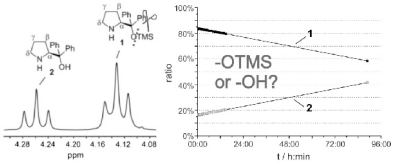
606 MO

WHAT IS YOUR ACTUAL CATALYST? TMS CLEAVAGE RATES OF DIARYLPROLINOL SILYL ETHERS STUDIED BY IN SITU NMR

Michael H. Haindl, Markus B. Schmid, Kirsten Zeitler and Ruth M. Gschwind

Institut für Organische Chemie – Universität Regensburg

Diarylprolinol silyl ethers are organocatalysts known for their broad application in various enamine and iminium-type synthetic transformations. However by losing the TMS protection group they can degradate to the corresponding diarylprolinols and subsequently form oxazolidines with aldehydes which may significantly affect their catalytic performance. A systematic NMR investigation on the loss of this silyl group is presented. By using in situ NMR we determined the cleavage rates of diarylprolinol silyl ether organocatalysts in various solvents depending on solvent properties, acidic/basic additives and the presence of water. Highly polar solvents with strong hydrogen bond acceptor properties and especially moderate acidic additives with pKa (DMSO) values around 10 accelerate the deprotection significantly, whereas basic and highly acidic additives are not promoting the cleavage process. In additional mechanistic studies silyl degradation products have been detected which explain the deprotection under dry conditions and show that the substitution reaction takes place at the silicon atom. We expect our findings to aid chemists working with diarylprolinol silyl ethers with the choice of reaction conditions to prevent unwanted cleavage of their catalyst.



Ref.: Michael H. Haindl, Markus B. Schmid, Kirsten Zeitler and Ruth M. Gschwind, *RSC Adv.*, 2012, **2**, 5941-5943

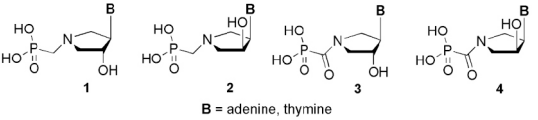
607 TU

CONFORMATION OF PYRROLIDINE NUCLEOTIDE ANALOGUES - NMR AND MOLECULAR MODELLING STUDY

Radek Pohl, Dominik Rejman, Miloš Buděšínský, Petr Kočalka, Ivan Rosenberg

Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo n. 2, 166 10 Prague, Czech Republic

Conformation of sugar ring in nucleosides and nucleotides influences their geometry, biological properties and self assembly in oligonucleotides and nucleic acids. Recently, we have prepared nucleotide analogues **1-4** where furanose ring is replaced by pyrrolidine with phosphonomethyl or phosphonoformyl moiety attached to pyrrolidine nitrogen.



We have found that the nature of nitrogen substituent determines physicochemical properties of the analogues, for example pD dependence of NMR spectra or conformation of pyrrolidine ring. The pyrrolidine ring conformation was investigated experimentally using vicinal coupling constants of ring protons and PSEUROT program, and theoretically using DFT B3LYP/6-31G* energy calculation of individual conformers covering whole pseudorotation pathway in 18 degree steps with constant sugar pucker 40 degrees. Subsequently, the most stable conformers were fully optimized and their geometries compared with those obtained by PSEUROT conformation analysis. Based on the results, we can conclude that pyrrolidine nitrogen substituent allows tuning of conformation of pyrrolidine ring in the pyrrolidine nucleotide analogues.

ACKNOWLEDGEMENT:
This work was supported by the Czech Science Foundation (Grant No. 13-24880S)

POSTER PRESENTATION

608 TH

COSMOS PROGRAM: A PROMISING RDC EVALUATION TOOL FROM RIGID TO FLEXIBLE ORGANIC MOLECULES

Pavleta Tzvetkova, Thomas Gloge, Ulrich Sternberg, Burkhard Luy

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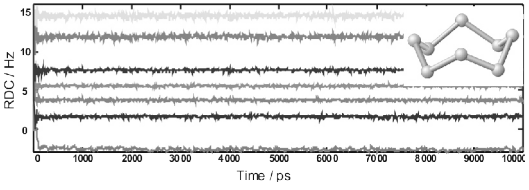

The program COSMOS uses molecular dynamics simulations with orientational constraints (MDOC). Such constraints are based on scalar and residual dipolar couplings (RDCs). MDOC simulations are performed in vacuum and allow the orientation and reorientation of the molecule to take place, so that the motional time averages would represent the tensorial NMR properties of the experimentally measured parameters. The full tensorial calculation performed allows improved characterisation of the orientation of the system. This feature is in contrast to other approaches for RDC analysis where an alignment tensor is calculated for a sterically fixed orientational model, therefore this makes the COSMOS approach in principle suitable for flexible molecules.
We have performed an evaluation of the application of the COSMOS program for a small number of organic molecules with varying flexibility: from rigid models as strychnine to a flexible organic compound with e.g. five-membered rings. The run is monitored with two parameters such as a quality factor for the correspondence of experimental and calculated data and the overall temperature of the MD simulation. The quality measure for the RDCs is defined as the summed squared difference of the experimental and calculated RDC divided by the respective error. High quality measure and low overall temperature during the simulation serve as an indication for the correct structure. Here, we present preliminary results obtained within the evaluation process.

609 MO

NMR STUDY OF CONFORMATIONALLY RESTRICTED ACYCLIC PHOSPHONATE NUCLEOTIDES

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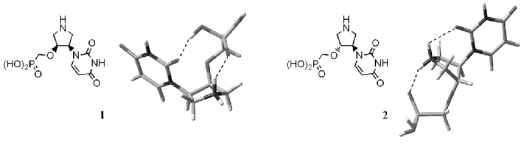
Acyclic phosphonate nucleotides represent an important group of biological active compounds used in treatment of HIV and hepatitis B. A systematic investigation in this area led us to synthesize nucleotide analogues **1** and **2**, where conformation of acyclic phosphonate part is locked *via* pyrrolidine ring.



The conformation preferences of pyrrolidine ring were determined by ^{1}H NMR spectra, particularly by extracting of ^{3}J(H,H) coupling constants of ring protons, which were used as an input for PSEUROT program. Due to the presence of both acidic and basic functionalities in the molecule we explored a pD dependence of ^{1}H, ^{13}C and ^{31}P chemical shifts changes. We have observed that the conformation of pyrrolidine ring doesn't significantly change up to pD 10. However at pD > 10 we noticed changes in ^{3}J(H,H) indicating conformation changes of pyrrolidine ring due to deprotonation of uracil and pyrrolidinium moiety. The experimental observation was completed by DFT molecular modeling studies. We have found that the most stable conformers obtained by B3LYP/6-31G* optimization correspond to those obtained by PSEUROT conformation analysis. In addition, as we supposed, the stabilities of particular conformers of both isomers were regulated by the presence of hydrogen bonds.

This work was supported by the Czech Science Foundation (Grant No. 13-24880S).

Poster Presentation

610 TU

DYNAMIC NMR INVESTIGATION OF N-ME GROUP ROTATION IN DIMETHYLATED ARGININES

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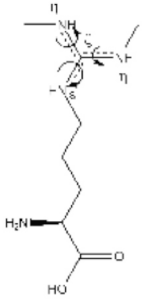
Symmetrically and asymmetrically N-dimethylated arginine (sDMA / aDMA) are known to act as recognition motifs for binding to Tudor protein domains. Published data suggest different binding modes of sDMA in different Tudor domains.

We have investigated the rotational barriers of the guanidine C-N partial double bonds by dynamic NMR over a wide temperature range. With methanol or a 50:50 methanol/water mixture as solvent, it was possible to take NMR data as low as 200 K and 230 K, resp., thus freezing out any high-barrier bond rotations.



For sDMA and aDMA only a single barrier was observed in the temperature range between 200 and 310 K, with a rotation barrier of ca. 12 kcal/mol for sDMA and ca. 9 kcal/mol for aDMA. Substitution of the HCl salt with the HABS salt (p-hydroxy-azobenzene-p'-sulfonate) could partially mimic the aromatic binding cage known from sDMA binding pockets in Tudor domains. Indeed, with sDMA·HABS in DMSO/water, a slightly higher barrier of ca. 13 kcal/mol was determined.

The observed barriers were assigned to rotations about the N^{ε}-C^{ζ} bond; for the C^{ζ}-N^{η} bond rotations (the one observed in the different Tudor binding modes), no barrier could be observed, i.e., these barriers should be clearly below 8 kcal/mol.

611 TH

STUDIES OF A PHOTOCHEMICAL MODEL SYSTEM USING A NEW LED BASED NMR ILLUMINATION DEVICE

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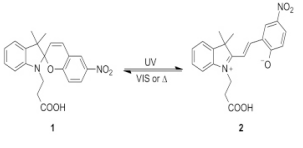
NMR spectroscopy is a non-destructive and quantitative method for in situ monitoring of reactions. For the study of photochemical reactions (like the one shown in the picture below) an irradiation source is essential.[1] So far there are several solutions for an illumination device inside the spectrometer, for example by modifying the probe itself or by using an optical fibre guiding the light.[2, 3] While several setups use laser as irradiation source, a new illumination setup was published this year using cheap and exchangeable LEDs and a sandblasted fibre tip.[4]

Using this new setup we study the photochemical equilibrium of spiropyrane **1** and merocyanine **2** by irradiation of light with varying wavelengths. The amount of merocyanine **2** in the photo-stationary-state obtained through this illumination method is compared to the amount obtained by illuminating outside of the spectrometer. By changing the wavelength of the irradiating light the equilibrium composition of the sample can be easily manipulated and the kinetics of these composition shifts can be monitored.



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Poster Presentation

612 MO

DETERMINATION OF THE CONFIGURATION IN SIX-MEMBERED SATURATED HETEROCYCLES (N, P, S, SE) AND THEIR OXIDATION PRODUCTS USING EXPERIMENTAL AND CALCULATED NMR CHEMICAL SHIFTS

Milos Budesinsky [a], Vaclav Vanek [a], Martin Dracinsky [a], Radek Pohl [a], Lenka Postova Slavetinska [a], Vladimir Sychrovsky [a], Jan Picha [a], Ivana Cisarova [b]

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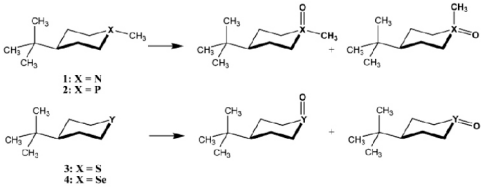

The six-membered saturated heterocycles (4-*tert*-butyl-1-methylpiperidine (**1**), 4-*tert*-butyl-1-methylphosphinane (**2**), 4-*tert*-butyl-tetrahydro-2*H*-thiopyran (**3**) and 4-*tert*-butyl-tetrahydro-2*H*-selenopyran (**4**)) were prepared as suitable model compounds with well-defined geometry for an NMR study of their oxidation products. The corresponding epimeric N-oxides, phosphinoxides, sulfoxides and selenoxides were obtained by standard chemical preparation (oxidation with hydrogen peroxide) and also by *in situ* oxidation of **1 – 4** with *meta*-chloroperbenzoic acid directly in the NMR tube. The experimental ^{1}H and ^{13}C chemical shifts were compared with corresponding calculated data obtained by GIAO approach with DFT and HF methods and various basis sets. The correlation of experimental *vs* calculated data showed the possibility to determine the stereochemistry of the epimeric oxidation products using fast DFT B3LYP/6-31G* method for geometry optimization and NMR chemical shifts calculation.

ACKNOWLEDGEMENT:
This work was supported by the Czech Science Foundation (Grant No. 13-24880S)

613 TU

SPIN-LABELING OF SMALL MOLECULE DRUGS AND DRUG TRANSPORT USING HUMAN SERUM ALBUMIN

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Besides globulins, serum albumin is the most abundant transport protein in human blood plasma. It thus regulates many endogenous processes of the organism and can serve as a drug carrier of many pharmaceutical ligands.
Our aim is to understand this complex transport process, specifically the transport connection from ligand to protein in detail by the selective elimination of specific functional groups of various pharmaceuticals. By comparison with unmodified pharmaceuticals, we are able to make more precise statements as to which functional groups contribute to protein binding. To this end, some selected pharmaceutical ligands were labeled with EPR active spin probes via Steglich synthesis.
In a screening approach with several spin-labeled pharmaceuticals we utilize continuous wave (CW) electron paramagnetic resonance (EPR) methods with human serum albumin (HSA) at physiological conditions and varying ratios of ligand to protein.
With aid of appropriate simulations of the recorded CW-EPR-spectra we extract association constants (K_A) of the modified pharmaceuticals. As indicated above, the comparison of the binding affinities from literature to our system, we aim at understanding the mechanisms of ligand-protein association.

Poster Presentation

614 TH

CHEMISTRY WITH BITE: TRACKING COLLABORATIVE ENZYME ACTIONS IN FOREST COBRA VENOM IN REAL TIME BY NMR SPECTROSCOPY

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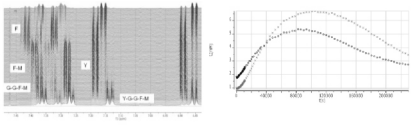
The venoms of snakes, scorpions and insects have attracted considerable scientific attention because of a) the danger they pose to humans and b) a curiosity as to how such powerful toxic effects can be achieved so quickly from such small amounts of material. A common feature is a rich content of different enzyme actions, many of which are not fully understood in terms of their contribution to toxicity once they enter the victim. In this study we show how whole venom from the Forest Cobra, *Naja melanoleuca* (a species of snake found in sub-Saharan Africa) initiates a cascade of linked enzymic reactions when challenged with certain peptide substrates. In vitro reaction monitoring by 1D ^{1}H and 2D [^{1}H, ^{13}C] HSQC/HMBC NMR spectroscopy using the model tripeptide YGG and the physiologically relevant pentapeptide Met-Enkephalin (YGGFM) was combined with the use of reference standards, metabolite databases and data handling software including Mestrelab's Mnova Reaction Monitoring Module, Bruker's Dynamics Centre and the AMIX data analysis application software. The results of our analysis reveal a succession of enzyme actions by which peptide breakdown yields a range of adducts, some unstable. In the presence of peroxide scavengers, or with the reaction vessel open to the atmosphere, the reaction cascade proceeds along alternative pathways leading to new ideas regarding the intended outcome of in vivo venom action. Understanding how the venom enzymes can collaborate with each other over a short time scale in physiological media allows deeper insight into the nature of the chemical impact following injection into the victim.



615 MO

DISCOVERING NEW BIOMARKERS IN INBORN ERRORS OF METABOLISM USING UNTARGETED NMR ANALYSIS

Konstantinos Tsiagkas[1], Vasilios Dimitroulis[1], Sarantos Kostidis[1], Efi Paramera[2], Evangelos Papakonstantinou[2], Emmanuel Mikros[1]

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NMR spectroscopy is a powerful non-targeted technique that has been successfully used in the analysis of body fluids especially in the field of metabonomics. Inborn errors of metabolism can be investigated thoroughly with NMR spectroscopy by detecting abnormal metabolites or abnormal concentrations of normal metabolites as a diagnostic tool. The aim of the study is to evaluate the efficacy of NMR spectroscopy in the analysis of newborn urine samples in comparison with other analytical techniques and to assess its capacity as a supplementary method in newborn screening. 120 newborn urine samples were collected and had been analyzed previously by a newborn screening lab (NEOLAB) using tandem-MS, HPLC and GC-MS methods. Spectra were acquired for each sample and about 70 metabolites were identified. The results of NMR analysis were compared with those obtained from the conventional analysis in order to confirm the detection of abnormal metabolites-biomarkers (3-hydroxybutyrate, glutaric acid, orotic acid, methylmalonate) in the diagnosis of inborn errors along with usual metabolites (glycine, acetate, creatinine). Metabolites usually not detected with other techniques (formate, dimethylamine, N-oxide trimethylamine) were typically identified and monitored with NMR. In several instances though, metabolites-biomarkers were detected by NMR spectroscopy where conventional screening techniques failed. Typical examples are: a) detection of increased urocanic acid in a patient with diagnosed methylmalonic aciduria, b) detection of increased 4-hydroxyphenyllactate in a patient with non-diagnosed disease, c) in samples of a patient with ornithine transcarbamoylase deficiency in addition to the expected increased levels of uracil and orotic acid, increased uridine has also been determined, d) gluconolactone, a metabolite that have never been described previously in inborn errors of metabolism, has been detected in samples of patients with different disorders. NMR spectroscopy is a vast and non-invasive technique that is applied in a holistic approach in metabolic profiling. NMR-based analysis in inborn errors of metabolism can be used as a complement to regular techniques employed in newborn screening and can offer rich insights on the diagnosis and treatment of metabolic diseases.

Poster Presentation

616 TU

INTERACTIONS BETWEEN CUCURBIT[N]URIL CAGE MOLECULES IN WATER STUDIED BY NUCLEAR MAGNETIC RESONANCE

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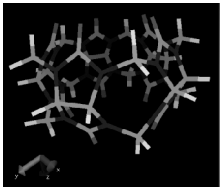

Cucurbit[n]uril molecules (CB[n]) consist of n=5 to 10 glycouril units linked by methylene groups to form a pumpkin shape cage with 2 hydrophilic rims and an apolar cavity. CB[n] molecules are able to encapsulate various gases. Chemical shift of Xe encapsulated in CB[5] depends on the proportion of cages filled with Xe[3]. This interesting result led us to this deeper study of interactions between CB[5] and Xe@CB[5] cages in water. In the solid state, CB[n] molecules are generally able to form crystals. An odd-even effect was reported[1] when CB[n] molecules with n being an even number form more stable crystals than CB[n] molecules with an odd n number. The number of intercucurbituril CH...O hydrogen bonds reflect the less efficient self-association for odd cucurbiturils. In water, the number of intercucurbituril hydrogen bonds determines the CB[n] solubility. NMR measurements of self-diffusion coefficients reveal that at high concentrations CB[7] partially aggregate[2]. It was also observed that the presence of substituents on methylene positions tends to increase the molecular solubility. We report a study of interactions between CB[5] cages and the influence of the presence of encapsulated xenon gas on these interactions.[3] We also synthesized CB[6] derivatives with up to 170 times higher solubility in comparison with CB[6].[4] ^{1}H NMR chemical shifts and T_2 relaxation times together with molecular dynamics and *ab initio* calculations were used.

[1] Baderlang et al., Cryst Growth Des 2011, 11, 5598-5614 [2] Wheate et al., J Phys Chem B 2008, 112, 2311-2314
[3] Huber et al., ChemPhysChem 2011, 12, 1053 – 1055 [4] Lewin et al., accepted in EurJOC

617 TH

STRUCTURAL ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS IN GLYCOSOMAL BIOGENESIS IN *TRYPANOSOMA BRUCEI*

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The protozoa *Trypanosoma brucei (Tb)* infects human and causes every year thousands cases of fatal sleeping disease in subtropical region of Africa. Trypanosomatids couple glycolytic and peroxisomal function in the same organelle, the glycosome. Since glycolysis is the only ATP source for the cell, glycosomes are essential for bloodstream-form of trypanosomes. Hence, we design a small molecule that causes glycosomal malfunction and ultimately ATP starvation of the parasite. Glycosomes are vesicle-like organelles that enclose enzymes involved in lipid and reactive oxygen species metabolism as well as glycolytic enzymes. Due to absence of protein synthesis within the glycosome, all lumen enzymes and membrane proteins have to be translocated post-translationally. Vesicles derived from the ER are gradually enriched with membrane proteins, thus assisting the final maturation of glycosomes by importing the enzymes into the lumen. The factors that govern the biogenesis and function of glycosomes/peroxisomes are named peroxins (Pex proteins). In human, malfunction of peroxins is associated with numerous severe disorders.

Pex5 and Pex14 are two of the conserved peroxins essential for peroxisomal and glycosomal biogenesis in humans and *T. brucei*, respectively. Pex5 recognizes cargo molecules in the cytoplasm and subsequently targets them to the organelle by forming a docking complex with Pex14 at the membrane. Although the Pex5/Pex14 interaction is conserved and functionally important in both humans and *T. brucei*, sequence variations suggest distinct structural features of the Pex5/Pex14 protein-protein interface.

The three-dimensional structure of TbPex14 was determined in solution and the binding to human and *T. brucei* derived Pex5 peptides was characterized using NMR-spectroscopy and fluorescent polarization assays.

Poster Presentation

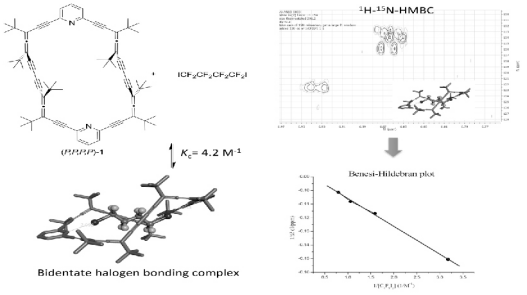
618 MO

CHARACTERIZATION OF A CHIRAL BIDENTATE PYRIDYLALLENOACETYLENIC HALOGEN BONDED COMPLEX BY NMR AND X-RAY CRYSTALLOGRAPHY

Armando Navarro-Vázquez, Silvia Castro, José Lorenzo Alonso Gómez, María-Magdalena Cid

Organic Chemistry Department. Facultade de Química. Universidade de Vigo. Spain

Chiral allenoacetylenic macrocycles present an outstanding chiroptical response, due to the presence of a largely conjugated p-systems and chromofore rigidity. Although synthesis of enantiopure allenoacetylene compounds have been reported, their role as chiral cyclophanic hosts has been scarcely evaluated. Examination of the morphology of chiral allenophane **1**, recently synthesized in our group, led us to evaluate their potential role as a bidentate host for halogen bonding complexation with diidooctafluorobutane. Strong shielding of the ^{15}N resonance, up to 10 ppm, was observed upon complexation in C_6D_6. Benesi-Hildebrand fitting of ^{15}N titration furnished a complexation constant K_c = 4 M^{-1}. Noteworthy, ^1H 1D spectrum shows that the C_2 symmetry of the host is broken upon complexation due the adoption of a chiral geometry by the diiodo compound guest inside the cavity, as beautifully shown by X-ray single crystal resolution of the complex structure.



619 TU

BINDING OF N6-ISOPENTENYLADENOSINE TO FARNESYL DIPHOSPHATE SYNTHASE: AN NMR INVESTIGATION

Mario Scrima[1], Sara Di Marino[1], Manuela Grimaldi[1], Alessandra Tosco[1], Gianluigi Lauro[1] Anna Ramunno[1] , Luciana Marinelli[2], Ettore Novellino[2], Maurizio Bifulco[3], Giuseppe Bifulco, Anna Maria D'Ursi[1]

[1]*Department of Pharmacy, University of Salerno*
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The enzyme farnesyl diphosphate synthase (FPPS) a key enzyme in the mevalonate, isoprenoid biosynthesis pathway, has been identified as an interesting target for anti-tumor and anti-infective drug leads. FPPS inhibitors, represented by bisphosphonates drugs are nowadays used in the treatment of malignant bone disease but their employment in different tumor or infective diseases is limited by their adverse pharmacokinetic properties. Therefore there is an increasing interest in the development of new antitumor or ant infective acting as FPPS inhibitors and endowed with improved pharmacokinetic properties. N6-Isopentenyladenosine (IPA) is a modified nucleoside exhibiting anti-tumor effects on human and murine cells. Increasing evidence show that this molecule is able to modulate pathways controlled by FPPS. However the mechanism by which IPA may control cancer cell growth and its potential biological target remain unknown. Here we present experimental data pointing to the identification of FPPS as IPA target. An NMR investigation based on Saturation Transfer Difference (STD) and Transfer NOE experiments shows that IPA binds FPPS in the active site generally occupied by biphosphonate molecules. New lead compound is also identified as ligand of FPPS allosteric site.
NMR data validate an "inverse virtual screening" procedure where a set of protein targets are screened in the search of IPA target.

Poster Presentation

620 TH

SPECIATION OF COBALT(II) IONS IN HUMAN SALIVA BY ^{1}H NMR ANALYSIS

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Objectives: Although cobalt-chromium (Co-Cr) alloys have been employed as oral implants in dentistry for many years, there remain many insights to be gained regarding their biological impact and *in vivo* corrosion. In this investigation we have evaluated cobalt(II) [Co(II)] ion-induced modifications to the ^{1}H NMR profiles of a wide range of potential biomolecular complexants for this metal ion in human salivary supernatants (HSSs), an experimental model which can provide much valuable information regarding the particular chemical nature of solution-phase Co(II) complexes liberated from cobalt-containing alloy oral implants or removable partial dentures during *in vivo* corrosion and wear episodes.
Methods: Unstimulated human saliva samples were obtained from a total of 16 healthy, non-medically-compromised participants, and HSSs were obtained from these via centrifugation. Microlitre aliquots of stock Co(II) solutions in HPLC-grade water were introduced to the HSS samples by micropipette. ^{1}H NMR measurements on were conducted on a Bruker Avance AX-600 spectrometer.
Results: Addition of Co(II) ions to isolated HSSs gave rise to its complexation by a variety of biomolecules therein. The relative efficacies of these complexants/chelators in this context were classifiable by the influence of increasing added Co(II) concentrations on their line-widths and chemical shift values. Those which were most affected by the addition of this metal ion were lactate > formate ≈ histidinate > succinate, this order reflecting the ability of these complexants to compete for the available Co(II) in terms of the thermodynamic equilibrium constants for the formation of their complexes and their HSS concentrations.
Conclusions: These observations provide evidence for the identity of particular Co(II) complexes in human saliva, and are of much significance regarding the *in vivo* corrosion of cobalt-containing metal alloy dental prostheses (e.g., Co-Cr alloys), trace metal ion research, and the molecular fate of ingested Co(II) ions in this biofluid.

621 MO

GLYCOSYLATED AND NON-GLYCOSYLATD CSF114 PEPTIDE AT MEMBRANE INTERFACE: NMR, EPR AND MOLECULAR DYNAMIC SIMULATIONS

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Glucosylations are fundamental determinants of antigen-antibody recognition. The majority of antigen-antibody reactions occur at interface between biological compartments and antibody surface, were the sugar moieties are critical for the right positioning of the epitopes and the correct binding to the antibody counterpart.
Inspired by the immunogenic glycosylated fragment of mielin oligodentrocyte glycoprotein (MOG) we have recently designed and synthesized the glycosylated peptide CSF114(Glc). In an innovative approach named "inverse chemical approach" it has been developed as peptide antigenic probe that accurately measures IgM autoAbs in the sera of a patients affected by multiple sclerosis. In an effort to improve the diagnostic and prognostic accuracy of CSF114 biomarker, we investigated the structural effect of the sugar and of a biomembrane compartment to the correct positioning of the autoantigen-autoantibody reaction. Accordingly we performed extensive molecular dynamic simulations using a funnel-shaped restraint potential (Limongelli et al. PNAS 2013). The results coming from dynamic simulations were validated through experimental data collected by means of NMR and EPR techniques in the presence of membrane models including spin labelled phospholipids. The results of the present analysis led to the identification of the main interacting sites between the glyco-peptides and the membrane, highlighting the key roles played by some residues and the sugar during the glycopeptide/membrane and the glycopeptide-antibody binding event.

Poster Presentation

622 TU

NMR MECHANISTIC STUDIES ON FLAVIN CATALYZED PHOTOOXIDATIONS OF BENZYL ALCOHOLS

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Using NMR spectroscopy we study the mechanism of photooxidations of benzyl alcohols to the corresponding aldehydes catalyzed by different flavin derivatives [1,2,3]. To apply all well-established NMR techniques to the study of photochemical reactions the NMR spectrometer was equipped with an LED based illumination device[4]. Different reaction intermediates were stabilized by different solvents and detected and characterized by 2D NMR techniques and EPR respectively. ^{1}H DOSY spectroscopy revealed aggregation trends depending on solvent composition and the structure of the flavins. The illumination device allows the implementation of well-defined light pulses into NMR pulse sequences and thus Photo-Chemical Induced Dynamic Nuclear Polarization (Photo-CIDNP) spectroscopy. With this technique radical paramagnetic intermediates were detected in their diamagnetic products by means of NMR. By the stabilization of the reduced form of flavin the Photo-CIDNP pattern of the singlet and triplet exit channel are distinguished and insights in the reaction mechanism and the influence of the solvent on the reaction pathway were gained.

[1] Schmaderer H.; Hilgers P.; Lechner R.; König B.; Adv. Synth. Catal. 351, 163-174, 2009
[2] Svoboda J.; Schmaderer H.; König B.; Chem. Eur. J. 14, 1854-1865, 2008
[3] U. Megerle, M. Wenninger, R.-J. Kutta, R. Lechner, B. König, B. Dick, et al., Phys. Chem. Chem. Phys. 13, 8869–8880 2011.
[5] C. Feldmeier, H. Bartling, E. Riedle, R. M. Gschwind, *J. Magn. Reson.* 2013, *232*, 39

623 TH

FINE TUNING A MULTICOMPONET REACTION TO PROCEED TOWARDS EITHER PYRROLO [1,2-A] QUINOXALINE OR PYRROLO [1,2-A] BENZIMIDAZOLE RING FORMATION

Calin Deleanu,[1] Alina Nicolescu,[2] Emilian Georgescu,[3] Florentina Georgescu,[3] Sergiu Shova,[2] Petru Filip.[1]

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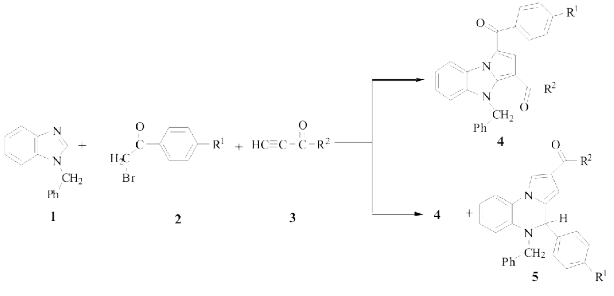
3 Oltchim SA, Research Center, Ramnicu Valcea, Romania.

A one-pot, three-components reaction involving a 1-benzylbenzimidazoles, a 2-substituted - bromocarbonyl derivative and an activated acetylenic derivative may be tuned to lead either a pyrrolo[1,2-a]bezimidazole (**4**) or a dihydropyrrolo[1,2-a]quinoxaline (**5**) depending on the reaction conditions.

Multinuclear NMR and X-crystallography have been used to prove the course of reaction.



Poster Presentation

624 MO

NMR CHARACTERIZATION OF DEXTRAN-MANNOSE DERIVATIVES AS CANCER RADIOPHARMACEUTICALS

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[2]Cátedra de Radioquímica, Facultad de Química, Montevideo, Uruguay
[3]Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany

Linear dextrans of MW 10-70 kDa are among the nanoparticle systems currently explored for cancer diagnosis and therapy being inexpensive, stable, easily derivatizable, and of low pharmacological activity and toxicity. In this work, the structural characterization of dextran derivatives (MW approx. 20 and 30 kDa) as potential radiopharmaceuticals for sentinel lymph node imaging is presented. The dextrans bear mannose moieties for targeting the mannose receptors of sentinel lymph nodes, the first site of tumor metastasis. In addition they bear isocyanide ligands for coordination to ^{99m}Tc, the most commonly used radioisotope in clinical practice. NMR is the only means of characterization of dextran nanoparticles that cannot be crystallized for X-ray analysis, and, in addition, are not amenable to MS analysis due to their polymeric nature that generates in each synthetic step mixtures of dextrans differing in the degree of substitution.
Detailed structural analysis of all synthetic intermediates leading to the desired dextran derivatives was achieved through a combination of NMR methods. For application in cancer radiodiagnosis complexation with the radioactive ^{99m}Tc was effected. The structural characterization of the complexed dextran derivatives was carried out with NMR through the employment of Re, the non-radioactive analogue of ^{99m}Tc. The study revealed the formation of "4+1" $Re^{III}(NS_3)$(CN-dextran) mixed-ligand complexes through the coordination of the isocyanide group of dextrans to the $Re(NS_3)(PPhMe_2)$ precursor, and allowed the quantification of the metal units per dextran molecule. This study is one of the few examples in the literature with well-documented structural characterization of dextran derivatives and provides a solid base for structure-based optimization of the biological properties of dextran radiopharmaceuticals.

625 TU

LOCKED AND UNLOCKED CONFORMATIONS IN CYCLODEXTRIN DIMERS

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IAMPPNM, NCSR "Demokritos"

Cyclodextrin oligomers are considered as versatile multicavity hosts that in principle can act as molecular carriers of higher payload, compared to natural cyclodextrins, known hosts/carriers of drugs. Furthermore, the higher molecular weight of cyclodextrin oligomers is envisaged as an advantage due to foreseen prolonged blood circulation times and augmented Enhanced Permeability and Retention (EPR) effect in drug delivery applications. The challenges associated with cyclodextrin oligomers are (i) the ease of preparation of monodisperse oligomers of well defined molecular weight (dimers, trimers, etc) (ii) their efficient purification and (iii) their structural characterisation. The later has proven to be of critical importance since it has been shown recently[1] that depending on the linker connecting the individual cyclodextrin moieties in a dimer or a trimer, self-inclusion, frequently associated with one glucopyranose unit inversion of one cyclodextrin moiety, totally incapacitates the cavities toward guest inclusion thus annihilating the utility of the oligomer.
We have worked out very efficient preparations of beta-cyclodextrin dimers utilizing the Staudinger ligation reaction for the first time based either on a known mono-functional phosphane linker[2] or on a difunctional (diphosphane) linker that we specifically developed and designed cyclodextrin azides.[2] The reaction conditions were optimized by monitoring the ^{31}P NMR signals. The structures of the products were analysed using a combination of NMR methods (2D ROESY/NOESY, ^{15}N and ^{31}P NMR, phase sensitive HSQC and Diffusion Ordered Spectroscopy) aided by quantum mechanical calculations. We have found that the initially semi-locked dimer structures due to intramolecular self-inclusion are readily unlocked by incoming guest molecules, as NMR titrations revealed, thus proving the full utility of the Staudinger cyclodextrin dimers as double molecular carriers.

[1] S. Menuel, N. Azaroual, D. Landy, N. Six, F. Hapiot, E. Monflier, *Chem. Eur. J.* **2011**, *17*, 3949. [2] F. L. Lin, H. M. Hoyt, H. van Halbeek, R. G. Bergman, C. R. Bertozzi, *J. Am. Chem. Soc.* **2005**, *127*, 2686. [3]N. Mourtzis, M. Paravatou, I. M. Mavridis, M. L. Roberts, K. Yannakopoulou, *Chem. Eur. J.* **2008**, *14,* 4188.

626 TH

MULTINUCLEAR SOLID-STATE NMR STUDY OF HEXAGONAL BORON NITRIDE

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Boron nitride has been studied by powder-XRD, ^{10}B, ^{11}B and ^{14}N solid-state NMR spectroscopy. Powder-XRD and ^{10}B solid-state NMR spectroscopy showed that the sample is hexagonal boron nitride. Spin-3/2 central line intensities were calculated for any ratio of the quadrupolar coupling constant to the radiofrequency field for the one-pulse and one-dimensional nutation NMR experiment. The sequence was then used to obtain the ^{11}B quadrupolar coupling constant on a powder sample of the compound. This technique is particularly useful when the NMR spectrum is featureless. A characterization by ^{14}N NMR of the sample will be presented. A magic angle spinning (MAS) lineshape has been investigated in order to determine the quadrupolar coupling constant Q_{cc} at the nitrogen site. A comparison will be made with similar compounds for which Q_{cc} was determined at the ^{11}B and ^{14}N atomic sites.

627 MO

USING THE DECRA METHOD TO DISTINGUISH BETWEEN SOLID PHARMACEUTICAL MATERIALS ACCORDING TO ^{1}H T_1 VALUES

A. S. Tatton, R. Wechselberger, C. Damblon.

Université de Liège, Janssen Pharmaceuticals

Many pharmaceutical solid formulations are delivered as a multi-component mixture, therefore correct identification of different components and/or phases within the mixture, in addition to identification of any potential molecular association between components, is an important regulatory and safety concern. Solid-state NMR has been demonstrated as important tool in the characterisation of pharmaceutical materials.,

Herein, different solid components are discriminated using chemometrics. The DECRA method,3 which in this study separates components according to differences in the ^{1}H longitudinal relaxation time, T_1, is applied to a range of solid mixtures. Firstly, investigations into detection limits and required differences in ^{1}H T_1 times are presented, using a combination of computation models and experimental applications to mixtures of amino-acids and pharmaceutical compounds.

A potential further application of this methodology is the capability to distinguish between distinct amorphous and crystalline regions, which often exhibit different ^{1}H longitudinal relaxation times. This is typically an important consideration during the development process of a pharmaceutical formulation. Herein, pharmaceutical crystalline/amorphous mixtures are discriminated between according to differences in respective ^{1}H T_1 values.

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Poster Presentation

628 TU

NEW TRITERPENE SAPONINS FROM THE TEA SEED POMACE (CAMELLIA OLEIFERA ABEL) AND THEIR INHIBITORY EFFECT ON THE PRODUCTION OF PRO-INFLAMMATORY CYTOKINES IN LIPOPOLYSACCHARIDE-STIMULATED BONE MARROW-DERIVED DENDRITIC CELLS

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Eight new triterpene saponins, oleiferasaponins A–H (1– 8) were isolated from Tea Seed Pomace (*Camellia oleifera* Abel) by various chromatographic methods. Their structures were elucidated through spectral studies including HR-ESI-MS, 1D NMR (^{1}H,^{13}C NMR, DEPT) and 2D NMR (COSY, NOESY, HSQC and HMBC), experiments. Compounds 1-8 moderately inhibited IL-12 p40 production in lipopolysaccharide (LPS)-stimulated bone marrow-derived dendritic cells (BMDCs) with IC50 values of 33.1,28.9.29.6,32.7,31.8,36.4, 31.6, and 22.8 μM, respectively.

629 TH

STRUCTURAL BASIS FOR SIGNALLING IN INNATE IMMUNITY VIA AUTOCATALYTIC CHANGES OF MAVS BY SOLID STATE NMR

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Upon virus infection, the innate immune response is triggered by RIG-I like helicases recognizing viral RNA. Signals are transduced by the mitochondrial antiviral signaling protein (MAVS), which forms high molecular weight, detergent insoluble aggregates upon activation, and in this conformation induces the dimerization of IRF3 and production of type I interferon. Previous studies showed that the N-terminal CARD domain (caspase activation and recruitment domain) of MAVS is responsible for aggregation. *In vitro*, MAVS CARD domain forms long, fibrillar structures.
Like activated MAVS aggregates isolated from infected cells, the *in vitro* formed CARD fibrils can also convert endogenous, unstimulated MAVS into functional aggregates, giving rise to IRF3 dimerization. However the mechanism of CARD domain mediated MAVS aggregation is still not understood. In this study, we have employed both solution NMR and magic angle spinning solid-state NMR to elucidate the structural basis of MAVS CARD domain activation in innate immune response. We present the solution NMR structure of soluble, monomeric MAVS CARD domain, and compare it to data derived from a solid state NMR analysis of the fibrillar form. This study allows us to provide first insights into the CARD-domain mediated activation of MAVS in antiviral signaling.

Poster Presentation

630 MO

QUADRUPOLE-RESONANCE MAS NMR SPECTROSCOPY

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Solid state NMR spectroscopy witnessed a remarkable progress towards efficient proton-detected NMR experiments, achieved by utilizing deuterated proteins. At moderate MAS frequencies ~30 % proton content at exchangeable amide sites is optimal for sensitivity, whereas 100 % proton content can be utilized at ultrafast MAS of ~60 kHz. These results have been demonstrated on model microcrystalline proteins, as well as on membrane proteins and fibrils.
For protein systems which can only be studied in their native environments, or which can not be unfold-refold to back-exchange with protons, there is an intrinsic problem of achieving sufficient amount of protons at amide sites for proton-detection based NMR experiments. For such systems, utilizing deuterium excitation is essential, which could be combined with proton detection schemes, e.g., at selectively protonated sites. This goal can be achieved only by a "*quadrupole-resonance NMR spectroscopy*" comprising suitable NMR probes with four RF channels (H, D, C and N), as well as, efficient deuterium excitation and polarization transfer schemes.
Here we demonstrated new types of proton detected NMR experiments utilizing initial deuterium excitation schemes. The possibility and perspectives will be shown. This new approach has particular importance, especially when combined with our already established "deuterium tool-package" for deuterated proteins (DONER, TCP, ^{2}H OC CP, ^{2}H RESPIRATION excitation and RADIP acquisition techniques), which were shown to result in superior initial magnetization and to sufficiently distribute magnetization between heteronuclei.

631 TU

A COMPETITIVE SOLID STATE NMR AND PAL SPECTROSCOPY STUDY OF BOND-CHANGE STRUCTURAL DEVIATIONS IN GLASSY AS-SE UNDER NATURAL PHYSICAL AGING

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Changes in chemical ordering associated with natural physical aging during prolonged time period of storage lasting over two decades are studied comprehensively and systematically in semiconductor $As_{30}Se_{70}$ glasses using solid state ^{77}Se NMR (measurements at room temperature using ASX 300 Bruker spectrometer operating at 57.3 MHz with a 2.5 mm Magic Angle Spinning probe rotating at 22 kHz) and PAL (positron annihilation lifetime) spectroscopy (measurements at room temperature using fast-fast coincidence system ORTEC of 230 ps resolution).
It is shown that switching of two bridge-like =As-Se-Se-As= atomic fragments into short =As-Se-As= and long =As-Se-Se-Se-As= units is energetically favourable in these glasses, testifying in a favour of thermodynamic possibility for such structural deviations under prolonged storage in the ambient conditions. In glasses under consideration,the observed aging effect reveals through character increase in the chemical shift of 580 ppm NMR line attributed to –Se–Se–As= atomic sites. This process is treated in terms of relatively quick ring-chain transformations of Se entities followed by subsequent atomic shrinkage. The latter is accompanied by slight increase in the content of –Se–Se–Se- and =As–Se–As= sites associated with NMR lines near 860 and 380 ppm, respectively, while the intensity of 580 ppm NMR line assigned to –Se–Se–As= sites is subsequently decreased. The PAL spectroscopy data for aged and just-rejuvenated samples testify in a favour of free-volume void fragmentation during natural physical aging. In final, we suggest that physical aging in the studied $As_{30}Se_{70}$glasses can be treated as slow low-temperature cooperative relaxation process involving atomic reconfiguration of homopolar Se-Se covalent bonds incorporated between pairs of neighbouring corner-shared $AsSe_3$ pyramids. *Ab initio* quantum chemical calculations with RHF/6-311G* basis set are performed to justify destruction-polymerization transformations in the studied As_3Se_7glasses possible during long-term natural physical ageing.

Poster Presentation

632 TH

HIGH-FIELD ^{17}O VARIABLE-TEMPERATURE MAS NMR REVEALS DYNAMIC AND STRUCTURAL EFFECTS FOR TETRAOXOANIONS IN INORGANIC SOLIDS

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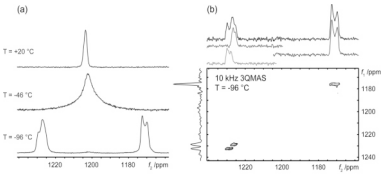
^{17}O variable-temperature (VT) MAS NMR studies of the MnO_4^- anion in $KMnO_4$ show that this anion exhibits unusual dynamics in the solid state. Similar interesting effects have also recently been observed for the XO_4^- tetraoxoanions in Cs_2WO_4, $KReO_4$ and NH_4ReO_4. The ^{17}O MAS spectra of these anions all change drastically at low temperatures compared to their corresponding room temperature (RT) spectra. This poster reports the results on the molecular dynamics, exchange times, and structural data obtained from ^{17}O VT 2D-EXSY, 1D-EXSY, 1D MAS and 2D MQMAS experiments for $KMnO_4$, Cs_2WO_4 and NH_4ReO_4 at temperatures down to –138 °C. Fig. 1a shows ^{17}O 1D VT MAS NMR spectra of $KMnO_4$ at three different temperatures. The narrow ^{17}O resonance (FWHM = 260 Hz) for the four O atoms broadens upon cooling and splits into two equal-intensity resonances at –96 °C. To characterize the ^{17}O resonances a 3QMAS experiment (Fig.1b) was performed at –96 °C. This shows that the high-frequency resonance resolves into two non-equivalent ^{17}O sites (~1230 and ~1227 ppm) and the low-frequency resonance exhibits one ^{17}O site (~1173 ppm) with double intensity, in agreement with the 1:1:2 ratio of oxygen sites in the *Pnma* crystal structure for $KMnO_4$. The ^{17}O exchange times (two-site jump) obtained from 1D-EXSY spectra fall in the range 20 to 100 ms for the temperature range –85 to –135 °C. For Cs_2WO_4 the ^{17}O exchange times range from 0.25 to 63 ms for temperatures from –10 to –60 °C. For NH_4ReO_4 we resolve the isotropic $^1J(^{17}O–^{187}Re)$ = –278 Hz at –132 °C.



633 MO

DIVERSE DYNAMICS OF WATER MOLECULES CONFINED IN CAGES OF FAUJASITES. DEUTERON NMR INVESTIGATION

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Study of dynamic behavior of water molecules in zeolites is a part of investigations aiming to elucidate also catalytic properties at molecular level. In a detailed microscopic model one expects features related to various interactions, such as electrostatic water-sodium cation, hydrogen bonding of water to framework oxygens, and water-water bonding, as well as their dependence on the loading and Si/Al ratio. There are two narrow components of different width in the spectra above 220K for all samples considered. Their relative weights change with temperature. We may conclude at this point that there are two phases and negligible exchange between them. Contribution of the narrow component undergoes thermally activated temperature dependence and can be attributed to water molecules forming clusters freely mowing in space, with O-D performing internal tetrahedral jumps. Other water molecules perform chaotic rotational jumps, and belong to bottom adsorption layers at sodium cations. Their contribution increases on decreasing temperature. All molecules become localized below 220K, as indicated by extreme broadening of the spectra, which in turn provide evidence for the symmetry of deuteron mobility. Three main components can be pointed out. Pake doublets, with the separation related to the quadrupole coupling constant, are attributed to immobile deuterons. The value of the quadrupole coupling constant, there are four similar values measured in all cases, allows to specify location of a deuteron on four chemically distinguishable oxygens in the zeolite framework. Twofold exchange of deuteron positions leads to the characteristic spectral shape. Gaussian spectral components, with the width decreasing on increasing temperature, represent chaotic reorientations leading to narrow spectra at high temperature. Contribution of these components depends on Si/Al ratio and loading. Pake doublets dominate at temperature below 70K, while twofold exchange was observed at the intermediate range.

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Poster Presentation

634 TU

PROBE FOR STATIC DNP AT 263 GHz AND LIQUID HELIUM TEMPERATURE

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DNP (Dynamic Nuclear Polarization) is a hyperpolarization technique in which electron spin polarization is transferred to nuclei by microwave irradiation, leading to substantial increase in signals compared to conventional thermal equilibrium NMR. The achievable spin polarization depends on various parameters such as temperature, magnetic field strength, and radical type and concentration. In order to study these dependencies at 9.4T around 4K, we have designed a probe suitable for DNP studies of static samples immersed in liquid helium, used in conjunction with a conventional 400MHz/263GHz Bruker DNP system utilizing a gyrotron.
The probe is based on a conventional low-temperature DNP-MAS probe frame, which has been modified for our purposes. It comprises a container for liquid helium, a quartz tube that contains the DNP sample, and a 4 mm solenoid coil with ^{1}H and ^{13}C local tuning and matching immersed in liquid helium to push arcing limits to higher power levels. The probe is remotely fine-tuned using an external box. Samples can be exchanged during cold operation using precooled helium gas for insertion/ejection, with flow rates controlled using a dedicated pneumatic setup. A smooth-wall stainless-steel waveguide is used for transfer of mm-wave energy to the sample. Temperatures in the sample region are monitored using various sensors, which also allow discrimination between liquid and gas state in helium.
Typical cool-down time to liquid helium temperature (4.3K due to a slight overpressure) is 15 minutes at flow rates between 5-9 l/h. RF fields of 70 kHz on both ^{1}H and ^{13}C are achievable, enabling efficient CP experiments. A maximum DNP enhancement of 100 on ^{1}H was obtained with TEMPOL radical on an acetate sample. CP transfer leads to 18% polarization on 13C. The probe was used in multiple experimental sessions and proved to be a stable and robust platform for reproducible DNP studies. Detailed results are given in a separate abstract "DNP at 9.4T and 4.3K. Prospects of Dissolution DNP at High Field" by Sami Jannin et al.

635 TH

COLLAGEN-LIKE PEPTIDES IMMOBILIZED ON POROUS SILICA MATERIALS CHARACTERIZED BY SS CP MAS NMR

Dipl.-Chem. Mayke Werner, Dipl.-Chem. Niels Rothermel, BSc. Tobias Roether, BSc. Sarah Bothe, Dr. Hergen Breitzke, Prof. Dr. Gerd Buntkowsky

Technical University of Darmstadt:
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Biological mineralization is one of the most interesting topics for material scientists. Nature manages to create hybrid materials made of an inorganic matrices and organic fibers to combine most desired and often reverse properties such as robustness and flexibility. In the case of mammal bones, it means a material made of hydroxyl apatite and collagen. To copy these properties, the material scientist must understand how the two materials are linked to each other and how they arrange to give these sorts of materials and consequently show these properties. Extensive studies have been done to understand the role of apatite, the collagen structure [1-4] and biomineralization processes in general[5].
Herein, we present silica-based organic-inorganic hybrid materials as model systems for bone-like materials which have been characterized by solid state NMR.

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Poster Presentation

636 MO

EFFICIENT BAND-SELECTIVE HOMONUCLEAR CO-CA CROSS-POLARIZATION TRANSFER IN PROTONATED PROTEINS

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Dipolar-based homonuclear cross polarization is an efficient method for band-selective magnetization transfer between CO and CA spins in deuterated samples in solid state MAS NMR(1). Here we show the advantages of the Band-Selective Homonuclear (BSH) CP method on fully protonated U-^{15}N,^{13}C-Ubiquitin. Similar to the results on deuterated samples, the most efficient recoupling is achieved when the sum of effective radio-frequency fields on CO and CA resonances equals two times the spinning rate. More than 30 % of the CO magnetization can be transferred to CA using BSH-CP, and the transfer efficiency is increased by up to 70 % compared to the most frequently applied spin diffusion (PDSD) procedure. This BSH recoupling method has been adapted for sensitivity-enhanced 2D $N_iCO_{i-1}CA_{i-1}$ and $CA_i(N_i)CO_{i-1}CA_{i-1}$ inter residual correlation experiments on a 20T spectrometer for fast and efficient backbone resonance assignment of protonated ubiquitin.

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637 TU

SIMULATED ANNEALING SPECTRAL FITTING APPROACH TO SIGNAL ASSIGNMENT AND SECONDARY STRUCTURE ANALYSIS OF BETA-2 MICROGLOBULIN (B2M) IN FIBRIL FORM

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Signal assignment forms a basis for all studies using NMR. In Solid-State NMR, successful signal assignment and structural analysis has been achieved so far for proteins with high-degree of short-range order, such as crystalline proteins, that exhibit exceptionally narrow resonances (e.g. <0.5~0.8ppm for carbon). Majority of non-crystalline proteins, however, usually exhibit carbon line width of more than 1ppm, and signal overlap often impedes obtaining objective signal assignment, thus subsequent structural analysis. The situation is especially severe for proteins with 100 residues or more.

Here we present our efforts on de novo signal assignment and secondary structure analysis of beta-2-microglobulin (b2m) in fibril form, using only one fully labelled sample. B2m has 100 amino acids (including an extra Met in the N terminus), and its fibril sample exhibits in our condition line width of ~1.2ppm for carbon. Although the line width suggests that the structure is relatively ordered, detailed structural model has not been given yet, mainly due to the difficulty of complete manual assignment [1].

Our approach involves simultaneous fitting of multiple intra- and inter-residue correlation spectra in parallel to the simulated-annealing molecular dynamics simulation. The method has been validated for resonance assignment and secondary structure prediction of membrane-bound peptide [2], and lyophilized proteins with 50 to 200 residues [3].

A good fitting was simultaneously obtained for four input spectra of b2m fibril, and thus a set of C^{α}, C^{β} and C' chemical shifts for all residues. Distribution of secondary structure along the primary sequence predicted from the chemical shifts obtained was roughly consistent to the previous report that was based on the partially (and manually) assigned signals in spectra of b2m fibril [1]. The present analysis extends knowledge on the secondary structure of b2m in fibril form to previously unassigned residues.

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Poster Presentation

638 TH

ZINC SALPHEN DYAD – A POTENTIAL CHARGE SEPARATOR FOR ARTIFICIAL LIGHT - HARVESTING SELF -ASSEMBLE IN TO ANTIPARALLEL STACKS

Brijith Thomas[a], Jeroen Rombouts[b], Karthick Sai Sankar Gupta[a],Gert T. Oostergetel[a], Ute Baumeister[c], Francesco Buda[a],Romano Orru[b], Huub J. M. de Groot[a].

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In the quest for nanodevices for solar to fuel conversion, analytical methods serve to guide the design of new nanosized materials for efficient light harvesting, charge separation and catalyst modules for the organic-inorganic tandem cell. The molecular structure and packing of self-assembled Zinc(II) 6,6'-((1E,1'E)-((4,5-dibromo-2,7-dioctyl-1,3,6,8-tetraoxo-1,2,3,6,7,8,9,14-octahydro-[3,8] phenanthrolino [1,10-abc] phenazine-11,12-diyl) bis (azanylylidene)) bis (methanylylidene))bis(3-(dimethylamino)phenolate) was studied in detail in the solid state which is a potential charge separator . Computational integration of EM, powder XRD, solid state NMR was used in this work to extend and connect the structure information from the molecular to supra molecular level to obtain a very detailed structure information.

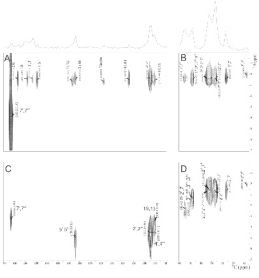

Fig:1, Contour plot sections of ^{1}H-^{13}C heteronuclear MAS NMR dipolar correlation spectra of dyad with CP contact times of 4 milliseconds (A and B) and 0.2 milliseconds (C and D). We propose an antiparallel stacking of the dyad by integrating the data obtained from Solid state NMR and TEM. This is similar to the stacking observed in chlorosome, supramolecular light harvesters that represent a paradigm for functional supramolecular light harvesting structures.

639 MO

SOLID-STATE NMR INVESTIGATION OF MEMBRANE-ASSOCIATED PEPTIDES

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Solid-state NMR spectroscopy has a proven record during the investigation of the structure and dynamics of membrane-associated polypeptides. In particular from oriented samples considerable details on the structure and topology of the protein is obtained. A solid-state NMR approach which allows for the accurate determination of the tilt and rotational pitch angles of peptides reconstituted into uniaxially oriented membranes will be presented. Proton-decoupled ^{15}N and ^{2}H solid-state NMR spectroscopy have been used to characterize the tilt and rotational pitch angle of several peptides in considerable detail. Furthermore, valuable information on the rotational diffusion constants in membranes and thereby the size of peptide complexes is obtained. Here we present solid-state NMR investigations of a number of peptides we have investigated recently including the antimicrobial peptides PGLa, magainin 2 and the Huntingtin N-terminal amphipathic membrane anchor.

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Poster Presentation

640 TU

SIMPSON: INTERPOLATION BY FAST WIGNER TRANSFORM AND OTHER FEATURES

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In our poster contribution we shall present the key new features implemented in a popular software package SIMPSON for numerical simulations of solid state NMR experiments[1] and application of optimal control methods[2] in this area.
The main new functionality involves interpolation in powder averages by fast Wigner transform[3] which substantially outperforms the standard ASG method[4]. Generally, smaller sets of crystallite orientations are needed for smooth converged powder spectra of wide lines, thus reducing the calculation time. Moreover, it is also applicable for non-diagonal Hamiltonians, or, can be combined with the ASG procedure. The method will be explained and documented on selected examples. We also improved implementation of optimal control tools for automatic design of NMR pulse sequences and introduced the BFGS-GRAPE optimization protocol[5]. This should lead again to shortening the overall calculation time in typical applications.

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641 TH

NMR AT 60 T: RELAXATION MEASUREMENTS IN PULSED MAGNETIC FIELDS

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Nuclear Magnetic Resonance (NMR) profits tremendously from the availability of high static magnetic fields. Unfortunately, these are limited to less then 25 T in case of efficient superconducting magnets. Up to 45 T can be achieved with hybrid magnets that use in part resistive magnets with high power losses and the high associated costs. Magnetic fields at up to about 100 T can only be achieved with pulsed magnets that are available in a few dedicated facilities around the world. Efforts to perform NMR experiments in such non-destructive magnets have been successful in Germany, Japan, and France, only recently. Here, we report on our recent advances at the Dresden High Magnetic Field Laboratory that houses such a pulsed magnet facility. We show that we were able to measure even the spin-lattice relaxation time T1 during a single magnetic field pulse using the changing magnetic field to perform an adiabatic inversion of the spin system. Subsequent small pulses are then used to monitor the relaxation process.
For the data analysis, off-resonance effects are taken into account and a field demodulation is applied to remove the effects of the changing magnetic field during the time of the field pulse.
With this technique, we were able to perform measurements on metallic aluminum at about 38 T and on metallic gallium at about 58 T, another step to show the potential for future applications in NMR and pulsed high magnetic field research.

Poster Presentation

642 MO

SOLID-STATE NMR EXPERIMENTS AT MAGIC-ANGLE SPINNING FREQUENCIES OF 90-100 KHZ

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Recent developments in probe technology allow one to achieve magic-angle spinning (MAS) frequencies of 100 kHz using rotors with a diameter of 0.8 mm and smaller. This has opened up new avenues for solid-state NMR, both in terms of methods and application. Generally, with higher spinning frequencies, low-power recoupling and decoupling sequences[1] become feasible, enabling the implementation of completely low-power based pulse sequences which reduces sample heating and allows for faster repetition rates. At 100 kHz MAS frequency it has become possible to record high-resolution proton spectra of solid-state samples without the need for deuteration or high-power homonuclear decoupling. Another advantage is that very small quantities (< 500 µg) of a 10 kDa protein are needed to record proton-detected heteronuclear correlation spectra in about 5 minutes with a signal to noise of ~60. This extends the range of biological systems, e.g. proteins and their complexes, that can be studied by solid-state NMR to systems where the expression of several milligramms is not practical.
In this presentation the bulk ^1H T_2's of a fully protonated ubiquitin sample are compared to the ones of the deuterated and 100% back-exchanged version of the protein as a function of spinning frequencies. In addition, a site-specific ^1H T_2' analysis at 90 kHz will be shown for the deuterated version of ubiquitin. The small sample quantity combined with proton detection, low-power CP and decoupling elements are used to record assignment-type triple-resonance experiments within a few hours. We compare the efficiency of dipolar and scalar-based transfers at the highest spinning frequency in deuterated and fully re-protonated protein samples. We also show that the coherence life times of carbons at 90 kHz MAS with low-power heteronuclear decoupling are comparable to those obtained with high-power decoupling at a field strength of 400 kHz at 10 kHz MAS frequency.
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SUPPRESSING SIGNALS *VIA* THE "EMMA METHOD"

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We have recently introduced a simple procedure, dubbed as Electronic Mixing-Mediated Annihilation (EMMA), in order to effectively suppress undesired background signals in NMR spectra, such as those arising from hardware components (e.g. probe heads, stators, rotors, inserts, tubes). The method relies on the ERETIC™ principle and uses an electronically generated time-dependent signal that is injected into the receiver coil of the NMR probe head during signal acquisition. In the original implementation of EMMA, the line shape, width and frequency of this electronic signal were determined by first deconvoluting the background signal in the frequency domain. Then, the so-obtained deconvoluted signal was converted into a time-dependent function (through inverse Fourier Transform), which was used to generate the shaped pulse that was ultimately fed into the receiver coil during the acquisition of the Free Induction Decay. The power of this shaped pulse was adjusted to match the intensity of the background signal, and its phase was shifted by 180° with respect to the receiver reference phase. This has been shown to be very effective in suppressing background signals both in liquid-state and in solid-state NMR experiments, yielding high suppression ratio while maintaining the quantitative character of the NMR data. In this work, we aim to show how this method can be extended to suppress other types of equally bothering signals, being either of instrumental or chemical origin.

Poster Presentation

644 TH

PHYSICAL PROPERTIES OF THE V-$Al_5Cu_6Mg_2$ COMPLEX INTERMETALLIC PHASE

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The cubic V-$Al_5Cu_6Mg_2$ phase with 39 atoms in the unit cell is an intermetallic phase with intermediate structural complexity between the simple Laves phase and the complex Bergman phase. Using ^{27}Al NMR spectroscopy, we have determined the electric-field-gradient tensors at the positions of three crystallographically nonequivalent Al sites in the unit cell and confirmed the local site symmetries of these sites, as predicted by the Samson structural model of the V-phase from 1949. The influence of structural complexity on the physical properties of a solid was studied by determining bulk electrical and thermal properties (electrical resistivity, thermoelectric power, Hall coefficient, thermal conductivity and specific heat) and local electronic properties of the V-$Al_5Cu_6Mg_2$ monocrystal by studying the ^{27}Al NMR Knight shift and the spin-lattice relaxation rate. The experiments reveal that free-electron picture is a good approximation to the V-$Al_5Cu_6Mg_2$ electronic structure, despite the structural complexity of the lattice. The positive thermopower and Hall coefficient reveal that V-$Al_5Cu_6Mg_2$ is a hole-type electrical conductor. Electrical resistivity shows linear temperature dependence with a positive temperature coefficient, typical of regular metals and alloys. The relatively large residual resistivity and the low thermal conductivity suggest the presence of quenched structural disorder, very likely intrinsic to the V-$Al_5Cu_6Mg_2$ structure. We did not find any experimental evidence of a pseudogap close to the Fermi energy in the electronic density of states.

645 MO

DIRECT MEASUREMENTS OF AVERAGE T_2 RELAXATION TIME SPATIALLY RESOLVED

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Magnetic Resonance measurements of the T_2 distribution have become very common and are a very powerful way to probe microporous fluid bearing solids. While the structure of the T_2 distribution, and changes in the structure, are often very informative, it is common to reduce the T_2 distribution to an average numeric quantity in order to provide a quantitative interpretation of the distribution. Magnetic Resonance Imaging measurements of the T_2 distribution have recently been introduced, but they are time consuming, especially for 2 and 3 spatial dimensions.
In this paper we explore a direct MRI measurement of the arithmetic average $1/T_2$, characterising the distributions by using the initial slope of the spatially resolved T_2 decay in a CPMG prepared centric scan SPRITE experiment. The methodology is explored with test phantom samples and realistic petroleum reservoir core plug samples. The arithmetic average $1/T_2$ is related to the harmonic average T_2 of the early CPMG decay. The average obtained from the early decay is explored through measurements of uniform saturated core plug samples and by comparison to other averages determined from the complete T_2 distribution. Complementary measurements were obtained using SE-SPI pulse sequence.
The utility of the arithmetic average $1/T_2$ is explored through measurements of centrifuged core plug samples where the T_2 distribution varies spatially. The harmonic average of T_2 obtained based on the initial slope was also used to estimate the irreducible water saturation for core plug samples.

Poster Presentation

646 TU

THE USE OF HIGH-FIELD DOSY AND LOW FIELD 2D-CORRELATED T2-D-NMR TO STUDY THYLAKOID MEMBRANE DYNAMICS IN CHLOROPLASTS

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Several techniques have been employed to increase the efficiency of solar cells. Bio-mimic is one of them since plants use solar energy more efficiently through photosynthesis. So, it is very important to understand the underlying mechanism of photosynthesis which takes place in a plant membrane called thylakoid. The thylakoid membrane switches its behaviour to prevent damage from unfavourable environmental conditions. Study of the membrane switch could also be useful for better crop production.
In this project we test the hypothesis that changes (reorganisation and composition) in thylakoid membranes in response to external perturbations are reflected in exchange kinetics over the thylakoid membranes (membrane permeability) and (restricted) diffusion behaviour of the bulk water, membrane lipid and inorganic phosphate molecules.
First, the (time dependent) diffusion behaviour of water and (membrane) lipid molecules and organic compounds in isolated chloroplasts and chloroplasts in leaf disks was studied by use of ^{1}H high field DOSY- and low field TD-NMR. Water/proton exchange between water pools outside and inside isolated chloroplasts was observed to be fast, limiting the possibility to study restricted water diffusion and membrane permeability. Lipid diffusion, however, can be studied in isolated chloroplasts. Diffusion constants as low as $5 * 10^{-13}$ m^2/s has been observed, in dependence of the diffusion labelling time. In algae, the two water pools can easily be discriminated, in addition to the lipid signals.
In intact leaf disks water in chloroplasts can clearly be discriminated from other proton pools, among which lipid fractions can be observed. The latter is especially manifest in leaf disks of Ficus benjamina, which show ^{1}H spectra that are orientation dependent, and (lipid?) resonances shifted from the water pool. For a better interpretation and assignment of the observed 1H pools we have now started to compare the results of ^{1}H NMR diffusion measurements on low field and higher field as a function of leaf disk orientation.

647 TH

SOLVENTS DYNAMICS IN GELS OF TEREPHTHAYLIDENE-BIS-GLUCOPYRANOSIDE DERIVATIVE PROBED BY PFG NMR DIFFUSOMETRY

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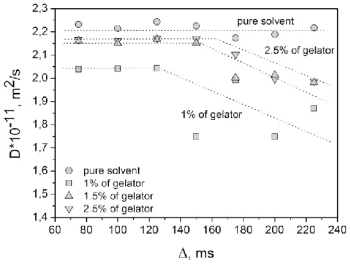
In this work we investigated diffusion phenomenon of solvents in a real porous material: a gel composed of 4,6,4',6'-O-Terephthylidene-bis(methyl alfa-D-glucopyranoside) (**1**) and glycerol derivatives by Pulse Field Gradient NMR techniques. The unique feature of one of the solvent in the gel of **1** was observed. With an increasing of gelator concentration in the gel the increase of diffusion coefficient of propylene glycol was detected (Figure). Such behaviour is attributed to the ability of gel matrix made by the self-assembly of gelator molecules to disrupt the intermolecular hydrogen network of propylene glycol. These results combined with UV-Vis, IR and Raman spectroscopy allowed us to detected the mode od gelator agregation (J or H-type).



Acknowledgments
Financial support for this work from the National Centre for Science (Grant N N202 1961 40) is gratefully acknowledged

Poster Presentation

648 MO

A COMBINED SPARSE SAMPLING OF TIME-GRADIENT DOMAIN FOR NMR DIFFUSOMETRY AND RELAXOMETRY

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Multidimensional NMR spectra can provide an information on structures of large molecules or complex chemical mixtures. However, because of time-consuming sampling of a multidimensional signal, they require days-long data collection process. This problem has been to some extent circumvented by an application of various sparse sampling techniques. Nevertheless, they were limited to time dimensions of NMR spectra. In this work, we show how to extend sparse sampling to gradient dimensions.
The procedure is based on a minimum l1-norm restrained optimization using Fourier and inverted Laplace transforms. We demonstrate the performance of the algorithm on simulated and experimental data.

649 TU

NMR PGSE FOR THE STUDY OF NANOSTRUCTURES

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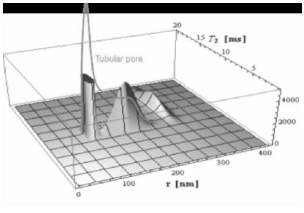
NMR pulse gradient spin echo is the most efficient method for non-invasive elucidation of molecular transport in heterogeneous media. With a proper interpretation of experimental data, the method can also be applied to investigate molecular self-diffusion in nano-pores. We show it by the analysis of restricted self-diffusion measurement of water molecules trapped in a porous polyamide membrane. PGSE measurement gives the spin echo dependence on the magnetic field gradient that exhibits the diffraction undulation of the decay that prevents the use of the inverse-Laplace transform method to extract the pore size distribution. The q-space cosine Fourier transform of data gives the propagator in the form of the sum of normal distributions[1]. Its decomposition gives three propagators with the amplitudes that decay due to the spin relaxation and with the second moments that remain fixed as the interval between gradient pulses increases. This indicates a motional narrowing regime of measurements, in which the size of pores can be obtained from the fourth root of the second moments[2]. 3-D plot of the spin-relaxation rate and pore size distributions shows the prevailing share of pores with the radius r = 100(1±0.1) nm (70%) and pores with the radius r = 175(1±0.17) nm (20%). Water in these two types of pores has almost identical spin relaxation T_2=10(1±0.2) ms, while water in the pores with the radius r = 282(1±0.03) nm (5%) have a broader distribution of relaxation times, T_2=14(1±0.36) ms. This approach[3] exposes the NMR PGSE technique as a useful tool in the nanotechnology.



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650 TH

DIFFUSION OF SMALL SOLUTES AS A TOOL TO STUDY LIQUID CRYSTALS

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Liquid crystalline (LC) phase is a phase between the liquid and the solid state; the molecules possess crystal-like order, but they can move around fairly freely, like molecules in the liquid state. Under the influence of electric or magnetic fields the LC molecules have a preferred orientation and the delicate interplay between the external electric field, orientational order and optical properties of the LC materials lays the foundation for the currently most valuable field of applications of these materials; liquid crystal display (LCD) technology. The current LCDs commonly utilize thermotropic uniaxial nematic LCs, which have a single preferred orientational axis. On the other hand, biaxial thermotropic nematic liquid crystals (BTN-LCs) have two distinct orientational axes and can presumably have, for example, faster response times than the conventional uniaxial nematics. The reduced symmetry of the biaxial system has also been postulated to give rise to new applications in the field non-linear optics. The ongoing debate on how to unambiguously identify the nematic biaxial phase has continued and still today the experimental evidence of the biaxiality of the alleged BTN-LC materials is questioned. Consequently, there is still demand for experimental methods that could unambiguously reveal the biaxiality of the nematic LCs. In this study we are investigating the possibility to utilize the anisotropy of the diffusion tensor of small solutes to identify the liquid crystalline phases.

651 MO

AN ULTRA-HIGH-POWER H/C/N NMR PROBE FOR MEMBRANE PROTEINS

F. David Doty, John Staab, George Entzminger, JB Spitzmesser, Daniel Arcos, Laura Holte, Paul Ellis

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Stationary (non-MAS) solids NMR high-power methods, such as PISEMA, have been fruitful in yielding structures of large, complex, helical membrane proteins. Researchers in macromolecule structure determination by these methods have voiced the need for major increases in RF field strength, as required for significantly improved spectral resolution, along with dramatically reduced RF sample heating, in triple-resonance $^{1}H/^{13}C/^{15}N$ stationary solids probes.

Doty Scientific has developed an ultra-high-power triple-resonance probe at 900 MHz with order-of-magnitude reduction in RF sample heating and substantial improvement in each of the remaining three most important and technically demanding specifications: RF field strength, spectral resolution, and S/N. The tests in the 900 MHz magnet at the NHMFL in Tallahassee included CP experiments for $^{1}H/^{13}C$ with 4.6 μs π/2 pulse widths at 111 W on ^{1}H and 180 W on ^{13}C, and CP experiments for $^{1}H/^{15}N$ with 4.3 μs π/2 pulse widths at 920 W on ^{15}N. The maximum power levels available were 320 W ^{1}H, 700 W for ^{13}C, and 1100 W for ^{15}N. The probe had no difficulty handling these powers under CP conditions, as the ^{1}H is on an outer resonator while the ^{13}C and ^{15}N are on a doubly balanced inner solenoid. The probe is expected to be able to handle at least 40% higher power on all channels. The probe included a gradient coil which was tested to 157 G/cm in the magnet and demonstrated excellent linearity, efficiency, and recovery time. The approach will be compatible with operation in narrow-bore (NB) magnets at the highest fields anticipated – to at least 1.0 GHz.

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MAS NMR MEASUREMENT OF C-H BOND EFFECTIVE CORRELATION TIMES IN MEMBRANE MODEL SYSTEMS

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Abstract: (Your abstract must use Normal style and must fit into the box)
The way lipid molecules orient and move in biological membranes is often studied by means of NMR spectroscopy and molecular dynamics (MD) simulations on model systems. Based on a previous NMR relaxation theory[1], we have derived a simple method to translate ^{13}C relaxation rates from lipid or surfactant bilayers into a well defined quantity of C—H bonds, the so-called C—H bond effective correlation time. C—H bond specificity is achieved by using magic angle spinning (MAS) NMR. The method enables a basic and quantitative interpretation of NMR relaxation rates since the effective correlation time quantity has a much simpler definition than the relaxation rates alone. Moreover, the derivation of the method also demonstrates that T1r relaxation times can be directly calculated from the most up-to-date MD simulations of lipid bilayers and therefore be used to validate dynamics occurring in the simulation models within time scales of 0.1 to 1ms.
We present all the principles behind this new MAS NMR protocol, and its importance both for experimental studies and MD simulations is illustrated with an example concerning the effect of cholesterol on a phospholipid bilayer. References:

[1] Wennerström H., Lindman B., Söderman O., Drakenberg B., and Rosenholm J. B., *JACS,* **101**(1979)

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PROTON CHEMICAL EXCHANGE IN AN ALKYL GLUCOSIDE SYSTEM IN THE "ALMOST DRY" REGIME

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In many different industries (pharmaceutical, detergency and so on) it is important to understand the effects of water interactions brought on by water sorption from the atmosphere for a number of "almost dry" systems. For instance, the physical and chemical stability may be greatly affected during not only long-time storage but also during the actual formulation processes.
Sugar surfactants such as alkyl glucosides serve as good model systems to study the "almost dry" regime. It has been shown that it is the strong inter-molecular hydrogen bonds between the hydroxyl groups in the neighbouring head-groups that gives rise to the interesting and complicated solid phase behaviour of alkylglucosides.[1]

In this study we investigate by nuclear Overhauser enhancement spectroscopy (NOESY) the chemical exchange between the hydroxyl groups of alkyl glucoside *n*-octyl-β-D-maltoside (C8G2) and the water incorporated in the liquid crystalline phase.

References:
[1] C. A. Ericsson, L. C. Ericsson, V. Kocherbitov, O. Söderman and S. Ulvenlund, Phys. Chem. Chem. Phys., 2005, 7, 2970–2977.

Poster Presentation

654 MO

MOBILITY OF CO_2 IN THE MOF $Zn_2(bdc)_2(dabco)$

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$Zn_2(bdc)_2(dabco)$ is porous coordination polymer (sometimes called also Metal-Organic Framework). The structure is anisotropic with 1-D parallel rectangular channels. It is well capable to absorb CO_2. We applied ^{13}C NMR spectroscopy and diffusometry in order to analyze mobility of adsorbed CO_2. Both methods were subsequently compared with molecular dynamics simulation.
Despite the fact, that diffusometry showed high diffusion coefficient (ca. 810^{-9} m^2 s^{-1}, e.g. higher than for water), there is a significant anisotropic broadening of adsorbed CO_2 spectrum. The broadening is caused by chemical shift anisotropy, but it is smaller than in solid CO_2 and temperature dependent.
MD revealed adsorption sites in the corners of Zn_2-paddle wheels. CO_2 prefers the orientation parallel to the channels when staying in those adsorption sites. The jumps between the adsorption sites are very frequent comparing to spectral timescale, but they do not change the preferred orientation of the molecule.

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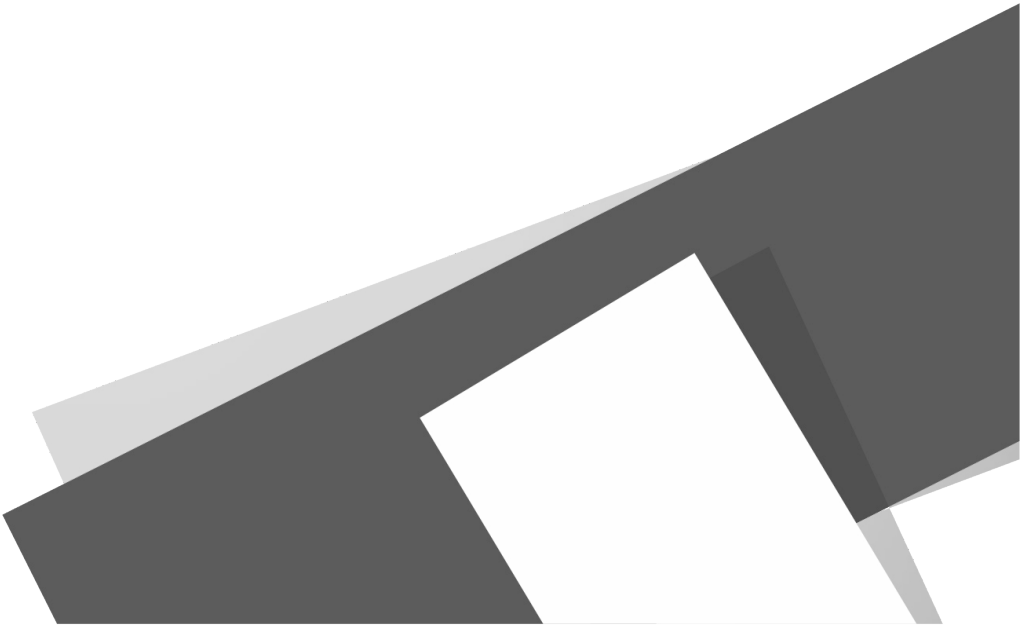

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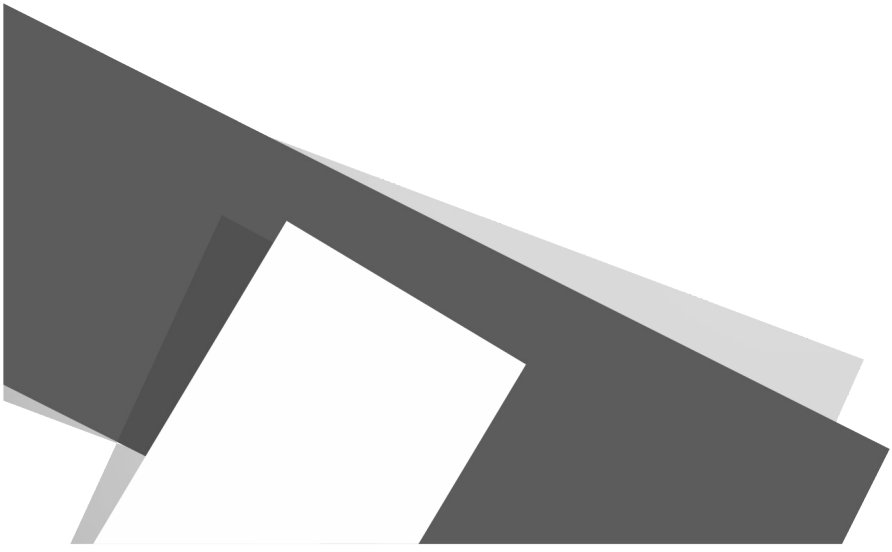

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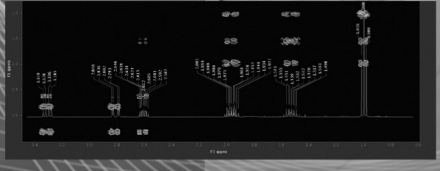

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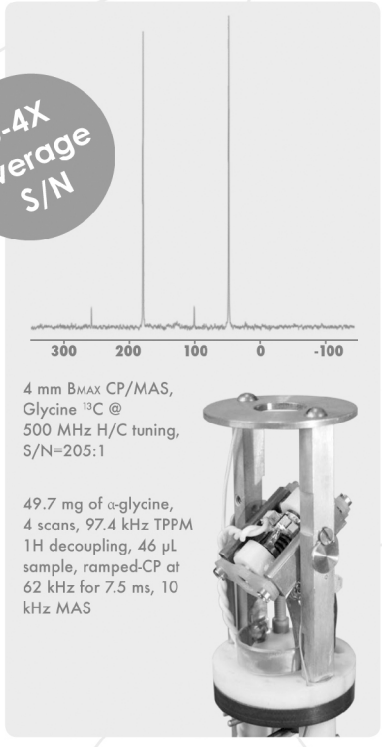
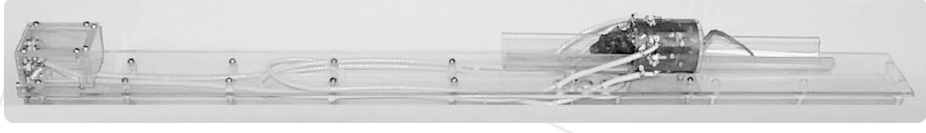


BMAX MAS Probes

BMAX series Magic Angle Spinning (MAS) Probes applies Doty Scientific's over 30 years of experience in the field of NMR.

- Highest S/N – by a wide margin
- DI-4: 205:1 on 50 mg of glycine, ^{13}C, 11.7 T
- DI-3: 258:1 on 18 mg of HMB, ^{13}C, 11.7 T
- Highest RF field strengths on X/Y
- Compare: 1.3 µs π/2 with only 640 W for ^{13}C, DI-3 at 11.7 T, H/X
- H-F/X/Y broad-band tuning
- Tuning options from ^{109}Ag to ^{31}P
- Three Year Limited Warranty!



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Isotopically labeled proteins

Giotto Biotech S.r.l. offers a wide range of recombinant proteins produced through expression in *E. coli*. **We specialize in proteins labeled with stable isotopes**, typically ^{13}C, ^{15}N, and ^{2}H in any combination: plus, we **can custom label proteins as desired!** All Giotto Biotech products undergo a **strict quality control process** including **mass spectroscopy and NMR**.

Some examples of Giotto Biotech's proteins

Calmodulin	SOD1
Ubiquitin	Synuclein
Calbindin-D9k	Matrix metalloproteinases
Maltose binding protein	S100 proteins

Custom production

Giotto Biotech also offers a **custom protein production service**, including custom labeling as desired, for proteins with reported expression and purification procedures.

NMR reference proteins

A wide range of selectively or uniformly labeled proteins produced in *E. coli* and ranging from 7 kDa to 40 kDa molecular weight are supplied in high quality **NMR tubes sealed under argon**, and are ready to use as NMR standards**.**

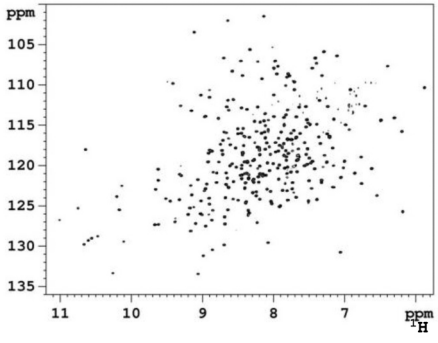
Diamagnetic proteins

Maltose binding protein (MBP) (40.3 kDa)	Ubiquitin (8.6 kDa)
Zn(II)-Carbonic anhydrase II (29.1 kDa)	MMP12 catalytic domain (17.6 kDa)

Paramagnetic proteins

Co(II)-MMP12 catalytic domain (17.6 kDa)	YbCa-Calbindin (8.5 kDa)

^{1}H–^{15}N HSQC-TROSY NMR spectrum of Maltose Binding Protein (1.2 mM; MW = 40.3 kDa) recorded at 900 MHz and 310 K, pH 7.2.



Contact us at info@giottobiotech.com for information and customized quotes.

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Ph: +39 (055) 457 4258 - 4237

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LAST MINUTE ABSTRACT

655 TU

ORDER AND DISORDER IN THE REPLICATION MACHINERY OF PARAMYXOVIRUSES

Guillaume Communie[1,2]; Malene Ringkjobing Jensen[1]; Damien Maurin[1]; Euripides Ribeiro[2]; Nicolas Martinez[2]; Loic Salmon[1]; Luca Mollica[1]; Marc Jamin[2]; Rob Ruigrok[2]; Martin Blackledge[1]

1: Institut de Biologie Structurale, Univ. Grenoble Alpes-CNRS-CEA, 41 rue Jules Horowitz, Grenoble, France
2: Unit for Virus Host-Cell Interactions, Univ. Grenoble Alpes-EMBL-CNRS, 6 rue Jules Horowitz, Grenoble, France.

Paramyxoviruses are single stranded RNA viruses. Their genome is encapsidated by multiple copies of the Nucleoprotein (N), forming helical nucleocapsids of molecular mass in the tens of megadaltons range.The proteins involved in the transcription and replication machinery of this family of viruses contain a high level of intrinsic disorder.We are using Nuclear Magnetic Resonance spectroscopy, in combination with electron microscopy, X-ray crystallography and small angle scattering, to characterize the nucleoprotein (N) as well as the phosphoprotein (P), a constituent of the polymerase complex that interacts with the disordered domain of the nucleoprotein to initiate viral transcription and replication.

EUROMAR 2013

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TIMETABLE EUROMAR 2013

Sunday 30		Time	Monday 1			Tuesday 2			Wednesday 3			Thursday 4			Friday 5		
		Chair	Malcolm Levitt			Claude Berthier			Geoffrey Bodenhausen			Sabine van Doorslaer					
		8:30-9:15	Daniel B. Vigneron			Anja Boeckmann			Isabella Felli			Daniella Goldfarb			chair:	Bernhard Blümich	
		9:15-10:00	Arno Kentgens			Stephen Hill			Graham Smith			Peter van Zijl			Dmitri Budker		
		10:00-10:40	Coffee			Coffee			Coffee			Coffee			Coffee		
		Session	Bioliquids I	MRI / In Vivo	EPR Methods I	NMR Methods I	Proteins & Nucleic Acids	Magnetism and Superconductivity	Biosolids I	New Methodologies & Instrumentation Advances	Intermetallic & Composite Materials	NMR Methods II	In Cell & In Vivo Studies	EPR Methods II	Biosolids II	Transport & Diffusion	Materials & Processes
		Chair	C. Hewage	A. Jerschow	M. Bennati	T. Meersmann	I. Gerothanassis	M. Hagiwara	G. Boutis	S. Caldarelli	J. Dolinšek	J. P. Amoureux	I. Felli	S. Misra	M. Pons	L. Gladden	S. Stapf
10:00-14:00	IDPbyNMR Workshop	10:40-11:10	A. Gronenborn	H. Degani	J. Morton	P. Madhu	L. Spyracopoulos	M. H. Julien	B. Reif	A. Samoson	H. Kim	J. Reimer	V. Dötsch	V. Petrouleas	M. Baldus	D. Topgaard	R. Schurko
		11:10-11:30	H. Meyer	P. Berthault	P. Baranov	N. Giraud	K. Pervushin	A. Potočnik	J. P. Amoureux	H. Ohta	U. Scheler	V. Shevelkov	M. Pons	J. Freed	G. Pintacuda	G. Guthausen	O. Poluektov
		11:30-11:50	G. Mas	E. Serrao	F. Mentink-Vigier	D. Carnevale	H. Schwalbe	P. Carretta	F. Larsen	J. Blanchard	M. Wencka	Y. Nishiyama	F.-X. Theillet	A. Marko	A. Jantschke	D. Bernin	E. Brunner
		11:50-12:10	D. Neuhaus	D. Fiat	G. Morley	M. Beaugrand	E. Guittet	V. Kataev	J. Lewandowski	N. Salvi	M. Požek	G. Zheng	S. Klippel	R. Narkowicz	A. Goldbourt	C. Bessada	Y.-Y. Hu
		12:10-12:40	N. Wolff	I. Koptyug	M. Fedin	K. Takegoshi	V. Sklenar	S. Zvyagin	T. Polenova	D. Sakellariou	M. Klanjšek	G. Veglia	M. Lerche	B. Bode	A. McDermott	J. Stepišnik	X. Xue
		12:40-14:30	Lunch			Lunch			Lunch			Lunch			chair:	Gunnar Jeschke	
		14:30-16:00	POSTER SESSION ONE AND COFFEE			POSTER SESSION TWO AND COFFEE						POSTER SESSION THREE AND COFFEE			Charalampos Kalodimos		
14:00-16:15	Tutorial Lectures	Session	Relaxation & Dynamics	Sensitivity Enchancement	Materials & Methods in the Nanoscale	Small Molecules & Metabolomics	Theory & Computation	Paramagnetic Systems				Bioliquids II	Industrial & Cultural Applications	Spatially Resolved NMR & EPR of Solids	CLOSING		
		Chair	C. Kalodimos	L. Frydman	H. Ohta	E. Mikros	A. Bagno	D. Fiat				C. Geraldes	F. Milia	J. Tritt-Goc			
		16:00-16:30	E. Meirovitch	C. Hilty	A. Tartakovskii	J. Prestegard	A. Bonvin	L. Banci				J. Christodoulou	D. Capitani	J. Ackerman			
16:30-19:15	Opening and Prize Session	16:30-16:50	D. Lee	H. Takahashi	O. Lafon	A. Wong	P. Lantto	J. Kowalewski				R. Pierattelli	A. Andreev	A. Jerschow			
		16:50-17:10	J. Tritt-Goc	A. Lesage	S. Mamone	E. Matei	T. Madl	M. Murakami				E. Lescop	F. Dalitz	T. Meersmann			
		17:10-17:30	D. Peat	V. Zhivonitko	C. Avalos	U. Guenther	N. Sgourakis	F. Mulder	Excursion			S. Dames	W. Zia	M. Katsiotis			
		17:30-18:00	N. Skrynnikov	S. Jannin	F. Shi	C. Larive	F. Neese	J. van Slageren				M. Akke	L. Gladden	A. Blank			
		Chair	Jürgen Haase			Beat Meier						Gil Navon					
		18:15-19:00	Dominique Massiot			Shimon Vega						Yi-Qiao Song					
19:00	Welcome party	19:00-22:00	Bruker Hospitality Suite									20:30	Dinner				