EURO MAR 2022

JULY 10-14 UTRECHT THE NETHERLANDS

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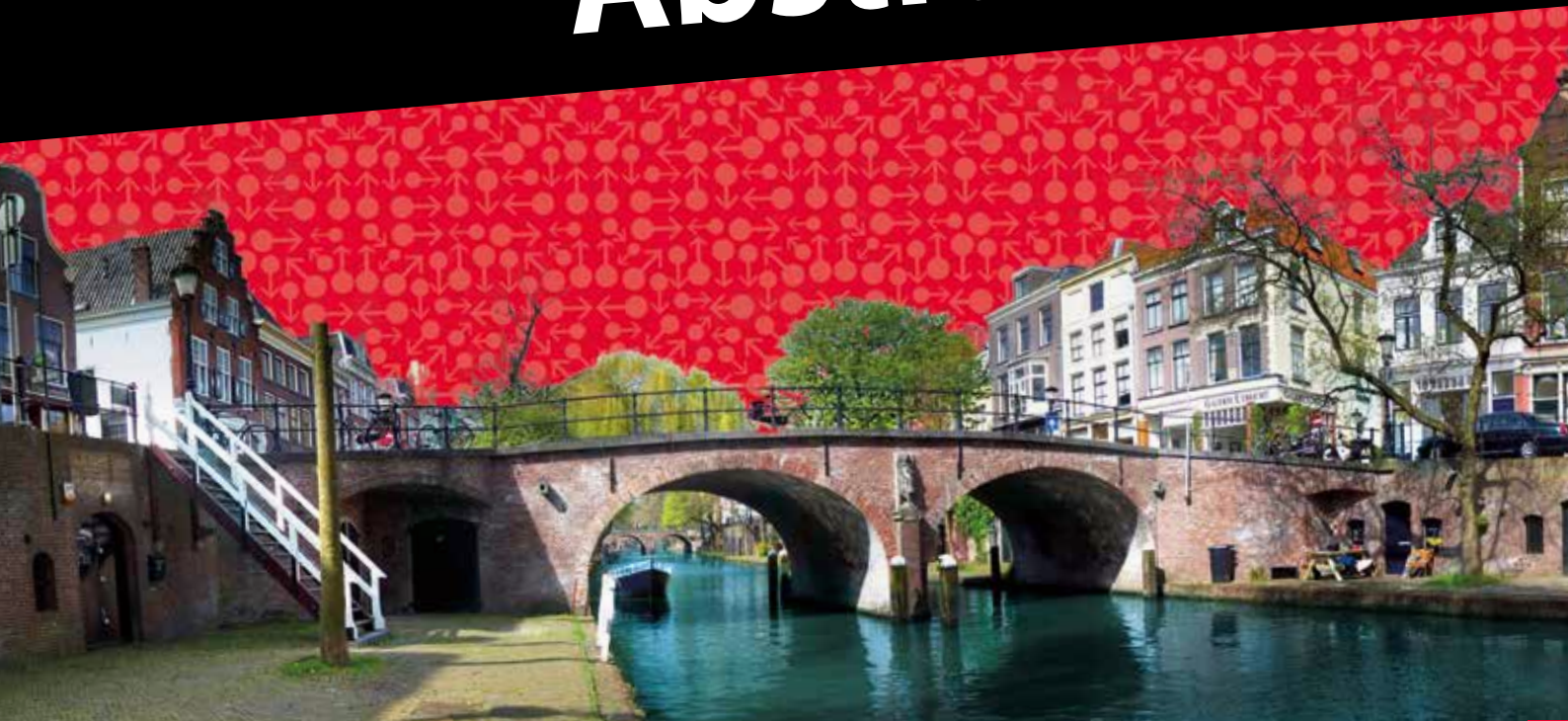# Abstractbook

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Dear Magnetic Resonance Enthusiasts, dear Colleagues,

As the Chair of the Board of Trustees of EUROMAR I am very happy to welcome you to the EUROMAR conference 2022 in Utrecht. After two years of only online meetings, I am looking forward to this in-person EUROMAR conference in Utrecht! It was good to have continuity with our annual EUROAR meetings by having them organized as online events in 2020 by Óscar Millet and in 2021 by Janez Plavez. It was nice to see that these and so many other online conferences and seminar series kept the scientific community in touch and up-to-date with the ongoing scientific progress in our research fields. Despite that, I am looking very much forward to the in-person EUROMAR meeting this year in Utrecht, organized by Marc Baldus and his team! Meeting many of you in person again is something I missed very much over the last 2 years. Only by the absence of such meetings within the last 2 years I realized how important such direct personal contacts indeed are! I think it is even more important for our young researchers on a PhD or Postdoc level. For them, such personal contacts are not only important for stimulation and encouragement for their own research and for their visibility to the senior researchers, but also crucial for the planning and successful continuation of their future academic careers. Of course, this 2 years interruption also allowed us to question some of our old habits. Is it really necessary to join so many of such meetings and conferences all-around the world each year? Cannot some of the online meetings and conferences substitute for that, also for the sake of a reduced climate footprint? Of course! But for me personally, the EUROMAR conference is one of these events I really want to go to in person, also in the future!

I wish all of us an exciting meeting and very much look forward to meet you all in Utrecht!

Thomas Prisner
Chair of the Board of Trustees of EUROMAR
Institute of Physical and Theoretical Chemistry
and Center of Biomolecular Magnetic Resonance,
Goethe University Frankfurt am Main, Germany

DEAR PARTICIPANTS OF EUROMAR 2022, DEAR COLLEAGUES,

On behalf of the Scientific Committees of the EUROMAR and AMPERE organizations, we are delighted to welcome you to EUROMAR 2022 in Utrecht, The Netherlands!

After more than two years, we are glad and grateful to be able to welcome so many of you in person to our meeting. Our "welkom" goes out to our many speakers and poster presenters who will present their latest science and trigger stimulating discussions. In addition, we are indebted to our sponsors that committed to our meeting. They will not only display their latest innovations but have also provided key financial support. Last but not least, this meeting would not be possible without the continuous help of our International Scientific Program Committee and the members of the local organizing committee.

Without doubt, the world has changed since the last "in-person" Euromar meeting in 2018 and has affected professional and private lives of all of us. Also, and especially in the last 12 months, the organization of our meeting involved many emotional highs and lows on how our meeting would look like, having to keep different scenarios open until the last minute.

In spite of the continuing turbulent times, our mission was always to set up a meeting in which personal interactions are possible again, fostering exchanging scientific ideas, meeting old and making new friends. We also did our best to support our youngest generation of scientists for which Euromar22 may be the first "in-person" scientific meeting in their careers. We are therefore extremely pleased to be able to welcome more than 600 participants in person on the premises of Utrecht University. Next to the excellent science ahead of us, we hope you will also find time to discover the beautiful city of Utrecht which celebrating this year 900 years of city rights!

We wish you all a stimulating and joyful meeting and look forward to welcoming you to Utrecht!

Local Organizers:

Marc Baldus – Alexandre Bonvin – Markus Weingarth – Hugo van Ingen – Andrei Gurinov – Geeske Badart

Organizing committees

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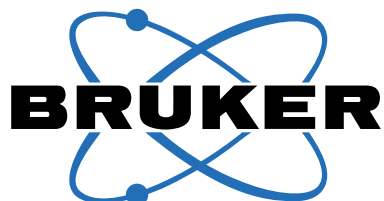
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OTHER

DSM

Program

Sunday 10 July

Time	Event	Location
12.00 - 19.00	**Registration**	*Educatorium Foyer*
13.00 - 16.00	**Bruker Symposium**	*Wit*
16.15 - 18.30	**Opening session and prizes**	*Theatron*

Time	Event
16.15	Welcome Utrecht University, Department Head Chemistry
16.20	Welcome AMPERE
16.25	Welcome EUROMAR
16.30	Welcome LOC EUROMAR 2022
16.40	Raymond Andrew Prize Intro
16.45	Raymond Andrew Prize Lecture: *Jakob Lindale (Duke University, USA)*
17.05	Varian Young investigator Intro
17.10	Varian Young investigator Lecture: *Rina Rosenzweig (Weiszmann Institute, Israel)*
17.40	Ernst Prize Intro
17.50	Ernst Prize Lecture: *Harald Schwalbe (Frankfurt University, Germany)*
19.00 - 21.00	**Welcome Mixer**

Monday 11 July

Time	Event	Location
08.30 - 19.00	**Registration**	*Foyer*
08.30 - 19.00	**Exhibition**	*RuppertHall*
08.45 - 10.15	**Plenary 1**	*Theatron*

Chair: Malcolm Levitt

Time		Event
08.45	PL001	Plenary Speaker 1: Dynamic Nuclear Polarization using High-Spin Radicals and Electron Spins Clusters *Songi Han, USA*
09.30	PL002	Plenary Speaker 2: Single Spins in Diamond: Technology and Applications. *Christian Degen, Switzerland*
10.15 - 10.45		**Break**

Program

10.45 - 12.45	Parallel Sessions

Parallel Session 1: Bio NMR 1   *Room: Blauw*
Chair: Tatyana Polenova

10.45	IN001	Structural polymorphism and dynamics of G-rich DNA repeats *Janez Plavec, Slovenia*
11.15	PT001	Phosphates form spectroscopically dark state assemblies in common aqueous solutions *Joshua Straub, USA*
11.35	PT002	Real-time NMR recording of fermentation and lipid metabolism processes in live microalgae cells *Anjali Pandit, The Netherlands*
11.55	PT003	Conformational transformation of the intrinsically disordered SARS-CoV-2 nucleoprotein on interaction with its viral partner nsp3 *Martin Blackledge, France*
12.15	IN002	Observation of conformational changes that underlie the catalytic cycle of the 100 kDa exoribonuclease Xrn2 *Remco Sprangers, Germany*

Parallel Session 2: EPR & Hyperpolarization   *Room: Wit*
Chair: Bjoern Corzilius

10.45	IN003	Unveiling the steps of the prepore-to-pore transition of a Tc toxin *Enrica Bordignon, Switzerland*
11.15	PT004	Water Concentration Gradients Across Lipid Bilayers Revealed by a High Resolution HYSCORE Method *Alex Smirnov, USA*
11.35	PT005	On-the-fly optimisation of ESR experiments *Jean-Baptise Verstraete, United Kingdom*
11.55	PT061	Utilizing EPR spectroscopy to resolve metal-sensitive transcription mechanisms. *S. Ruthstein, Israel*
12.15	IN004	Hydrogenative and Non-Hydrogenative Parahydrogen Induced Polarization for Precision Measurement and Molecular Imaging Applications *Thomas Theis, USA*

Parallel Session 3: Single Molecular / NV   *Room: Rood*
Chair: Susumu Takahashi

10.45	IN005	Prospects of Diamond Solid-State Quantum Sensors *Mutsuko Hatano, Japan*
11.15	PT007	Three-dimensional Fourier imaging of thousands individual NVs with sub-micron resolution *Aharon Blank, Israel*
11.35	PT008	Nitrogen-vacancy center as a terahertz source *Sándor Kollarics, Hungary*
11.55	PT009	Surface NMR spectroscopy using NV-centers in diamond *Dominik Bucher, Germany*
12.15	IN006	Title: t.b.a. *Tjerk Oosterkamp, The Netherlands*

12.45 - 13.45	Lunch

Lunch Symposium JEOL   *Megaron*

13.45 - 15.45	Poster Session 1 - All posters with an even number will be presented	*Alfa, Beta*
15.45 - 17.45	Parallel Sessions	

Program

Parallel Session 4: Solid-state NMR 1   *Room: Blauw*
Chair: Yoshitake Ishii

15.45	IN007	Innovations in Protein Solid-state NMR using Ultra-fast MAS and its Applications to Amyloid-beta Fibrils *Yoshitaka Ishii, Japan*
16.15	PT010	Nitrogen (14N/15N)-Hydrogen MAS NMR Two-Dimensional Correlation Spectroscopy: Developing Methods for Pharmaceutical Applications *Steven Brown, UK*
16.35	PT011	Structure and dynamics of photochromic rare-earth oxyhydrides *Shrestha Banerjee, The Netherlands*
16.55	PT012	Recent developments in NMR of quadrupolar nuclei in solids *Jean Paul Amoureux, France*
17.15	IN008	Studying structure, dynamics, and inhibition of intra-membrane proteases in a native-like environment by solid-state NMR *Adam Lange, Germany*

Parallel Session 5: Computation 1   *Room: Wit*
Chair: Jeff Hoch

15.45	IN009	Beyond the piecewise-constant approximation: efficient simulation of shaped pulses *Ilya Kuprov, United Kingdom*
16.15	PT013	Evaluation of Simulated RDC, NOE and 3J Data to Determine the Configuration of Flexible Molecules *Ulrich Sternberg, Germany*
16.35	PT014	The accuracy of protein structures in solution determined by AlphaFold2 and NMR *Nicholas Fowler, United Kingdom*
16.55	PT015	Automated chemical shift assignment and protein structure determination with the deep learning method ARTINA *Piotr Klukowski, Switzerland*
17.15	IN010	Versatile simulations for better understanding of NMR experiments *Thomas Vosegaard, Denmark*

Parallel Session 6: Bemchtop / low field   *Room: Rood*
Chair: Camilla Terenzi

15.45	IN011	Dissolution dynamic nuclear polarization opens new perspectives for metabolomics *Patrick Giraudeau, France*
16.15	PT016	Ultralow-field NMR detection of photochemically induced dynamic nuclear polarization *Kirill Sheberstov, France*
16.35	PT017	Study of zeolite anti-caking effects for fertilizers by 1H low-field NMR *Patrik Galvosas, New Zealand*
16.55	PT018	Zero- and Ultralow-Field NMR Relaxometry *Seyma Alcicek, Poland*
17.15	IN012	Title: t.b.a. *Dimitrios Sakellariou, Belgium*

17.45 - 18.15	**Break**
18.15 - 19.00	**Plenary 2** *Theatron*

Program

Monday 11 July continued

Chair: Chantal Tax	
PL003	Plenary Speaker 1: Low field MRI: hardware, data acquisition, image processing, sustainability and in vivo applications *Andrew Webb, The Netherlands*

TBC	**Bruker dinner**	*Galgen-waard Stadium*

Tuesday 12 July

08.30 - 19.00	**Registration**	*Foyer*
08.30 - 19.00	**Exhibition**	*Ruppert Hall*

08.45-10.15	**Plenary 3**		*Theatron*
	Chair: Enrica Bordigon		
08.45	PL004	Plenary Speaker 1: Microresonators for EPR spectroscopy of nanoliter solutions *Veronika Szalai, USA*	
09.30	PL005	Plenary Speaker 2: Title: t.b.a. *Gregg Siegal, The Netherlands*	
10.15 - 10.45	**Break**		

10.45 - 12.45	**Parallel Sessions**		
	Parallel Session 7: Materials 1 Chair: Sharon Ashbrook		*Room: Blauw*
10.45	IN013	Operando 7Li NMR Characterization of Electrochemical Cells Using an Optimized Parallel Plate Resonator *Gillian Goward, USA*	
11.15	PT065	Designing biobased polyamide adhesives: predicting functional properties with solid-state NMR spectroscopy *Marianne Gaborieau, France*	
11.35	PT020	59Co Internal Field NMR: Using the magnetic properties of cobalt nanoparticles to study catalytic processes *Pascal Scholzen, France*	
11.55	PT021	Revealing defects in nanoparticles using very-high-field NMR of quadrupolar nuclei *Olivier Lafon, France*	
12.15	IN014	Solid-state and in situ NMR spectroscopic studies of flexible metal-organic frameworks *Eike Brunner, Germany*	

Program

Parallel Session 8: Solution 1   *Room: Wit*
Chair: Laura Pietratelli

10.45	IN015	*Exploring the Dynamic World of Membrane Systems and Biocatalysis* *Manuel Etzkorn, Germany*	
11.15	PT022	Methods for exploring non-Fourier dimensions - from small molecules to proteins *Krzysztof Kazimierczuk, Poland*	
11.35	PT023	New NMR methods for structural analysis of fluorinated systems, *Coral Mycroft, UK*	
11.55	PT024	Activation and allosteric regulation of HtrA proteases revealed by solution NMR spectroscopy *Björn Burmann, Sweden*	
12.15	IN016	Structural studies of intrinsically disordered proteins with cross-correlated relaxation *Anna Zawadzka-Kazimierczuk, Poland*	

Parallel Session 9: Exotica   *Room: Rood*
Chair: Huub de Groot

10.45	IN017	Spin noise, RASER, radiation damping in solution NMR *Norbert Mueller, Austria*	
11.15	PT025	Light-coupled NMR spectroscopy: NMRtorch and its applications *Alexander Golovanov, United Kingdom*	
11.35	PT026	Towards applications of β-NMR at CERN *Beatrice Karg, Switzerland*	
11.55	PT028	Adaptive Magnetic Resonance *Assaf Tal, Israel*	
12.15	IN018	Delocalized long-lived states in aliphatic chains excited by polychromatic spin-lock induced crossing (poly-SLIC). *Geoffrey Bodenhausen, France*	

12.45 - 13.45   **Lunch**

Lunch Symposium JEOL   *Megaron*

13.45 - 15.45   **Poster Session 2 - All posters with an odd number will be presented**   *Alfa, Beta*

15.45 - 17.45   **Tutorials**   *Theatron*

Chair: Enrica Bordigon

15.45	TU001	Tutorial Speaker 1: MRI hardware and image reconstruction *Lawrence Wald, USA*	
16.25	TU002	Tutorial Speaker 2: Tissue microstructure imaging with diffusion MRI *Chantal Tax, The Netherlands*	
17.05	TU003	Tutorial Speaker 3: Imaging metabolism with 13C and 2H labelled substrates *Kevin Brindle, United Kingdom*	

17.45 - 18.15   **Break**

18.15 - 19.00   **Plenary 4**   *Theatron*

Chair: Hartmut Oschkinat

	PL006	Plenary Speaker 1: NMR spectroscopy to study dynamics of small molecules and proteins *Christian Griesinger, Germany*	

Program

Wednesday 13 July

08.30 - 19.00	Registration		*Foyer*
08.30 - 19.00	Exhibition		*Ruppert Hall*

8.45 - 10.15	**Plenary 5**		*Theatron*
	Chair: Alexandre Bonvin		
08.45	PL007	Plenary Speaker 1: NMR experiments and biomolecular simulations: A perfect match? K*resten Lindorf Larsen, Denmark*	
09.30	PL008	Plenary Speaker 2: A tale of broad and narrow lines – fast MAS solid-state NMR of viral proteins *Anja Bockmann, France*	

10.15 - 10.45	**Break**

10.45 - 12.45	**Parallel Sessions**		
	Parallel Session 10: Hyperpolarization Chair: Sammi Jannin		*Room: Blauw*
10.45	IN019	Decoding the Structural Complexity of Supported Molecular Catalysts by DNP Surface Enhanced Solid-State NMR *Anne Lesage, France*	
11.15	PT029	Fine optimization of a dissolution-DNP experimental setting for NMR of metabolic samples at natural abundance *Arnab Dey, France*	
11.35	PT030	Isotopological fingerprinting via 1H/D scrambling identifies SABRE hyperpolarization catalysts *Ewoud Vaneeckhaute, Belgium*	
11.55	PT031	The AsymPol family: a whole set of highly efficient DNP polarizing agents. *Sabine Hediger, France*	
12.15	IN020	Mixed-valence Polarizing Agents for Overhauser Effect DNP in Insulating Solids S*vetlana Pylaeva, The Netherlands*	
	Parallel Session 11: Computation 2 Chair: V. Orekhov		*Room: Wit*
10.45	IN033	The Enduring and Emerging Value of BMRB *Jeff Hoch, USA*	
11.15	PT050	Quadratic spacing of effective gradient area for spatially encoded diffusion NMR *Rituraj Mishra, France*	
11.35	PT051	RApid Metabolic Identifier for 1D PM-TOCSY (RaMIT) *Shankararama Sharma, India*	
11.55	PT052	Dynamical Xe NMR modelling in molecular and materials cavities *Perttu Lantto, Finland*	
12.15	IN034	Analysis of sidechain dynamics using slow-relaxing methyl quadruple-quantum coherences *Chris Waudby, United Kingdom*	

Program

Wednesday 13 July continued

		Parallel Session 12: Para NMR *Room: Rood*
		Chair: M. Ubbink
10.45	IN023	Lessons from intrinsic paramagnetic tensors and application to high-energy sparsly populated protein states *Daniel Häussinger, Switzerland*
11.15	PT035	Machine learning-based refinement of the metal coordination sphere in paramagnetic metalloproteins by pseudocontact shifts *Giacomo Parigi, Italy*
11.35	PT036	Utilization of solid-state NMR to determine the local magnetic susceptibility *Ridvan Ince, France*
11.55	PT037	Solution state NMR reveals insights into the gelation mechanism of paramagnetic metal-coordinated hydrogels. *Valeria Gabrielli, Italy*
12.15	IN024	Site-specific labelling of proteins with phenylsulfonyl-pyridine tags for paramagnetic NMR *Xun-Cheng Su, China*

12.45 - 13.45	**Lunch**	
	Lunch Symposium Magritek	*Megaron*

13.45 - 15.45	**Poster Session 3** **All posters will be presented**	*Alfa, Beta*
15.45 - 17.45	**Parallel Sessions**	

		Parallel Session 13: Materials 2 *Room: Blauw*
		Chair: Arno Kentgens
15.45	IN025	Investigating Structure and Dynamics in Solar Thermal Fuels by Solid-State NMR *John Griffin, United Kingdom*
16.15	PT038	Formation and evolution of nanoscale calcium phosphate precursors under biomimetic conditions *Ludovica Martina Epasto, Austria*
16.35	PT039	Revealing Carbon Capture Chemistry by 17O NMR Spectroscopy *Suzi Pugh, United Kingdom*
16.55	PT067	Insights into Novel Supported Ionic Liquids Phase Catalysts by Solid-State NMR spectroscopy *Dorotheau Wisser, Germany*
17.15	IN026	Title: t.b.a. *Luis Mafra, Portugal*

		Parallel Session 14: Solution 2 *Room: Wit*
		Chair: Oscar Millet
15.45	IN027	Improved pulse sequences with respect to sensitivity, resolution, and bandwidth *Burkhard Luy, Germany*
16.15	PT041	GEMSTONE: ultra-selective NMR methods for complex spectra *Emma Gates, United Kingdom*
16.35	PT042	Broadband effects of radiation damping during homonuclear total correlation mixing *Philippe Pelupessy, France*
16.55	PT043	Long-lived states and coherences for magnetisation transfer via Overhauser and exchange effects in biomolecules *Paul Vasos, Romania*
17.15	IN028	*Ultrafast relaxation and diffusion correlation and exchange measurements* *Ville-Veikko Telkki, Finland*

Program

		Parallel Session 15: Metabolomics Chair: John van Duijnhoven	*Room: Rood*
15.45	IN029	Protein Biophysics by NMR in Silico and in Silica *Rafael Brueschweiler, USA*	
16.15	PT044	Deuterium metabolic imaging of human gastric emptying and hepatic and renal glucose metabolism at 7T *Ayhan Gursan, The Netherlands*	
16.35	PT045	Parahydrogen hyperpolarization in chemical analysis of biological samples *Indrek Reile, Estonia*	
16.55	PT046	COVID-19 metabolic progression and disease prognosis as investigated by NMR metabolomics *Oscar Millet, Spain*	
17.15	IN030	Title: t.b.a. *Alia Matysik, The Netherlands*	
17.45 - 18.15		**Break**	
18.15 - 19.00		**Plenary 6**	*Theatron*
		Chair: Kendra Frederick, Germany	
	PL009	Plenary Speaker 1: Time Domain and High Frequency DNP Experiment *Bob Griffin, USA*	

Program

Thursday 14 July

08.30 - 19.00	Registration	*Foyer*
08.30 - 16.30	Exhibition	*Ruppert Hall*

8.45 - 10.15	**Plenary 7**		*Theatron*
	Chair: Guido Pintacuda		
08.45	PL010	Plenary Speaker 1: Integrative Structural Biology of Protein Assemblies: Challenges and Opportunities for Magnetic Resonance *Tatyana Polenova , USA*	
09.30	PL011	Plenary Speaker 2: Design and construction of mobile, small scale devices for MRI and NMR of plants in the field *Carel Windt, Germany*	

10.15 - 10.45	**Break**

10.45 - 12.45	**Parallel Sessions**

Parallel Session 16: Bio NMR 2 *Room: Blauw*
Chair: Manuel Etzkorn

10.45	IN031	Towards in-cell NMR spectroscopy in physiologically defined cellular states *Lukas Trantirek, Czech Republic*
11.15	PT047	Protein- and ligand-observed 19F NMR spectroscopy in human cells *Enrico Luchinat, Italy*
11.35	PT048	Atomic interrogation of proteins within intact nuclei by DNP-supported solid-state NMR *David Beriashvili, The Netherlands*
11.55	PT049	High sensitivity NMR for structural determination of neurodegenerative disease-associated proteins inside cells *Kendra Frederick, USA*
12.15	IN032	Unspinning chromatin: studying nucleosome structure, dynamics and interactions by NMR *Hugo van Ingen, The Netherlands*

Parallel Session 17: Hardware *Room: Wit*
Chair: Jennifer Mathies

10.45	IN021	Designing artificial materials for efficient local signal increase at ultra-high field human MRI *Rita Schmidt, Israel*
11.15	PT032	HYPNOESYS: Hyperpolarization in Liquid-State NMR Spectroscopy using Optically Polarized Crystals *Tim Eichhorn, Germany*
11.35	PT033	Nuclear Magnetic Resonance over nine orders of magnitude in the magnetic field *Laurynas Dagys, United Kingdom*
11.55	PT034	Optimizing hairpin coils for metabolomic analyses *Bing Wu, The Netherlands*
12.15	IN022	Integrating Dissolution DNP - Hyperpolarized computation to characterize complex systems *Dennis Kurzbach, Austria*

Program

Parallel Session 18: MRI *Room: Rood*
Chair: Daniel Topgaard

10.45	IN035	Non-local rheo-MRI of industrially-relevant particulate fluids *Camilla Terenzi, The Netherlands*
11.15	PT053	In-vivo Diffusion Tensor Imaging of the mouse abdomen using a driven-equilibrium approach to spatial encoding *Sónia Gonçalves, Portugal*
11.35	PT054	Massively multidimensional diffusion MRI: from concepts to restriction sensitive and sparsely-sampled acquisition *Maxime Yon, Sweden*
11.55	PT055	Clinical applications of sodium TQ/TPPI spectroscopy and microimaging: The case of Type 2 Diabetes Mellitus *Galina Pavlovskaya, United Kingdom*
12.15	IN036	Prospectively triggering cardiac MRI by sensing the modulation of a magnetic Pilot Tone *Peter Speier, Germany*

12.45 - 13.45	**Lunch**
13.45 - 15.45	**Parallel Sessions**

Parallel Session 19: Solid state NMR 2 *Room: Blauw*
Chair: Patrick van der Wel

13.45	IN037	Using solid-state NMR spectroscopy to understand biological tissues in health and disease *Melinda Duer, United Kingdom*
14.15	PT056	DNP on membrane proteins: Channelrhodopsin-2, the Cannabinoid Receptor 2 and application of AsymPolPOK *Johanna Becker-Baldus, Germany*
14.35	PT057	Diamond Rotors for DNP MAS NMR *Natalie Golota, USA*
14.55	PT058	Fast-MAS NMR structure elucidation of fully protonated proteins via innovations for assignment and distance information *Rasmus Linser, Germany*
15.15	IN038	A continuous approach to Floquet theory for pulse-sequence optimization in solid-state NMR *Mathias Ernst, Switzerland*

Parallel Session 20: Hyperpolarization & EPR *Room: Wit*
Chair: Veronika Szalai

13.45	IN039	Shining a Light on Electron Spin Resonance: Light-induced Pulsed Dipolar Spectroscopy *Alice Bowen, United Kingdom*
14.15	PT059	The C-terminal domains of yeast Hsp90 in vitro and in cells *Angeliki Giannoulis, Israel*
14.35	PT060	The predicted structure of a pathogen surface protein validated by pulse dipolar EPR *Bela Bode, United Kingdom*
14.55	PT061	Solid-like Dynamic Nuclear Polarization Observed in the Fluid Phase of Lipid Bilayers at 9.4 T *Andrei Kuzhelev, Germany*
15.15	IN040	Nuclear pair electron spin echo envelope modulation *Gunnar Jeschke, Switzerland*

Program

Thursday 14 July continued

		Parallel Session 21: Small molecules & drug discovery Room: Rood
		Chair: Laura Castanar
13.45	IN041	Novel Multifrequency STD NMR Tools to gain 3D Structural Information on Weak Protein-Ligand Complexes *Jesus Angulo, Spain*
14.15	PT062	Enabling high throughput fragment screening with hyperpolarized NMR *Felix Torres, Switzerland*
14.35	PT063	Unravelling the novel mode of action of Teixobactin using solid-state NMR *Rhythm Shukla, The Netherlands*
14.55	PT064	Binding of the clinical drug candidate anle138b to lipid-induced α-synuclein fibrils *Leif Antonschmidt, Germany*
15.15	IN042	Automated peak picking, and uSTA *Andy Baldwin, United Kingdom*
15.45 - 16.15		**Break**
16.15 - 18.30		**Plenary and closing** *Theatron*
		Chair: LOC & Beat Meier
16.15		*Tatyana Polenova JMR*
16.20		IES president, *Songi Han*
16.25		Ampere video prize, *S. Hiller*
16.30		Concluding remarks, *Thomas Prisner*
16.35		Concluding remarks, *Anja Bockmann*
16.40		Concluding remarks, LOC EUROMAR 2022
16.50		EUROMAR 2023
17.00	PL012	ONLINE Plenary Speaker 1: Optically-pumped NMR of CdTe – a System for Studying the "Spin Bath" of Dilute Spins *Sophia Hayes, USA*
17.45	PL013	Plenary Speaker 2: Classical and modern ways of exploiting the information content of paramagnetic observables *Claudio Luchinat, Italy*
18.30		**Closing**
19.30 - 00.00		**Gala dinner** *Tivoli Vredenburg*

Program

Friday 15 July

09.00 - 15.00		Satellite meeting **Magnetic Resonance at Ultra-High Field**	*Theatron*
	09.00	Opening	
	09.05	*Beat Meier (ETH Zurich)*	
	09.30	*Nils Alexander Lakomek (Düsseldorf University /BMFZ Julich)*	
	09.55	*Siva Veeramuthu Natarajan (Leiden University)*	
	10.10	*Jeanine Prompers (Utrecht University) & Rico Singer (Leiden University)*	
10.10 - 10.45		**Coffee Break**	
	10.45	*Enrico Luchinat (CERM, Florence Universiy)*	
	11.10	*Christian Griesinger & Loren Andreas (MPI Göttingen)*	
	11.35	*Jennifer Gomez (Nijmegen University)*	
	11.50	*Salima Bahri (Utrecht University)*	
12.15 - 12.45		**Festive Program**	
12.45 - 13.45		**Lunch**	
	13.45	*Maksim Mayzel (Bruker)*	
	14.10	*Robert Griffin (MIT)*	

Topics

1. Bio NMR
2. Computation
3. EPR/ESR
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Lexture & Poster Index

POSTERS

EUROMAR 2022

EUROMAR 2022

Ernst Prize Lecture

Development and application of real-time NMR to study (re)-folding of proteins, RNAs and DNAs

In this presentation, I will discuss the development of time-resolved NMR spectroscopy and application to biologically relevant examples. From studying protein and RNA folding, our group has been focusing on the investigation of kinetic aspects on RNA-based regulation and on how speed of translation can be linked to 3D protein structure.

H. Schwalbe

Center for Biomolecular Magnetic Resonance (BMRZ)
Institute for Organic Chemistry and Biological Chemistry
Johann Wolfgang Goethe-University Frankfurt

Understanding and Optimizing Dynamics in Hyperpolarized Magnetic Resonance

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Signal Amplification By Reversible Exchange (SABRE) is an exciting hyperpolarization method that derives spin polarization from parahydrogen during transient interactions mediated by an organometallic catalyst. SABRE exists in a regime where the resonance frequencies, dominant couplings, and exchange are all on the same order of magnitude, complicating analysis of the system. Improved understanding of the SABRE dynamics is critical to improving the efficiency of this technique. We developed a robust theoretical framework to describe SABRE dynamics, which was facilitated by addressing fundamental limitations of the conventional theory for chemical exchange that has been used for over fifty years. This had the practical effect of improving the speed of chemical exchange simulations by an order of magnitude and facilitated the development of our exhaustive model. Recently, this treatment for exchange has been fully generalized within the Lindblad formalism and permitted extensions to finite lifetimes of intermediate exchange states.

The insights gained from our theoretical framework have significantly improved SABRE performance, as validated experimentally. For example, sequences that do not approach the traditional (level anti-crossing) matching condition for polarization transfer, either instantaneously or on average, generate significantly improved magnetization. Utilizing a combination of perturbation theory, average Hamiltonian theory, and numerical simulation is effective in both motivating new excitation schemes as well as optimizing hyperpolarization of these new experiments. This culminated in the first demonstration of a phase-coherent and simultaneous hyperpolarization of multiple targets at high magnetic field with the development of new excitation methods with large bandwidths, and the development of multi-axis excitation geometries that yield improved hyperpolarization transfer.

PL001

Dynamic Nuclear Polarization using High-Spin Radicals and Electron Spins Clusters

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The direct transfer of polarization from isolated electron spins to a surrounding nucleus is inefficient at high B0 field. It is understood that utilizing coupled electron spins (e-e) can remedy this problem. Two distinct DNP mechanisms (Cross Effect (CE) and Thermal Mixing (TM)) rely on e-e couplings (1's-100's MHz) to induce triple-flip transitions between two coupled e spins and a hyperfine coupled nucleus. The resonance condition is satisfied when the difference of the EPR frequencies of the coupled electron spins matches the nuclear Larmor frequency. In bis-nitroxides, this condition is met by its large g-anisotropies, where the CE is predominant and highly efficient at moderate B0 such as 9T. However, contemporary NMR benefits from higher B0, routinely 18 T, but typically the DNP efficiency is significantly lower at these high fields. A promising mechanism is TM DNP that exploits a strongly coupled electron spin network, in which the difference of the EPR frequencies of the coupled electron spins matching the 1H Larmor frequency is generated from dipolar and exchange coupling between the coupled electron spins. In this talk, I will present new diagnostics and conceptual advances to enhance DNP effects by utilizing high-spin radicals in conjunction with spin ½ sensitizer radicals to satisfy CE conditions at high magnetic fields, as well as utilizing strong multi-electron spin coupling to maximize the TM DNP effects. The key to these studies is the concurrent EPR diagnostics relying on CW EPR, pulsed EPR relaxation measurements and pump-probe Electron double resonance (ELDOR) measurements performed under DNP conditions.

PL002

Single spins in diamond: Technology and Applications

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Isolated spin defects in diamond have become an exciting playground for NMR and EPR experiments, because optical detection allows measuring them at the single-spin level. One of these defects, the nitrogen-vacancy (NV) center, preserves its excellent longitudinal and transverse relaxation times up to room temperature. Our group is harnessing the properties of NV centers for a variety of high-resolution magnetic sensing applications.

In this talk, I will give an introduction into magnetic resonance experiments with single diamond spins. After discussing the basic physics and experimental platform, I will highlight two on-going efforts in our laboratory: A first effort is the detection of individual nuclear spins in the vicinity of the NV center, with a long-term goal of enabling 3D atomic imaging of single molecules. In a second part, I will discuss the integration of single NV centers into scanning tips, allowing us to perform scanning probe microscopy of magnetic surfaces with sub-100 nm spatial resolution.

Low field MRI: hardware, data acquisition, image processing, sustainability and in vivo applications

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[1]_Leiden University Medical Center, Leiden, The Netherlands_

Commercial magnetic resonance imaging (MRI) systems cost millions of euros to purchase, require large shielded spaces to house, are expensive to maintain and require highly trained technicians. These factors means that their distribution is confined to centrally-located medical centres in large towns and cities. Globally over 70% of the world's population has absolutely no access to MRI, and clinical conditions which could benefit from even very simple scans cannot be treated. In the financially developed world, although MRI is diagnostically very important, the high cost and fixed nature prohibits any type of role in widespread health screening, for example. The magnetic fields typically used are very high, which means that there are severe contraindications so that, for example, MRI cannot currently be used in the emergency room. From the considerations above it is clear that if low-field MRI could be made more portable, accessible and sustainable then it would open up new opportunities in both developed and developing countries.

Rather than designing a highly sophisticated and expensive piece of equipment that can be used for all types of scanning, we use the philosophy of tailored design, such that we can design much more inexpensive systems for specific medical applications. In order to achieve portability, we design systems that use thousands of very small low-cost permanent magnets, arranged in designs which have no fringe field and therefore very easy siting requirements. The low magnetic fields allow scanning of patients with implants, and the scanner could potentially be transported on an ambulance for differentiation of hemorrhagic or ischemic stroke, for example.

This talk will cover aspects of magnet, gradient and RF coil design for low fields (~50 mT) , as well as corrections for gradient- and B0-distortions, and present the latest in vivo results as well as an outlook on future developments.

PL004

Microresonators for EPR spectroscopy of nanoliter solutions

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In this talk, I will focus on our work to develop microresonators for EPR spectroscopy. Resonators are at the heart of every EPR spectrometer and are a main enabling technology. Microresonators, which are miniaturized versions of resonators, offer both benefits and challenges in their implementation for inductive-detection EPR spectroscopy. We have reported a planar inverse anapole microresonator design for EPR spectroscopy for continuous wave (CW) measurements at room temperature [Abhyankar et al. Sci. Adv. 2020]. Adding microfluidic wells to our microresonator, we have collected CW spectra of a spin-labeled protein and used serial dilution to experimentally determine the solution spin concentration sensitivity. The evaluation of the performance of our microresonator design for pulsed EPR measurements on solutions at room temperature is in progress. In addition to highlighting recent experimental results, I will present considerations for the design and fabrication of microresonators. To conclude, I offer our perspective on current challenges and prospective directions for microresonator development and applications for EPR spectroscopy.

PL005

NMR (and Biophysics) in Drug Discovery: Effectiveness vs Elegance

Siegal G[1]

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Despite the explosion of therapeutic modalities such as gene therapy and mRNA vaccines, small molecule drugs remain the backbone for therapeutic intervention in most human diseases. In the last 10 years there has been wide spread appreciation of the value of biophysical techniques in many aspects of small molecule drug discovery including: hit discovery, mode of action assessment, triage of non-druglike interactions and provision of low resolution structural information. However, of the many possible biophysical techniques, perhaps the most information rich, that is NMR, remains the least valued. In this talk, I will provide a number of ways in which NMR can contribute mission critical information in real world, commercial drug discovery projects in both a timely and cost-effective manner. Many of these examples make use of simple 1D homonuclear experiments which can be performed in a relatively high throughput manner. These examples are culled from the more than 17 years of experience accrued at ZoBio

PL006

NMR spectroscopy to study dynamics of small molecules and proteins

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Dynamics in various systems is presented that can be detected by NMR spectroscopy. We studied the domain motion of the two Calcium binding domains of Calmodulin (CaM) in complex with an IQ and a Munc-13 derived peptide using the CaM-N60D mutant to bind a single lanthanide. Pseudocontact shifts and residual dipolar couplings observed for the two CaM domains provide a rich source of information whose conversion into converging ensembles will be described. While the CaM/IQ complex exhibits small amplitude motion (order parameter close to 0.9), the CaM/Munc13 shows large amplitude motion with an order parameter of 0.16.

Kinetics of conformational conversions in the microsecond range can be assessed by high power relaxation dispersion making lifetimes down to 1 digit microseconds accessible. The measurements are based on the time dependent chemical exchange induced variance of the isotropic chemical shift which depends on the static magnetic field quadratically. Ubiquitin shows two distinct modes of motion involving the whole protein. They occur on microsecond time scales and can be easily distinguished kinetically. The N-terminal domain of p53 forms a transient helix on a one digit microsecond time scale. The kinetics can be slowed down dramatically by the introduction of a proline residue.

Finally, the kinetics of a protein protein recognition process will be presented in which ubiquitin and a SH3c domain bind to each other. Based on the concentration dependent measurement of high power relaxation, we find profiles for ubiquitin, but not SH3c, indicating that conformational selection prevails and analyse the conformational transitions for ubiquitin from free to bound with molecular modelling and Markov state analysis (Chakrabarty and Olsson, Nature Comm. In press).

PL007

NMR experiments and biomolecular simulations: A perfect match?

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Biological macromolecules are dynamic entities, and I will discuss methods and applications for how simulations and experiments can be used synergistically to study the structure and dynamics of macromolecules.

Functional protein motions are often described as an exchange between a ground state structure and minor states. The structural and biophysical properties of these transiently and sparsely populated states are, however, difficult to study, and an atomic-level description of those states is challenging. Using proteins with extensive NMR data available as test systems, we have shown how enhanced sampling simulations can be used to capture accurately complex conformational changes in proteins, and I will briefly discuss such examples.

Despite recent progress, one may still find that a simulation does not quantitatively match experiments. Then, experiments and simulations may be combined in a very direct fashion to provide a description of the molecular motions that combines the details of atomic simulations with the accuracy afforded by experiments. The resulting conformational ensembles may provide novel insight into biomolecular systems that are not obtainable by simulations of experiments alone. I will discuss how this may be achieved and give examples of the application of such approaches using to describe proteins or RNA, and extensions to include timescales of motion and NMR relaxation measurements.

PL008

A tale of broad and narrow lines – fast MAS solid-state NMR of viral proteins

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Fast MAS has paved the way for sensitive proton detection, enabling the analysis of small amounts of protein. This in turn has made possible the combination of often low-yield eukaryotic protein expression approaches, typically successful for complex proteins, with solid-state NMR. We will report on cell-free NMR sample preparation procedures that can be done in favorable cases in as few as 2 days, and present the resulting high-resolution spectra for a variety of viral protein assemblies. On the other hand, we will present cases where proton, but fortunately not carbon and nitrogen lines, remain broad. For both cases, NMR approaches will be discussed and applications to proteins of different viruses will be presented.

PL009

Time Domain and High Frequency DNP Experiment

<u>**Griffin R**</u>

Dynamic nuclear polarization (DNP) has become an invaluable tool to enhance sensitivity of magic angle spinning (MAS) NMR, enabling the study of biomolecules and materials which are otherwise intractable. In this presentation we explore some new aspects of time domain DNP experiments and their applications.

One of the main thrusts of DNP was to provide increased sensitivity for MAS spectroscopy of membrane and amyloid protein experiments. A problem frequently encountered in these experiments is the broadened resonances that occur at low temperatures when motion is quenched. In some cases it is clear that the proteins are homogeneously broadened, and therefore that higher Zeeman fields and faster spinning is required to recall the resolution. We show this is the case for MAS DNP spectra of Ab1-42 amyloid fibrils where the resolution at 100 K is identical to that at room temperature. Furthermore, we compare the sensitivity of DNP and 1

H detected experiments and find

that DNP, even with a modest $\mathcal{E}$=22, is ~x6.5 times more sensitive.

We have also investigated the frequency swept-integrated solid effect (FS-ISE) and two recently discovered variants – the stretched solid effect (SSE) and the adiabatic solid effect (ASE). We find that the latter two experiments can give up to a factor of ~2 larger enhancement than the FS-ISE. The SSE and ASE experiments should function well at high fields.

Finally, we discuss two new instrumental advances. First, a frequency swept microwave source that permits facile investigation of field profiles. It circumvents the need for a B0 sweep coil and the compromise of field homogeneity and loss of helium associated with such studies. This instrumentation has permitted us to elucidate the polarization transfer mechanism of the Overhauser effect, and also revealed interesting additional couplings (ripples) in field profiles of cross effect polarizing agents. Second, to improve the spinning frequency in DNP experiments, we have developed MAS rotors laser machined from single crystal diamonds. Diamond rotors should permit higher spinning frequencies, improved microwave penetration, and sample cooling

PL010

Integrative Structural Biology of Protein Assemblies: Challenges and Opportunities for Magnetic Resonance

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I will discuss recent advances in MAS NMR methods for atomic-resolution structural analysis of large biological assemblies. Using examples of distinct systems studied by our lab, kinesin motor domain assembled with polymerized microtubules, cofilin assembled with filamentous actin, and HIV-1 protein assemblies bound with small-molecule maturation inhibitors, I will illustrate the unique information revealed by MAS NMR about atomic-level 3D structures, dynamics, and drug binding, inaccessible by other techniques. I will demonstrate the power of integrating MAS NMR with medium-resolution cryo-EM and data-driven MD simulations, and discuss the challenges and opportunities for magnetic resonance in integrative structural biology.

PL011

Design and construction of mobile, small scale devices for MRI and NMR of plants in the field

Windt C

In this contribution we present the development of a mobile, fully integrated MR plant imager that can be used in greenhouse and field. To allow imaging of branches and stems an open C-shaped permanent magnet was constructed. In the design of the magnet, pole gap width and resilience against handling were prioritized over homogeneity and field strength. To overcome the adverse effects of short T2* that may result from these design choices multi-spin echo imaging strategies were employed, using short echo times and high spectral widths [1]. To still achieve microscopic resolution under these constraints requires fast switching field gradients, driven by strong and fast gradient amplifiers. While small-scale spectrometers and RF amplifiers are readily available, appropriate small-scale gradient amplifiers or designs thereof currently are not. We therefore constructed a small, 3-channel gradient amplifier on the basis of a conventional current-controlled AB amplifier design. Tailored to our small low-impedance gradient coils the amplifiers could remain small, suitable for battery driven operation and still meet our requirements regarding switching speed, power and duty cycle. The finished device weighs 5 kg and is capable of delivering 40 A gradient pulses of up to 6 ms in duration, sufficient for micro imaging and flow mapping. With all components built onto an aluminum hand trolley, the entire imaging setup weighs 45 kg and is small enough to fit into a car[2]. We demonstrate the mobility and utility of the device imaging quantitative water content and T2, first of an apple tree in an orchard; second, of a beech tree during spring leaf flushing in a greenhouse. Without imaging such systems can also be used as NMR relaxometer. Utilizing a reinterpretation of the solid fat content method, dry matter deposition into growing cereal spikes and bean pods can be monitored in a straightforward, sensor-like manner [3].

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[2] Meixner, M., et al., An integrated magnetic resonance plant imager for mobile use in greenhouse and field. Journal of Magnetic Resonance, 2021. 323: p. 106879

[3] Meixner, M., et al., An integrated magnetic resonance plant imager for mobile use in greenhouse and field. Journal of Magnetic Resonance, 2021. 323: p. 106879

PL012

Optically-pumped NMR of CdTe – a System for Studying the "Spin Bath" of Dilute Spins

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A grand challenge in quantum technologies is the preservation of spin coherence lifetimes. Spin systems that coherently couple to light offer key capabilities for quantum technologies. Here we report on long nuclear spin coherence lifetimes of ^{113}Cd in CdTe that have been polarized through coupling to optically-oriented electrons. In single crystal CdTe, these are ^{113}Cd -^{125}Te 2-spin (directly-bonded) pairs that interact via heteronuclear dipole-dipole and scalar couplings. Optically-pumped NMR (OPNMR) is a hyperpolarization scheme where optically-pumped (and polarized) conduction electrons in a semiconductor serve to polarize nuclear spins and overcome very long spin-lattice T_1 relaxation times. The natural abundances of the NMR-active species in CdTe are not large, providing an opportunity to study the nuclear spin bath in detail, including ^{113}Cd -^{125}Te spin pairs and "solo" ^{113}Cd spins, as well as the complex cross-polarization behavior that results. We also have findings to rectify optical/nuclear quantization conventions with each other, as well as with NMR phasing conventions by comparing CdTe and GaAs results.

PL013

Classical and modern ways of exploiting the information content of paramagnetic observables

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Among structural techniques, NMR works close to biological conditions. The presence of a paramagnetic metal ion provides additional information[1], at a level hardly accessible with other techniques. Paramagnetic observables (CS, PCS, PRE, RDC) can provide structural refinement starting from, e.g., crystallographic models, and determine the internal arrangement of domains with known structures[2]. Conformational variability can be addressed to reveal conformations with largest possible weights or lowly populated states[3]. Recent theoretical and computational advancements in QC calculations of paramagnetic NMR observables are opening new routes in structural biology[4,5,6,7]. Hyperfine shifts can drive the refinement of protein structures at the metal coordination site to an unprecedented resolution[8,9].

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Tutorial Lectures

MRI hardware and image reconstruction

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MRI technology has traditionally concentrated on the considerable engineering challenges of rapidly applying the magnetic fields needed to polarize and excite the spins, rapidly encode the image spatial information, and sensitively detect the MR signal. Image reconstruction has been historically un-coupled from this effort and mainly consisted of applying the Fourier transform. Thus, for the first half of MRI history, the burden was on the hardware and acquisition to create data from which an image could be formed with a simple and computationally fast "inverse transform". This subservient relationship has now reversed due to the enormous "Moore's law" leap in computational power and advances in signal processing. The hardware constraints can be relaxed and more importantly used in new ways. Progress has been made in regularizing the inversion of detailed/complex forward models with missing information and perhaps without unique inverses.

These image reconstruction advances first changed how we process MR raw data, then they impacted the collection of the data itself. Namely the data sampling is now jointly designed with the reconstruction method to facilitate faster, more "under-sampled" acquisitions and to be more robust to biological constraints such as patient motion. In the final chapter of this arc, we have come full circle and the same signal processing, cast as an optimization problem now impacts how we design the hardware. This talk will show some examples and discuss the impacts of the "signal processing revolution" on MRI hardware and acquisition methods, including how the gradient fields no longer play a primary role in image encoding, and how we approach some of the biological barriers to MRI performance such as patient motion, patient induced non-uniformities of the static magnetic field, and peripheral nerve stimulation by the switching gradient coil fields.

Tutorial Lectures

TU002

Tissue microstructure imaging with diffusion MRI

Tax C[1,2]

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Water molecules diffuse in the order of micrometers during the typical time scale of a spin echo experiment, remarkably coinciding with the characteristic size of cellular structures in biological tissues. As such, diffusion MRI has been extensively used to probe the architectural configuration of tissue in health and disease. This talk will cover some notable advances in the measurement of diffusion with NMR – from the use of spin echo techniques by Hahn, Carr and Purcell in the 1950's and the development of pulsed-field gradients by Stejskal and Tanner over a decade later, to emerging and more exotic encoding techniques today. We will discuss promising applications in healthcare including tissue modelling and fiber tractography, and open challenges in the field.

Tutorial Lectures

TU003

Imaging metabolism with 13C and 2H labelled substrates

Brindle K[1]

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Metabolic networks typically operate in a dynamic steady state, where the rate of synthesis of metabolic intermediates are equal to their rates of breakdown and their concentrations are thus time invariant. To measure metabolic fluxes in this situation we need to introduce an isotopically labelled substrate, where in magnetic resonance studies this has usually involved a 13C-labelled substrate. The introduction of dissolution dynamic nuclear spin polarization, which increased the sensitivity of 13C label detection by 104 – 105-fold has allowed imaging of the metabolism of hyperpolarized 13C-labelled substrates in vivo, including in the clinic. Hyperpolarized 13C studies however are limited by the short lifetime of the nuclear spin polarization, which means that imaging studies are restricted to a few minutes of data acquisition and can only be used to follow relatively fast metabolic processes. Clinical translation has also been hindered by the complexity of the polarization process. Although studies using 2H-labelled substrates were first reported in the 1980s this was a relatively neglected isotope for studies of metabolism in vivo until the publication of a landmark paper in 2018, which reported 2H MRSI studies of 2H-labelled glucose and acetate metabolism in brain and liver and in an animal model of glioblastoma and, most importantly, in glioblastoma patients in the clinic. The low sensitivity of detection is compensated by the frequently short T1 relaxation times, which means that signal can be acquired rapidly in the absence of saturation. The limitation is the narrow chemical shift range, which combined with the low g, makes the spectrum crowded and often poorly resolved, particularly at the clinically relevant magnetic field strength of 3T. In this presentation I will describe recent studies using 2H-labelled and hyperpolarized 13C-labelled substrates and discuss potential clinical applications, particularly in oncology.

Structural polymorphism and dynamics of G-rich DNA repeats

**Plavec J**[3]

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It is the nature of nucleic acids, including DNA and RNA, that they function to store, transmit, and express genetic information. Alternative secondary structures to the Watson-Crick duplex, including quadruplexes and i-motifs, are associated with many biological functions. The best-studied noncanonical DNA structures are G-quadruplexes with the G-quartet as the basic structural unit. However, our studies show that the structure and folding landscape of DNA single strands is much more complex than previously thought. New principles of folding of G-rich oligonucleotides that could be applied to the prediction of natural and the design of artificial recognition elements, as well as to the design of ligands with specific targeting features.

A G-rich sequence d(G3TAG3AGCG3AGAG3), a G4T analog of the wild-type sequence in the regulatory region of the RANKL gene associated with bone metabolism homeostasis, folds into a basket-type G-quadruplex. Remarkably, a G-quadruplex with two G-quartets forms in the presence of K+ ions, although three G-quartet fold would be expected based on its sequence alone consisting of four GGG tracts.

Sequences containing d[(GGGNn)3GGG] repeats, where Nn stands for AGCGA, adopt topologies characterized by a tetrahelical core of AGCGA repeats connected by edge-like loops of different lengths stabilized by G-G base pairs. GGGAGCG repeats occur in the regulatory regions of genes responsible for neurological disorders, cancer, and abnormalities in bone and cartilage development.

The DNA oligonucleotide d(GTGTGGGTGTG), corresponding to the most abundant sequence motif in Saccharomyces cerevisiae (yeast) irregular telomeric DNA, adopts a G-hairpin stabilized by dynamic G:G base pairs alternating between N1-carbonyl symmetric and N1-carbonyl, N7-amino base-pairing arrangements.

IN002

Observation of conformational changes that underlie the catalytic cycle of the 100 kDa exoribonuclease Xrn2

Sprangers R[1]

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The development of nuclear magnetic resonance (NMR) methods that quantitatively probe motions on a molecular and atomic level has propelled the understanding of biomolecular processes for which static structures cannot provide a satisfactory description. Here, we study the structure and dynamics of the essential 100 kDa eukaryotic 5'-3' exoribonuclease Xrn2. A combination of complementary fluorine and methyl-TROSY NMR spectroscopy reveals that the apo enzyme is highly dynamic around the catalytic center. These observed dynamics are in agreement with a transition of the enzyme from the ground state into a catalytically competent state. We show that the conformational equilibrium in Xrn2 shifts significantly towards the active state in the presence of substrate and magnesium. Finally, our data reveal that the dynamics in Xrn2 correlate with the RNA degradation rate as a mutation that attenuates motions also impacts catalytic activity. In that light, our results stress the importance of studies that go beyond static structural information.

IN003

Unveiling the steps of the prepore-to-pore transition of a Tc toxin

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Tc toxins are 1.7 MDa protein complexes that are found in insect- and human-pathogenic bacteria. The complex consists of three subunits: the 1.4 MDa TcA pentamer, which mediates target cell association, membrane insertion and toxin translocation, and two smaller subunits, TcB and TcC, which form a 250 kDa cocoon that encapsulates the toxic enzyme. After endocytosis, Tc uses a syringe-like mechanism to penetrate the membrane of the host's cells and translocate a deadly enzyme into the cytosol[1,2]

However, the exact sequence and the kinetics of the prepore-to-pore transition are unknown. Here we present continuous wave EPR and DEER data of spin-labeled TcA protomers which unveiled the kinetics of shell opening and channel ejection of TcA triggered by high pH. The EPR data, corroborated by single molecule fluorescence analysis, allowed the identification of intermediate conformations in the transition from the prepore to the pore, which were structurally characterized by cryo-EM. We also identified point mutations in the TcA subunit, which accelerate pore formation and/or modify the pH-dependency of the reaction such that pores are also formed at physiologically relevant acidic pH values. The cryo-EM analysis revealed the structural origin of the effects observed, shedding light on the allosteric coupling mechanism in the conformational transition of this toxin.

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[2]   Roderer et al., PNAS 2019 116(46):23083

IN004

Hydrogenative and Non-Hydrogenative Parahydrogen Induced Polarization for Precision Measurement and Molecular Imaging Applications

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Parahydrogen induced polarization (PHIP) is cherished for its relative simplicity and ease. Here we report on the most recent progress with PHIP in hydrogenative addition and non-hydrogenative reversible exchange reactions to attain (a) Radiofrequency Amplification By Stimulated Emission of Radiation (RASER) for precision measurements of J-couplings and chemical shifts[1–4] and (b) molecular imaging with a cryogen-free, variable field MRI system after SABRE-SHEATH hyperpolarization of 13C-pyruvate to above 10% polarization with a temperature cycling approach.[5,6] Simultaneously, we continue the substrate scope expansion to other metabolites and drugs, for example antifungals.[7]

We use both hydrogenative and non-hydrogenative (SABRE-SHEATH) methods to achieve large degrees of hyperpolarization on substrates. In application to precision measurements we use these methods to establish population inversions (i.e. negative hyperpolarization) that is below the critical RASER threshold. Below this negative threshold (i.e. at large population inversions), the RASER sets in and leads to NMR signals that can be acquired for an arbitrarily long time. In turn arbitrarily narrow lines are obtained in the NMR spectra with unprecedented precision.

In application to molecular imaging, 1-13C-pyruvate is hyperpolarized at microT magnetic fields at lower temperatures of -5 C. This efficiently hyperpolarizes catalyst bound pyruvate. By elevating the temperature slowly, the polarization is transferred to the free form, which can then be imaged with a cryogen-free MRI system with sub mm resolution.

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Invited Lectures

Prospects of Diamond Solid-State Quantum Sensors

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Diamond is an excellent host for spin-based qubits, and the spin in diamond has excellent properties. Nitrogen-vacancy (NV) center in diamond is one of the most promising qubits for quantum sensing and quantum communication. The NV center preserves quantum coherence in a wide temperature range under atmospheric pressure. The energy levels of NV centers are sensitive to magnetic fields, electric fields, strain, and temperature, enabling scalable applications from the atomic to the macroscopic range.

In this talk, core technologies on material, devices, quantum control technology, sensor systems, and applications for life science and energy electronics are introduced.

- Materials and sensor devices:

Selectively-aligned NV ensemble formed by CVD growth for scalable applications.
The heteroepitaxial growth of NV-contained diamond on Si substrate for a large area and on-chip integration.

Sensing devices using pn junctions.

- Sensor systems and applications:

Multi-scale and multi-modal sensor systems for life-science and energy electronics applications.

- -High precision simultaneous measurement of current and temperature in EV batteries
- -High-resolution magnetocardiographic imaging of rats
- -Probing into living cells by tip-type NV sensor.

IN007

Innovations in Protein Solid-state NMR using Ultra-fast MAS and its Applications to Amyloid-beta Fibrils

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First, we discuss our progress in structural examination of misfolded amyloid-β (Aβ) by solid-state NMR (SSNMR). Increasing evidence suggests that formation and propagation of misfolded aggregates of 42-residue Aβ42, rather than more abundant Aβ40, provokes the Alzheimer's cascade. Our SSNMR study recently revealed a unique S-shaped structure of Aβ42 amyloid fibril for the first time.[1] Here, we discuss our ongoing efforts to examine the feasibility of characterizing the structure of trace amounts of brain-derived and synthetic amyloid fibrils by sensitivity-enhanced 1H-detected solid-state NMR (SSNMR) under ultra-fast magic angle spinning (UFMAS). We also present the first case of complete signal assignments on fully-protonated amyloid fibrils for Aβ42 fibril by 1H-detected 3D and 4D SSNMR. The data reveals the presence of new polymorphs for Aβ42 fibrils.[2] Other data on cryoEM and SSNMR analysis of Aβ fibril will be also presented.

Second, we discuss our recent progress in resolution and sensitivity enhancement in 1H-detected biomolecular SSNMR under UFMAS conditions ($\geq$ 80 kHz) in a high magnetic field (1H frequency: 750-900 MHz).[2,3] Our data on protein microcrystal GB1 and Aβ fibril show that traditionally time-consuming 3-5D biomolecular SSNMR is feasible for signal assignments in a nano-mole-scale with this approach. We also discuss novel concepts for drastic sensitivity enhancement by various polarization-transfer schemes using ultra-fast MAS[4].

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IN008

Studying structure, dynamics, and inhibition of intra-membrane proteases in a native-like environment by solid-state NMR

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Our research group uses solid-state NMR in combination with other structural and biophysical techniques to study protein structure and dynamics. Solid-state NMR allows to investigate proteins under native-like conditions. Recent applications in the group concern flexible supramolecular assemblies (1) as well as membrane proteins such as ion channels (2) and rhomboid proteases in the context of liposomes at room temperature and under physiological conditions. Here we will present recent results on the rhomboid protease GlpG using tailored proton-detected solid-state NMR experiments developed in our group (3). Our data reveal a previously unobserved kink in the central part of the gating helix TM5 (4). Dynamics measurements revealed increased dynamics of GlpG at the N-terminal part of TM5 and the adjacent loop L4, indicating that this region is important for gating. In addition, relaxation dispersion experiments suggest that TM5 is in conformational exchange between an open and a closed state. More recently, we have characterized the interaction of GlpG with a range of inhibitors (5). Currently, we are extending our studies to full-length GlpG, including the cytosolic domain, and to other rhomboid proteases such as PARL. We have also started to screen for novel rhomboid protease modulators in cooperation with our in-house screening unit using a liposome-based assay (6).

References:

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IN009

Beyond the piecewise-constant approximation: efficient simulation of shaped pulses

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Of the many ways to simulate density matrix dynamics under a time-dependent Hamiltonian, the popular piecewise-constant approximation used in many simulation and optimal control packages is actually the least efficient one in the class of exponential action integrators [1]. It is also awkward in optimal control settings because real-life spectrometers (particularly in EPR spectroscopy) struggle with piecewise-constant control sequences – those are distorted by the response functions of electromagnetic circuits.

In this communication we report an implementation and practical performance benchmarks for multi-point geometric integrators [2] that were recently added to Spinach time propagation and optimal control modules. High-order geometric integrators are, loosely speaking, group-theoretical product equivalents of trapezium and Simpson quadratures. The presence of matrix-matrix products in those integrators makes them prohibitively expensive in Liouville space unless Krylov-type propagation algorithms [3], which are free of matrix-matrix operations, are also used. That is hard but possible; an implementation is now available in Spinach.

Apart from simply improving the simulation efficiency, geometric integrators allow the popular GRAPE algorithm for quantum optimal control [4] to be migrated from piecewise-constant control sequences to the more instrument-friendly piecewise-linear ones.

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IN010

Versatile simulations for better understanding of NMR experiments

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This presentation will give an overview of basic concepts of numerical simulations in solid-state NMR as well as give examples on more advanced simulations. The examples will give examples on simulations of pulse sequences and fitting of experimental spectra using full-density simulations and simplified and faster models. A new web-based workflow for efficient simulations will be presented, which provides a simple manner to work with experimental data, apply advanced processing and analysis algorithms, and provide easy sharing of data and data-analyses.

IN011

Dissolution dynamic nuclear polarization opens new perspectives for metabolomics

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Most NMR-based metabolomics studies are based on [1]H 1D spectra which are sensitive and high-throughput, but severely impacted by peak overlap. Heteronuclear [13]C 1D/2D experiments would be ideally suited to tackle the complexity of complex metabolite mixtures, but they are barely used in practice due to their low sensitivity. To tackle such limitations, we recently started to explore the potential of dissolution dynamic nuclear polarization (D-DNP) for ^{13}C NMR metabolomics. Such exploration raises a number of methodological and analytical challenges, since D-DNP is still a recent method relying on complex experimental settings, whose potential to study complex biological mixtures at natural abundance remains unexplored. We recently developed a complete untargeted NMR-based metabolomics workflow based on D-DNP. Using a D-DNP prototype dedicated to analytical chemistry applications, we showed the ability of this approach to statistically distinguish two group of plant sample extracts, and to highlight relevant biomarkers. We also explored the potential of D-DNP to analyze biofluids at natural abundance, making it possible to detect and identify several tens of relevant metabolites from ^{13}C hyperpolarized spectra of freeze-dried urine samples.

In parallel with these promising metabolomics applications, we are constantly improving the performance of the D-DNP analytical workflow to increase its applicability for the analysis of complex metabolic mixtures. Thanks to a fine optimization of the many parameters involved in the D-DNP experiment, we have been able to significantly increase the sensitivity, the precision and the robustness of our experimental setting. In addition, we are exploring the potential of ultrafast 2D NMR methods to provide single-scan homo- and hetero-nuclear correlations following D-DNP, in order to improve the separation of overlapped metabolite signals. We will describe these recent developments, highlighting the potential of D-DNP for metabolomics, as well as the questions raised by the development of this new analytical approach.

IN013

Operando 7Li NMR Characterization of Electrochemical Cells Using an Optimized Parallel Plate Resonator

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Li-ion batteries are considered as the main source of energy for an electric vehicle application; however, the significantly longer "refueling" time compared to standard internal combustion engine vehicles is a substantial disadvantage from the perspective of the end-user. The development of optimal fast charging protocols requires detailed information about the lithium inventory in the battery. A natural choice for acquiring this information is the use of nuclear magnetic resonance (NMR), which is isotope-specific, quantitative, non-reliant on crystallinity, and importantly, non-destructive. NMR spectra thus can be acquired while the battery is repeatedly charging/discharging, permitting the quantification of various phases existing within the battery in real time. The main downside of NMR is its low inherent sensitivity, which can restrict the time resolution of these studies. Herein we report the development of a parallel-plate resonator (PPR) RF probe and a cartridge-type single layer cell of improved designs.1 The probe has excellent B1-field homogeneity,2 and exhibits approximately three-times higher sensitivity than a comparable operando NMR probe that uses a solenoidal coil. Using this probe assembly, operando 7Li NMR measurements on a graphite//NMC622 cell were acquired. At low (C/10) charging rates the usual sequencing of LixC6 phases from dilute to concentrated is exhibited, while at high (1C) rates a direct transition to, and propagation of concentrated phases throughout the graphite electrode is observed, with no evidence for the formation of dilute phases. Concurrently, a peak assigned to plated Li metal forms. Thus, we can conclude that Li accumulates on the surface of graphite at high charging rates. Such real-time observations of electrochemical cells will contribute to optimizing performance.

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IN014

Solid-state and in situ NMR spectroscopic studies of flexible metal-organic frameworks

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NMR spectroscopy is a unique tool for studying structure, dynamics, and flexibility of metal-organic frameworks (MOFs) [1]. Adsorption processes as well as adsorption-induced structural changes in flexible MOFs, i.e., host-guest interactions, are also accessible for NMR spectroscopy. In situ studies of host-guest interactions are particularly powerful [2,3]. Special equipment allows in situ high-pressure NMR spectroscopic studies by the application of variable gas pressures up to a relative pressure of 1 at variable temperatures down to 190 K inside the NMR spectrometer [2]. This allows following adsorption/desorption isotherms by observing the signals of the adsorbed gases and to correlate the NMR-derived parameters with volumetric adsorption/desorption isotherms. Gases like xenon, carbon dioxide, and methane were already used for our investigations. Xe-129 NMR spectroscopy is a well-established technique since it provides characteristic, structure-sensitive parameters like the chemical shift, the chemical shift anisotropy, signal intensities, and relaxation times. Gas exchange processes can be studied by 2D exchange spectroscopy (EXSY). The described in situ NMR technique could for example be used to characterize the xenon-induced phase-transitions [2] in the recently discovered pressure-amplifying framework material DUT-49 with its unique negative gas adsorption transitions. Xenon is capable of inducing mesopore closure at 200 K. Liquid-phase adsorption-induced phase transitions of MOFs were recently visualized by solid-state NMR spectroscopy [3].

In summary, the combination of in situ high-pressure NMR spectroscopy and solid-state NMR spectroscopy provides deeper insight into the adsorption and phase transition behavior of MOFs.

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IN015

Exploring the Dynamic World of Membrane Systems and Biocatalysis

*Borggräfe J[1,2], Chu C[1], Elter S[1], Escobedo F[1], Gopalswamy M[4], Klein A[1], Victor J[1], Viegas A[1], Viennet T[1,2], Gohlke H[4], Heise H[1,2,3], Schiemann O[5], **Etzkorn M**[1,2,3]*

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To understand life on a molecular level, it is of fundamental importance to study structure interactions, and dynamics of life's key players – proteins, nucleic acids, and lipids – in their native environment. NMR spectroscopy has a unique potential to provide high spatial and temporal resolution of the target molecules in complex environments. However, sample preparation, low sensitivity, and ensemble averaging pose clear limitations in increasingly complex systems.

During the last years we contributed a number of methods that aim to overcome central restrictions and that are tailored to further exploit the inherent strengths of NMR spectroscopy. This includes optimized isotope-labelling(1), sample-preparation(2), and polarization-usage(3) strategies as well as selective hyperpolarization via functionalized ligands(4).

Furthermore, we could show that the application of the developed methods can provide new insights into: (i) membrane-induced aggregation of the Parkinson's associated protein α-synuclein(5), (ii) signalling of central membrane receptors(6,7), and (iii) DNA-mediated biocatalysis(8).

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IN016

Structural studies of intrinsically disordered proteins with cross-correlated relaxation

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Under physiological conditions intrinsically disordered proteins (IDPs) lack a rigid three-dimensional structure; they only transiently adopt various structural motifs. This high mobility enables them to play a variety of roles, especially those related to signaling and regulation. 1 Therefore, the proportion of IDPs in organisms increases with organism complexity. The relation of IDPs to many civilization diseases, makes them important objects to study. NMR is a powerfull tool for such research, providing atomic-scale information on structure, dynamics or interactions of IDPs. Importantly, IDPs do ot behave like a random coil, but exhibit certain structural propensities that can be related to their function.

Cross-correlated relaxation (CCR) rates are a valuable source of protein structural information, as their values are related to dihedral angles.2 Such measurements were first proposed for folded proteins, as two- or three-dimensional (2D or 3D) experiments. But CCR rates can be also used for IDPs, where they report on the residual structure. Then, the dimensionality of the experiments should be increased, as an inherent flexibility of IDPs causes their chemical shift range to be significantly narrower than in the case of folded proteins. Also the data analysis is more complicated than in the case of folded proteins. Here, instead of a single value of each dihedral angle (φ and ψ) per residue, we expect a distribution of angles. To this end, we can use maximum entropy approach 3, yielding a set of probablities, each corresponding to a different conformation.

Here, we present the first application of the set of seven 4D CCR experiments, to an IDP called osteopontin.

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IN017

Spin noise, RASER, radiation damping in solution NMR

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Radiation damping, the feedback between the resonance circuit and the spin ensemble inside the rf-coil, is inseparably linked to both spin noise and stimulated emission phenomena (MASER or more appropriately RASER - Radio-frequency Amplification by Stimulated Emission of Radiation). Starting from our group's long-time research on the spin noise phenomena, which will be briefly reviewed, related RASER phenomena have recently come into our focus.

Trying to observe spin noise phenomena after inversion of magnetization, i.e. establishing negative spin temperature in a sufficiently polarized spin ensemble within a strongly coupled rf-circuit RASER emissions can be observed easily, sometimes unexpectedly. Hyperpolarization techniques, of course, are very efficient for that purpose. A spontaneous RASER emission requires sufficiently negative polarization P

$P < -4/(n\, Q\mu_0 \hbar \eta \gamma^2 T_2^*)$

(for n spins with gyromagnetic ratio γ at an apparent transverse relaxation time T_2^* in a probe with a quality factor Q at a filling factor η).

We have obtained first examples of RASER detected NMR coherence transfer spectra on cryogenically cooled probes starting from thermal polarization. Random variation of the timing of the spontaneous coherent emission is a characteristic of these experiments as every emission requires a trigger event, which can originate from spin noise or circuit (Nyquist) noise. The timing of such triggers is not entirely random and it has been shown that discrimination of resonances based on their characteristic trigger delays is possible.

For coherence transfer experiments it is more desirable to control the triggering of a RASER event, e.g. by applying a very short rf-pulse. Thus the time domain data can be aligned and first 2D RASER detected NMR spectra could be obtained. New processing protocols avoid artefacts in the indirect dimension of 2D RASER-detected NMR spectra due to the non-linear dependence (threshold effect) of the emitted signal on polarization.

IN018

Delocalized long-lived states in aliphatic chains excited by polychromatic spin-lock induced crossing (poly-SLIC)

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It is shown that one can excite and observe delocalized long-lived states encompassing 2n proton spins in (CH2)n chains in a variety of molecules in isotropic solution without isotopic labelling. The magnetic equivalence of pairs of protons in CH2 moieties in aliphatic chains is broken when the rotamer populations are unequal. For n = 3, the 6 protons constitute an AA'MM'XX' system. Delocalized long-lived states such as IA. IA' + IM. IM' + IX. IX' can be excited by polychromatic spin-lock induced crossing (poly-SLIC), i.e., by simultaneously applying weak selective spin-locking fields with appropriate radio-frequency amplitudes at 1, 2 or 3 different chemical shifts. The efficiency of the transfer of magnetisation to long-lived states and vice-versa depends on the irradiation scheme, the rf amplitudes and durations, and can be on the order of 10%. The resulting experimental multiplets are in good agreement with simulations. The lifetimes TLLS of these long-lived states are significantly longer than T1 and are strongly affected by binding to macromolecules.

IN019

Decoding the Structural Complexity of Supported Molecular Catalysts by DNP Surface Enhanced Solid-State NMR

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Over the last twenty years, solid-state NMR spectroscopy, often in combination with X-ray diffraction techniques, has emerged as a unique analytical method to probe the atomic-scale structure of active sites in heterogeneous catalysts. The recent advent of high-field dynamic nuclear polarization (DNP) has further reinforced the analytic power of solid-state NMR on surfaces (1).

In this presentation we will demonstrate unique methodologies to disclose, with atomic resolution, individual surface structures in complex multi-site environments, a long-standing challenge in the field of heterogeneous catalysts, while revealing new, unexpected structural features of the supported substrates. These approaches will be illustrated on isolated Ir- N-heterocyclic carbene (NHC) sites (2) as well as on Pt-NHC complexes supported on silica. In the latter case, we will discuss new experimental DNP NMR approaches at fast MAS frequencies (40 kHz) that rely on the indirect detect with high sensitivity of the 195Pt NMR parameters. We will show how these approaches in combination with DFT calculations performed on an extensive library of molecular models, allowed one to unambiguously elucidate in a site-specific way the nature and location of the ligands coordinating the metal center.

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IN020

Mixed-valence Polarizing Agents for Overhauser Effect DNP in Insulating Solids

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Overhauser effect DNP in insulating solids[1] has a number of beneficial properties for applications: it requires less microwave power than other methods, the enhancement was shown to scale favourably with magnetic field strength.[2]

Recently, we have investigated BDPA radical using state-of-the-art theoretical methods. Our findings allowed to classify the radical as a mixed-valence compound, also known as a Jan-Teller system.[3] For such compounds fluctuations electronic and vibrational degrees of freedom are strongly coupled. We showed that the BDPA radical belongs to a specific class of mixed valence compounds: spin density is localized on one part of the molecule, but can tunnel to the other side with a frequency in a range of hundreds of GHz.[3]

Later, we have identified more mixed valence molecules of the same class as the BDPA radical. We investigated those molecules by theoretical methods, and then synthesized and studied by high field EPR and DNP NMR.[4] We have observed large signal enhancement for all three radicals when irradiated on their EPR resonance.

In the current work we continue our studies of mixed valence radicals, focused on improving their stability and solubility in water.

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IN021

Designing artificial materials for efficient local signal increase at ultra-high field human MRI

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Ultra-high field human MRI offers increased signal-to-noise (SNR) and sensitivity, which can be translated into increased resolution of the acquired images. Inspired by the metamaterials design in optics, but driven by MRI requirements and constrains, we developed and optimized set of MRI-viable artificial materials. Among new directions are utilization of high permittivity dielectric materials and new hybrid metamaterial-based structures. The design of metamaterial-based structures becomes especially relevant, since it can offer compact design. Our work includes several designs of dielectric resonators as well as hybrid metamaterial-based structures to improve local imaging sensitivity. Our study showed a feasibility of artificial material to improve local sensitivity at ultra-high-field (7T) human brain MRI. We examined what are the key parameters required to optimize an efficient RF magnetic field while minimizing the electric field. Another topic of interest for MRI is a dual-nuclei design. We designed a dual-nuclei metamaterial-based structure for calf imaging, for phosphorous and proton imaging. This type of structure relies on a combination of long and short copper strips. We also examine new setups that can offer an improved patient comfort in a tight setup of brain imaging.

IN022

Integrating Dissolution DNP - Hyperpolarized computation to characterize complex systems

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Dissolution Dynamic Nuclear Polarization (DDNP) is a powerful method to boost signals in NMR spectra over 10.000-fold.[1-2] However, the obtained spectra do not always provide as much information as multidimensional NMR in thermal equilibrium, albeit excellent sensitivity. Indeed, DDNP data must be recorded within 1-2 minutes after preparation of the hyperpolarized spin states since the hyperpolarization decays to naught after dissolution and transfer to the detection spectrometer. Hence, typically only series of 1D or low-resolution 2D spectra are recorded.

This contribution aims to highlight a route towards overcoming this limitation by integrating DDNP with computational techniques.

We demonstrate the potential of this combination with two examples:

(i) Nuclear Overhauser effects (NOE) between a hyperpolarized solvent and a target molecule. We show that MD simulations combined with innovatove modes of analysis can accurately predict the direct solvent NOE for small molecules and large peptides – a phenomenon that is typically relatively weak and hence hard to observe but that can be substantially amplified through spin hyperpolarization. Thus, we could quantify the contributions of direct and exchange-relayed hyperpolarization transfer. This is important for understanding the recently proposed approach to use hyperpolarized water as a sensitivity booster in biomolecular NMR.

(ii) Protein structural dynamics through hyperpolarized single-shot detection. In this example. We show that the conformation of a protein can be determined from a single hyperpolarized spectrum when DDNP is combined with structure prediction and MD simulation tools. Since the protein spectra are boosted by over two orders of magnitude, data in the physiological (low micromolar to nanomolar) concentration regime become accessible. Thus, we could identify and characterize a structural transition of the major transcription factor MAX, when changing from high-concentration in-vitro to low-concentration conditions.

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Lessons from intrinsic paramagnetic tensors and application to high-energy sparsly populated protein states

**Häussinger D[1]**, Rieder P[1], Vogel R[1], Stiller J[2], Otten R[2], Kern D[2]

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Paramagnetic restraints like pseudocontact shifts (PCSs), residual dipolar couplings (RDCs) and paramagnetic relaxation enhancement (PRE) are well established NMR tools in structural biology for the elucidation of structures, dynamics and interactions of proteins and other biomolecules.

While lanthanide chelating tags (LCTs) provide an excellent and general way of exploiting these effects also for non metal-binding proteins, the prediction of the performance of new LCT architectures remains mostly empirical. We accomplished an experimental determination of the intrinsic anisotropy of the

magnetic susceptibility tensors for the full series of lanthanoids in a DOTA-based LCT [1]. The unusual size and modulation of the anisotropy parameters obtained, provide new insights in the dynamic properties of this type of LCTs. In addition, a highly remarkable correlation between the orientation of the principle magnetic axis of the tensor and the oblate or, respectively, prolate ground state electron distribution of the lanthanoid was found, resembling ligand field effects for d-transition metals.

The obtained knowledge of the LCT mobility relative to the protein of interest allowed the selection of suitable LCT candidates for a new method of PCS-enhanced relaxation dispersion, termed PCS-CPMG [2]. With the combination of PCS and relaxation dispersion it is possible to charcterise the structures as well as kinetic and thermodynamic parameters of high-energy protein conformations. These sparsely populated and often short-lived protein states are often crucial areas on the free-energy landscape that reveal details of enzyme catalysis.

PCS-CPMG has the potential to close a methodological gap, as these transient protein states are very difficult to study with other methods.

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IN024

Site-specific labelling of proteins with phenylsulfonyl-pyridine tags for paramagnetic NMR

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Site-specific tagging proteins with paramagentic tags for NMR applications in biological systems has made great progress in recent years, however, few paramagnetic tags are suitable for the in-cell studies. We recently developed an effecive way of site-specific labeling proteins via the reaction between the phenylsulfonated pyridine derivatives and the solvent exposed cysteine (Scheme 1), which generates a stable, short and rigid thioester bond between the protein and tag.1,2 In the present study, we will summarize the high performance of the paramagentic tags containing phenylsuolfonyl pridine moiety in a number of proteins. In addition, the 3D structure determination of RAS-GTP complex under real time conditions by PCSs.

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IN025

Investigating Structure and Dynamics in Solar Thermal Fuels by Solid-State NMR

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Solar thermal fuels (STFs) are an emerging class of materials that store light energy in strained bonding configurations of photoresponsive molecules and release it on demand as heat. They have potential applications as source of heat energy in technology and architecture. A key requirement for STFs to function is the availability of free volume for the photoresponsive molecules to change structure in response to light. For this reason, many STFs have been developed in the solution state, although this can present limitations in terms of storage, containment and energy density. For some applications solid-state STFs would be desirable, although these are challenging to design owing to the lack of steric freedom in dense phases.

In this work, we have been developing solid-state STFs based on molecular photoswitches such as azobenzene confined within metal-organic frameworks (MOFs). Using a combination of X-ray diffraction and solid-state NMR we are able to monitor guest-induced breathing upon loading the well-known framework DMOF-1 with azobenzene. Although this causes the MOF structure to contract, 13C CPMAS and 2H NMR measurements reveal the trans-azobenzene exhibits pedal motion and ring-flipping dynamics within the pores. The observed dynamics suggest that the azobenzene molecules have increased free volume as compared to bulk crystalline azobenzene, and indeed when the composite is exposed to 365 nm light we observe isomerisation to the cis isomer which is also highly mobile. In addition to the guest dynamics, 2H NMR has been used to study ring flipping dynamics in the framework linkers. We find that the activation energy for ring flipping shows a complex dependence on both the nature of guest species within the pores and the degree of guest-induced contraction. These results provide insight into mechanism of energy storage in MOF-based STFs, as well as into host-guest interactions in the wider context of breathable MOFs.

IN027

Improved pulse sequences with respect to sensitivity, resolution, and bandwidth

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Improved pulse sequences from different fields are reported using novel or improved concepts.

The first topic concerns the detection of intrinsically disordered proteins at physiological pH and temperature. The SHACA-HSQC with BASEREX homodecoupling allows detection with equal resolution and sensitivity as HN-detected experiments for conventional proteins. Several extensions of the concept are shown. Novel, effective HN-homodecoupling is also presented, as well as broadband selective pulses for spectrometer frequencies >1 GHz.

Effective fast pulsing ASAP and LowCOST-type experiments are shown for HSQC-type experiments, including CLIP-HSQC, HSQC-TOCSY and more. Finally, a fast-pulsing DOSY-type experiment based on isotropic mixing will be presented.

IN028

Ultrafast relaxation and diffusion correlation and exchange measurements

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Relaxation and diffusion NMR experiments allow elucidation molecular dynamics and obtaining chemical information complementary to conventional spectra. Multidimensional experiments enable one to correlate relaxation and diffusion parameters as well as observing molecular exchange through relaxation or diffusion contrast. Standard multidimensional experiments are slow due to incremented evolution delay in separated experiments.

We have shown that multidimensional relaxation and diffusion experiments can be accelerated by one to three orders of magnitude by spatial encoding. These single-scan ultrafast experiments facilitate also significantly the use of nuclear spin hyperpolarization to enhance sensitivity by several orders of magnitude. We have shown that both ultrafast correlation and exchange type experiments are feasible, and contrary to conventional multidimensional experiments relying on spectral information, the experiments are feasible also with low-field, single-sided magnets with inhomogeneous field.

This presentation focuses on recent progress of the development of ultrafast relaxation and diffusion experiments and their applications in different fields. For example, the principles of novel diffusion and T2 relaxation exchange experiments are described. An alternatively very simple version of D-T2 correlation experiment based on variable echo time CPMG measurements is also introduced. Various applications, ranging from porous materials and diary products to cellular metabolism and atmospheric surfactant solutions, are described as well.

IN029

Machine-learning in 2D NMR-based Metabolomics with Application to P. aeruginosa Biofilms

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The workflow in NMR-based metabolomics is slowed down considerably by qualitative and quantitative analysis of cohorts of spectra, which are often performed manually. We recently developed the deep neural network "DEEP Picker" toward the automated analysis of 2D NMR spectra, which is accompanied by quantitation using Voigt Fitter software. These new tools have been implemented in our latest and most advanced web server COLMARq. I will discuss these new methodologies and their application to Pseudomonas aeruginosa in its biofilm and planktonic phenotypes for the identification and quantification of metabolites and biochemical pathway differences as a function of treatment.

IN031

Towards in-cell NMR spectroscopy in physiologically defined cellular states

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Over the past 20 years, in-cell NMR spectroscopy has become a unique tool for characterizing biological macromolecules in their native cellular environment. Nowadays, in-cell NMR applications deliver valuable insights into biomolecules' 3D structure and thermodynamics in living prokaryotic and eukaryotic cells. Recently, in-cell NMR has also emerged as a promising tool in drug screening: Real-time in-cell NMR measurements allow an early assessment of drug potency in human cells by providing unique information on the kinetics and dynamics of ligand-to-target binding under physiologically relevant conditions.

Nonetheless, currently established in-cell NMR approaches applied to human cells have remained restricted to basic "asynchronous" 2D cellular models, a suspension of cells in various physiological states, with limited biological relevancy.

In my lecture, I will present the results of our outgoing effort to adapt the in-cell NMR approach for investigating proteins and nucleic acids in human cells in defined physiological states.

IN032

Unspinning chromatin: studying nucleosome structure, dynamics and interactions by NMR

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The fascinating thing about chromatin is that fulfills two conflicting roles: on one hand it compacts the DNA, represses unwanted transcription, and protects our genetic information from damage, on the other hand it attracts a myriad of proteins to regulate DNA damage repair, replication, and gene transcription. How these proteins recognize, modify, assemble, remodel or disassemble nucleosomes, the fundamental building block of chromatin, is one of the key questions in chromatin biology.

Our lab focusses on solving the molecular mechanisms underlying chromatin function using an NMR-driven integrative structural biology approach. In this talk I will highlight our recent results in the study of nucleosome assembly during DNA repair. We uncovered that intrinsically disordered histone chaperone APLF can single-handedly assemble the histone octamer and transfer it to the DNA to form nucleosomes, in contrast to the prevailing model of stepwise assembly. I will further present our recent work on the mechanism of nucleosome remodeling by ATP-dependent chromatin remodeler ISWI. This protein machine can translocate the DNA in the nucleosome to alter the spacings between nucleosomes within chromatin. We show that the ISWI ATPase domain (75 kDa) is an intrinsically dynamic machine that causes wide-spread conformational changes and alteration of histone-DNA contacts in the nucleosomes. These data provide crucial support for the twist-diffusion mechanism of remodeling and highlight the nucleosome as a plastic, allosteric unit.

IN033

The Enduring and Emerging Value of BMRB

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The Biological Magnetic Resonance Data Bank (BMRB) is the open, international repository for biomolecular NMR data. The enduring value of BMRB is apparent through the tools that are used every day by bioNMR spectroscopists that were developed from data in BMRB. Prominent examples are TALOS, SPARTA, PINE, PECAN, HIFI, DANGLE, and CSI. These tools are derived from chemical shift trends correlated with structural or dynamical propensities derived by federation with PDB. As BMRB grows, extreme values of chemical shifts that were once ignored as outliers gain statistical significance, with the potential to reveal insights not available from overall trends. An example based on extreme amide proton shifts will be described, revealing not only the surprising frequency of amide – aromatic hydrogen bonds, but also novel details of their geometry. Growth of BMRB will enable additional insights into biomolecular structure and dynamics, through both hypothesis-driven surveys and applications of machine learning. New resources for federation of BMRB with PDB, NCBI, and AlphaFold databases will accelerate these applications.

IN034

Analysis of sidechain dynamics using slow-relaxing methyl quadruple-quantum coherences

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Transverse nuclear spin relaxation is a sensitive probe of chemical exchange on timescales on the order of microseconds to milliseconds. Here we present an experiment for the simultaneous measurement of the relaxation rates of two quadruple-quantum transitions in ^{13}CHÐ-labelled methyl groups. These coherences are protected against relaxation by intra-methyl dipolar interactions and so have unexpectedly long lifetimes within perdeuterated biomacromolecules. However, these coherences also have an order of magnitude higher sensitivity to chemical exchange broadening than lower order coherences and therefore provide ideal probes of dynamic processes. We show that analysis of the static magnetic field dependence of zero-, double- and quadruple-quantum Hahn echo relaxation rates provides a robust indication of chemical exchange and can determine the signed relative magnitudes of proton and carbon chemical shift differences between ground and excited states [1]. We also demonstrate that this analysis can be combined with established CPMG relaxation dispersion measurements, and report new CPMG relaxation dispersion experiments exploiting quadruple-quantum coherences to provide increased sensitivity and improved precision in parameter estimates, particularly in the determination of ^{1}H chemical shift differences. Finally, we will report applications of these methods to the study of dynamics within the 45 kDa metastable serpin αÐ-antitrypsin [2].

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IN035

Non-local rheo-MRI of industrially-relevant particulate fluids

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Since the development of rheo-NMR/-MRI techniques more than two decades ago [1], flow in wide-gap geometries has been investigated in various complex fluid systems, such as worm-like micelles, liquid crystals, polymeric fluids and granular media. Thus far, the main focus has been on detecting shear banding or shear-induced formation of anisotropic ordered structures, polymerization kinetics, or particle migration.

Recent developments in both rheo-MRI setup design and data modelling have made narrow-gap rheo-MRI studies of spatially-heterogeneous non-local flow possible [2]. Non-local, or cooperative, flow of particulate fluids may occur when the flow gap size approaches the size of particle aggregates, yielding an enhanced macroscopic fluidization which cannot be predicted by standard local rheology equations [3]. The complex phenomenology of non-local flow is still poorly understood, largely due to its overlap with other spatial flow heterogeneities, e.g. due to shear banding, and to the lack of high-resolution velocity data acquired under strong confinement on non-model, optically-opaque, particulate materials.

By collecting rheo-MRI velocity profiles in mechanically-stable sub-mm geometries with spatial resolution up to below 10 μm, and by using suitable analytical or numerical modelling for each specific flow geometry, the spatial extent of flow cooperativity can be quantified in soft particulate gels and emulsions, including industrially-relevant optically-opaque food colloids and dispersions [4,5]. Narrow-gap rheo-MRI, and further ongoing developments in microcapillary flow-MRI, can substantially advance our understanding of flow cooperativity, and ultimately aid in optimizing the production conditions to account for enhanced velocities under strong confinement.

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IN036

Prospectively triggering cardiac MRI by sensing the modulation of a magnetic Pilot Tone

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Routine clinical MRI relies on ECG triggering despite its well-known drawbacks of complex patient preparation and B0 and gradient related interferences. Optical pulse sensors are used as fall back only due to the pulse wave delay, which makes the trigger time being patient state dependent.

We present an alternative contact-less trigger method, based on the modulation of a magnetic Pilot Tone (PT). It is generated close to the heart by a small loop integrated in a surface coil (Biomatrix 12 or 18, Siemens Healthcare, Erlangen, Germany). Amplitude and frequency are selected to allow the MR receive system to detect the PT in parallel with the MR signal, in a typical cardiac set-up by 20-30 receive elements.

The PT generates eddy currents in the body that vary with tissue conductivity. The associated secondary fields change the amplitude and phase detected by receiving coils. Thus, tissue shifts modulate the received PT without delay. Typical modulation depths are ~5% for respiration and ~1% for cardiac.

Cardiac and respiratory signals are formed by weighted linear channel combination, with weights trained using blind source separation on 20s of free breathing data. Artefacts from RF pulses are learned on a 12s training scan and suppressed by eliminating the artefact subspace during coil combination.

The cardiac signal is denoised and the inverted derivative calculated for trigger detection and visualization using a constant velocity Kalman filter. Cardiac triggers are detected in the acceleration phase of the contraction, i.e., early in the mid-systolic phase.

We developed a PT triggering prototype with the described processing for all standard cardiac sequences and ran complete cardiac exams at 1.5T and 3T using cardiac PT triggering in a group of volunteers. We observed stable triggering. This suggest that cardiac PT triggering is feasible and could be developed into an attractive alternative to ECG.

IN037

Using solid-state NMR spectroscopy to understand biological tissues in health and disease

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The extracellular matrix (ECM) forms the bulk of our structural tissues and provides them with their particular mechanical properties. At the microscopic level, it provides the scaffold which supports cells but more intriguingly, at the molecular level, it provides a dynamic communication system between the cells in the tissue and the signals that drive the behaviour of cells. Ultimately, if we can understand how the extracellular matrix structure dictates the behaviour of cells, then we can develop ways to treat diseases such as cancer, by changing the extracellular matrix to drive the necessary change in cell behaviour. However, understanding the molecular level properties of the extracellular matrix has been hampered by the lack of methods to study tissues at the atomic scale. In this talk, I will describe some of the solid-state NMR spectroscopy approaches my group has taken to tackle these complex questions. Both molecular structure and dynamics are important in defining communication between the ECM and cells. Thus, I will focus on the structure and dynamics of collagen (the major component of most ECMs), the consequences of non-enzymatic chemistry on collagen structure and mechanical disruption on collagen structure.

IN038

A continuous approach to Floquet theory for pulse-sequence optimization in solid-state NMR

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Floquet theory uses a Fourier series expansion of the time-dependent Hamiltonian to transform the Hamiltonian into a time-independent representation. The price to pay for this transformation is the transition from a finite-dimensional to an infinite-dimensional Hilbert space. Today, Floquet theory is often used in combination with analytical operator-based Primas-van Vleck perturbation theory to generate effective time-independent Hamiltonians.

We present a modified Floquet framework that uses a continuous frequency space to describe and design solid-state NMR experiments using time-dependent Hamiltonians. The approach is similar to the well established Floquet treatment for NMR, but is not restricted to periodic Hamiltonians and allows the design of experiments in a reverse fashion. The framework is based on perturbation theory on a continuous Fourier space, which leads to effective, i.e. time-independent, Hamiltonians. This continuous Floquet approach simplifies the description of sequences with effective fields and correctly describes the finite width of resonance conditions for pulse sequences with finite mixing time.

It allows, in certain cases, the back calculation of the pulse scheme from the desired effective Hamiltonian as a function of spin-system parameters. We show as an example how to back calculate the radio-frequency irradiation in the MIRROR experiment from the desired chemical-shift offset behavior of the sequence. It also enables us to optimize pulse sequences based on effective Hamiltonians without requiring the use of density-operator simulations. We will show first preliminary applications to frequency-selective recoupling in solid-state NMR under MAS.

Shining a Light on Electron Spin Resonance: Light-induced Pulsed Dipolar Spectroscopy

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Electron Paramagnetic Resonance (EPR) Pulsed Dipolar Spectroscopy (PDS) methods utilizing the photoexcited triplet-state of porphyrins, combined with nitroxide radicals, have previously been demonstrated.[1,2] Light-induced Double Electron–Electron Resonance (LiDEER) uses the triplet-species for detection, and traces benefit from enhanced signal intensity resulting from the non-Boltzmann population of the photoexcited triplet. Laser Induced Magnetic Dipole (LaserIMD) spectroscopy uses time-dependent optical-pumping of the triplet-state, resulting in larger modulation depths. Recently we have demonstrated that orientation selection effects in these porphyrin triplet–nitroxide experiments can be used to extract additional conformational information in model peptides.[3] These experiments have now been expended to consider additional chromophores, namely diiodo-BODIPY and Erythrosin B. Using simulations and DFT calculations, we extract distance distributions and relative orientations of the two spin-bearing moieties, allowing the dominant conformations to be identified. We also exploit frequency swept pulses generated to yield frequency-correlated LaserIMD, monitoring the complete orientation-dependence in a single experiment. Combining both ideologies of detection and pumping of optically-activated chromophores, we present a new technique Light-Induced Triplet–Triplet Electron Resonance (LITTER) spectroscopy,[4] enabling both the distance and angular distributions between the two triplet moieties to be determined in a model peptide. LITTER removes the requirement to have a permanent paramagnetic moiety within the system. This renders exciting possibilities for future applications; including in-cell measurements and potentially makes distance determination in unmodified macromolecular systems containing photo-excitable moieties accessible. Careful choice of chromophores may also make LITTER measurements directly comparable with FRET and allow combination with microscopy inside cells.

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IN040

Nuclear pair electron spin echo envelope modulation

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Electron spin echo envelope modulation (ESEEM) due to pairs of like nuclear spins usually dominates decay of electron spin coherence in the low-temperature limit. The appearance of a stochastic relaxation process arises from random configuration of the nuclei around individual electron spins. For a given electron spin, decay is governed by a moderate number of nuclear spin pairs, for which nuclear dipole-dipole coupling is similar to the difference of the two hyperfine couplings and sufficiently large. We explore specifics of nuclear pair ESEEM for the Hahn echo, the stimulated echo, and for Carr-Purcell trains with a small number of inversion pulses, as they are used in the context of dynamical decoupling. Insight into the phenomenon arises from analytical expressions derived by product operator formalism. Such expressions allow for fast numerical computations within a pair factorization approximation. On the example of an electron spin in a water box , we discuss limitations of this approximation and of any numerical treatment.

IN041

Novel Multifrequency STD NMR Tools to gain 3D Structural Information on Weak Protein-Ligand Complexes

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STD NMR is a powerful ligand-based NMR technique for weak ligand screening of protein targets and to gain quantitative structural information from biologically relevant protein-ligand complexes of interest in drug discovery. The approach is appropriate for small/medium-sized molecule binders of medium-weak affinity (dissociation constants from high nM to low mM), there is no upper limit for protein size, and labelling is not required. In this presentation, the investigation by STD NMR of molecular recognition processes of ligands by biologically relevant protein receptors will be presented with a focus on the application to specific cases of recognition of glycans by proteins. Protein-glycan interactions are very relevant protein-ligand interactions in Nature and are processes typically falling within the range of fast chemical exchange (weak affinity) but still showing high specificity. Along this communication the novel methodological developments in STD NMR produced in our lab will be presented, as the development of novel multifrequency STD NMR approaches, with a focus on the "DiffErential EPitope mapping STD NMR (DEEP-STD NMR)" method that allows for the first time to identify the nature of the protein-ligand contacts in the bound state from STD NMR approaches, facilitating ligand orientation in the binding pocket, of great value for the rational design of improved binders in drug discovery efforts.

IN042

Automated peak picking, and uSTA

Baldwin A, Bonvin A, Buchannan C, Casablancas-Antras V, Papadakis G

NMR's role in biochemistry has perhaps been diminished over the last decade as techniques such as cryoEM come into view. One of the reasons for this is simply the training and time required to analyse our data to get biochemical results. Peak picking is typically the first stage of all NMR analyses. There are many peak pickers one can use, but unless the problem is relatively simple, those of us that does this regularly know that there is no substitute for a trained user. To deal with this, we've created UnidecNMR, a program that in our hands, peak picks as well as a skilled user in 1-4D spectra. We have this program available with a nice GUI that let users inspect the results, and allows comparison of the raw data with data simulated from the calculated peak list. We show this off its performance in a variety of applications from 3D datasets for backbone and side chain assignment, and 4D methyl-methyl NOE data.

Armed with a good peak picker, there are a few new problems that we can tackle. I will focus on one of these, that was recently accepted for publication in Science where we have adapted older STD methods to make something we've called uSTA (universal saturation analysis). Please forgive me for this rebranding. This was not my idea. Nevertheless: our uSTA pipeline does enough new things to warrant a new name. It takes in FIDs, of ligand, protein and their mixtures, does a range of background subtractions, and automatic peak picking. It then requires a user to type in assignments of the ligand resonances, and calculates a heat map which can be interpreted directly as a $1/r^6$ distance map of contact points between ligand and protein. Finally, it takes data obtained over a range of ligand/protein ratios and returns you forward and backward rates, which include a Kd.

We demonstrate this on a range of systems but notably with sugar interactions with variants of COVID19 spike proteins (original, alpha,beta, delta, omicron) and find out that certain types of relevant sugars were recognised by Spike in early strains, but this recognition was lost as the virus evolved. We demonstrate our our ligand poses from uSTA are identical to poses we get from cryoEM structures (which also show good agreement with calculations from the excellent HADDOCK software). Reliably peak picking spectra of ligands containing many sugars, where peaks are heavily overlapped, would be extremely challenging without UnidecNMR and our pipeline.

We cross validate our uSTA Kds with orthogonal measurements from SPR and ITC to convince you that this method basically works. Our analysis to obtain Kds requires a Bloch-McConnell treatment, which draws attention to the similarities between the STD, the selective NOE, and the CEST experiments. In doing so, we demonstrate that a number of common assumptions associated with the STD method are incorrect, including for example that it is not at all a 'fast exchange' method. uSTA then is very neat method is applicable in a broader range of interaction space than previously recognised. Our software is available for download for anyone to have a go with it.

Phosphates form spectroscopically dark state assemblies in common aqueous solutions

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Phosphates and polyphosphates play ubiquitous roles in biology as integral structural components of cell membranes and bone, or as vehicles of energy storage via adenosine triphosphate and phosphocreatine. The solution phase space of phosphate species appears more complex than previously known. We present NMR and cryogenic transmission electron microscopy (cryo-TEM) experiments that suggest phosphate species including orthophosphates, pyrophosphates and adenosine phosphates associate into dynamic assemblies in dilute solutions that are spectroscopically 'dark'.

We performed 31P NMR to investigate the native state of phosphate species as a function of temperature with the initial intent to subsequently study the formation processes of calcium phosphate clusters. In this process, we encountered peculiar 31P NMR line broadening with increasing temperature of aqueous solution of pure phosphates that cannot be explained by typical dynamical processes of small molecules. We present experimental results showing that phosphate containing species, including orthophosphate, pyrophosphate, and adenosine diphosphate assemble into hitherto unreported spectroscopically 'dark' species, whose fractional population increases with increasing temperature.

This observation is shown to be consistent with the dehydration entropy-driven formation of dynamic phosphate assemblies. 31P NMR Chemical Exchange Saturation Transfer (CEST) reveals that phosphates assemble into species with broad spectroscopic signatures, whose population is in exchange with NMR-detectable phosphate species. 31P Diffusion Ordered Spectroscopy (DOSY) indicates dehydration of phosphates at elevated temperatures, and the addition of depletants and additional counterions are found to facilitate assembly formation in line with the trends of the Hofmeister series.

The discovery that common phosphate-containing molecules can readily assemble into higher order species in water under physiological conditions in the absence of biologically activated processes should be relevant to a variety of biological and biochemical processes that use phosphate containing species as building blocks, energy sources or reactants in an aqueous environment.

PT002

Real-time NMR recording of fermentation and lipid metabolism processes in live microalgae cells

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Non-invasive and real-time recording of processes in living cells has been limited to detection of small cellular components such as soluble proteins and metabolites. Here we report a multiphase NMR approach using Magic-Angle Spinning NMR to synchronously follow microbial processes of fermentation, lipid metabolism and structural dynamic changes in live microalgae cells. Chlamydomonas reinhardtii green algae were highly concentrated, introducing dark fermentation and anoxia conditions. Single-pulse NMR experiments were applied to obtain temperature-dependent kinetic profiles of the formed fermentation products. Through dynamics-based spectral editing NMR, simultaneous conversion of galactolipids into TAG and free fatty acids was observed and rapid loss of rigid lipid structures. This suggests that lipolysis under dark and anoxia conditions finally results in the breakdown of cell and organelle membranes, which could be beneficial for recovery of intracellular microbial useful products.

PT003

Conformational transformation of the intrinsically disordered SARS-CoV-2 nucleoprotein on interaction with its viral partner nsp3

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The processes of genome replication and transcription of SARS-Cov-2 represent important targets for viral inhibition. Beta-coronaviral nucleoprotein (N) is a highly dynamic cofactor of the replication-transcription complex (RTC), whose function depends on an essential interaction with the N-terminal Ubiquitin-like domain of nsp3 (Ubl1). Here we describe this complex (affinity 30-200nM) at atomic resolution using NMR spectroscopy. The interaction implicates two linear motifs in the intrinsically disordered linker domain (N3), a hydrophobic helix (219LALLLLDRLNQL230) and a disordered polar strand (243GQTVTKKSAAEAS255), that mutually engage to form a bipartite interaction, folding N3 around Ubl1. This results in substantial collapse in the dimensions of dimeric N, forming a highly compact molecular chaperone, that regulates binding to RNA, suggesting a key role of nsp3 in the association of N to the RTC. The identification of distinct linear motifs that mediate an important interaction between essential viral factors provides future targets for development of novel strategies against COVID-19.

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PT004

Water Concentration Gradients Across Lipid Bilayers Revealed by a High Resolution HYSCORE Method

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Organization, dynamics, and function of cellular membranes is governed by the hydrating water molecules. The hydrophilic nature of the lipid head groups and a hydrophobic environment of the alkyl chains are expected to result in a highly heterogeneous distribution of water molecules across the lipid bilayers. Previously, spin labeling methods including continuous wave (CW) EPR, electron spin echo envelope modulation (ESEEM), and Overhauser dynamic nuclear polarization (ODNP) provided data on local polarity and water penetration across the bilayers. However, these methods have limitations in terms of spatial (typically 1-1.5 nm) and suffer from spectral resolution and ambiguity in data interpretation. Here, we report an EPR method capable of measuring local water concentration in biological systems with a superior 0.2-0.3 nm spatial resolution. The method is based on detecting reversible hydrogen bonds (H-bonds) between water and nitroxides by hyperfine sublevel correlation (HYSCORE) spectroscopy. HYSCORE allows for unambiguous detection of H-bonded deuterons, while a series of calibrations in bulk mixed solvents relate the fraction of H-bonded nitroxides to local water concentration rather than empirical polarity or ODNP parameters reported previously. Profile of water concentration across bilayers formed by DOPC lipids was measured by employing a series of membrane-spanning α-helical WALP peptides labeled with MTSSL at 15 different sites. The data were analyzed by accounting for the flexible nature of the nitroxide tether and differences in H-bonding equilibria in the polar and apolar bilayer regions. The high spatial resolution data obtained for the first time revealed much steeper gradients and a slight but measurable increase in water concentration right in the bilayer center vs. interior regions just 0.5 nm away. The reported method is expected to be broadly applicable to probing local hydration in complex heterogeneous biological and chemical systems.

PT005

On-the-fly optimisation of ESR experiments

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Manual optimisation of experimental parameters is time-consuming and depending on the number of parameters, sometimes impractical. An effective alternative is an on-the-fly optimisation routine that evaluates the goodness of experimental parameters at each measurement and adjusts the parameters accordingly in a feedback loop. By taking advantage of one of the available optimisers [1-3], this approach can give access to an optimised set of parameters in a fully automated fashion and is remarkably faster than a full grid search.

Unfortunately, due to the lack of accessible tools, on-the-fly optimisation is heavily underused in magnetic resonance, with only a handful of related publications in nuclear quadrupole resonance (NQR) [4], nuclear magnetic resonance (NMR) [5] and Electron Spin Resonance (ESR) [6].

Here we present ESR-POISE (Parameter Optimisation by Iterative Spectral Estimation), a user-friendly Python package to conduct on-the-fly optimisation of ESR experiments. It allows for fully automated optimisation of routine operations, and compensation of ESR instrumental imperfections which affect a majority of experiments. This can not only deliver much faster operation of ESR experiments with superior spectral quality, but can also unlock new experimental procedures, unapproachable before due to tedious, manual experimental optimisation.

We demonstrate capabilities of ESR-POISE with examples ranging from simple signal phase adjustment and hard pulse calibration to optimisation of a DEER experiment using shaped pulses and compensation of resonator response for broadband chirped excitation [7].

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PT006

Solid-like Dynamic Nuclear Polarization Observed in the Fluid Phase of Lipid Bilayers at 9.4 T

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Dynamic nuclear polarization (DNP) is a powerful method to enhance NMR sensitivity. Much progress has been achieved recently to optimize DNP performance at high magnetic fields in solid-state samples, mostly by utilizing the solid or the cross effect. In liquids only the Overhauser mechanism is active, which exhibits a DNP field profile matching the EPR lineshape of the radical, distinguishable from other DNP mechanism. Here, we observe DNP enhancements with a field profile indicative for the solid effect and thermal mixing at ~320 K and a magnetic field of 9.4 T in the fluid phase of DMPC lipid bilayers doped with the radical BDPA (1,3-bis(diphenylene)-2-phenylallyl). This interesting observation might open up new perspectives for DNP applications in macromolecular systems at ambient temperatures [1].

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PT007

Three-dimensional Fourier imaging of thousands individual NVs with sub-micron resolution

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Nitrogen vacancies in diamond (NVs) are many times considered as possible candidates for spin-based quantum computers. The main caveats in this approach are the lack of reliable process for accurately placing many NVs in close proximity (~10-20 nm) and the inability to efficiently read out and manipulate the quantum state of many closely spaced NVs. Our approach to overcome these issues is to: (i) Make use of native NVs in the diamond in their original 3D position. (ii) Map out their location in a 3D manner with high accuracy using Fourier imaging MRI-like techniques. (iii) Employ MRI-techniques for selective spin manipulation, based on the known 3D locations of the NVs and (iv) Make use of Fourier imaging to read out the quantum state of the NVs. This presentation will outline this general concept for NV-based quantum computing and specifically focus on our recent work for enabling high resolution 3D imaging of thousands of individual NVs, with sub-micron resolution, using MRI Fourier techniques with fast and powerful pulsed magnetic field gradients.

PT008

Nitrogen-vacancy center as a terahertz source

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Defects in solid state matter have been in the focus of research for a long time. Nitrogen-vacancy centers in diamond are one of the most prominent examples with possible applications in quantum communication and magnetometry. Their unique electronic level scheme allows the almost exclusive population of the m=0 spin state just by optical pumping. This nature comes in handy when we aim to build a quantum bit, however it holds other opportunities. Putting the NV centers in external magnetic field and exploiting the above-mentioned light induced spin polarization one can achieve population inversion. Previously, continuous masing was achieved by putting the NV centers in an X-band microwave resonator and applying resonant external magnetic field [1]. Here we propose that this effect could be achieved in higher magnetic fields resulting in higher frequencies up to the THz regime [2]. Furthermore, we show that in the absence of a resonator, the frequency can be tuned by rotating the diamond crystal. We hope that this work could help to take a step toward the application of magnetic resonance phenomena in the field of terahertz technology.

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PT009

Surface NMR spectroscopy using NV-centers in diamond

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Nitrogen-vacancy (NV) point defects in diamonds have become a promising platform in the field of magnetic resonance spectroscopy. The spin state of these solid-state qubits can be optically polarized, coherently manipulated with microwave pulses, and read out via its spin-dependent photoluminescence. In this talk, I will report our recent progress on using these defects to perform NMR spectroscopy at surfaces and interfaces. In our approach, we combine the nanoscale-sensing capabilities of the NV-center with a high-precision diamond surface modification by atomic layer deposition (ALD). This allows us to place thin layers of alumina oxide on the diamond surface, which provides a platform for studying surface chemistry. In a proof-of-concept study, we grow a self-assembled monolayer (SAM), based on phosphonate chemistry, on this support. Shallow NV-centers allow us to detect NMR signals from (sub)monolayers of the self-assembled molecules with femtomole sensitivity. The optical readout enables us to detect these NMR signals spatially resolved on a tens of micrometer length-scale. Due to the intrinsic quantum sensing scheme, the molecular coverage of the alumina oxide surface can be deduced quantitatively. Moreover, the NV-surface NMR operates under chemically relevant conditions, demonstrating the detection of the SAM formation at the liquid-solid interface in real-time. Although still limited by dipolar broadened lines, the linewidth of the resonance can give information about local dynamics and interactions. In the outlook I will discuss the next steps of this technology, in particular how to improve spectral resolution in future.

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Promoted Lectures

Nitrogen (14N/15N)-Hydrogen MAS NMR Two-Dimensional Correlation Spectroscopy: Developing Methods for Pharmaceutical Applications

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Two-dimensional nitrogen-hydrogen magic-angle spinning (MAS) NMR correlation spectra are invaluable for characterising hydrogen bonds that are key structure-directing interactions for the solid-state structures adopted by moderately sized organic molecules such as pharmaceuticals [1-3]. We present here spectra recorded (in some cases at 850 MHz and 1 GHz at the UK High-Field Solid-State NMR Facility) using advanced 1H-detected pulse sequences applicable under fast MAS (60+ kHz) for both the spin I = 1/2 15N and the spin I = 1 14N nucleus at natural isotopic abundance, 0.4% for 15N and 99.6% for 14N.

15N-1H correlation spectra are recorded using a CP-refocused INEPT pulse sequence, employing windowed supercycled PMLG homonuclear decoupling to increase the spin-echo dephasing times. Optimum sensitivity is found for a Lee-Goldburg element with duration of less than half the ideal value, corresponding to a theta value, equal to arctan(nutation frequency/ LG offset), i.e., arctan(nutation frequency* tau_LG* sqrt(3)), that is much less than the magic angle, namely at ~30 degrees. This corresponds to a high scaling factor (for the chemical shift and heteronuclear 15N-1H J coupling) of ~0.8.

A comparison is presented of 14N-1H heteronuclear multiple-quantum coherence (HMQC) correlation spectra recorded using different recoupling methods, notably phase-inverted R3 and the SR4 2 1 methods that both apply rf at a 1H nutation frequency of twice the MAS frequency and TRAPDOR recoupling.

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PT011

Structure and dynamics of photochromic rare-earth oxyhydrides

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Oxyhydrides of rare earth metals such as Yttrium, Scandium and Gadolinium, are mixed-anion compounds where both oxygen and hydrides are anions. These materials show a reversible change in color and transparency on irradiation with UV light that can be applied for smart windows. Compounds with mobile hydrides form an interesting class of novel electrolytes. Moreover, compounds with mobile hydrides form an interesting class of novel electrolytes.

The underlying mechanism of this intriguing phenomenon is not well understood. The empirical formula of these systems is ReOxH(3−2x). It has been observed that the arrangement and composition of the anions, particularly the hydrides, dictate the efficiency of the photochromic behaviour in these materials. Nevertheless, the exact arrangement of the anions in the FCC cation sublattice is not known in detail. A better insight into the local environment of the ions is key to understanding the underlying mechanism of the colour change. We use 1H, 89Y and 45Sc and 17O solid-state NMR spectroscopy and DFT calculations to unravel different hydride, yttrium and scandium and oxygen local environments in YHO and ScHO films. The limited sample amounts (1 um films) is challenging for NMR, but nonetheless the hydrogen content of the samples could be determined. The lattice structure and mobility of hydrogen species are investigated in detail. The theoretical results point to an anion-disordered structure for H-rich oxyhydrides. Solid-state NMR experiments confirm the existence of substantial disorder in the material but nevertheless indicate the presence of distinctly different local coordinations which need to be unravelled in more detail. Finally, we are exploring in-situ illumination in view of a better understanding of the structure-function relationship.

PT012

Recent developments in NMR of quadrupolar nuclei in solids

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Over 74% of NMR active nuclei have a spin I ≥ 1 and are subject to quadrupole interactions. The observation of these nuclei in solids often remains challenging since the spectral resolution is decreased by the second-order quadrupole interaction, and the quadrupole interactions exceed the rf-field amplitude. We present new techniques to facilitate the observation of these quadrupolar isotopes.

We have analyzed the performances of the cosine MQMAS sequence.[1] For spin-3/2, this technique is more efficient than STMAS and requires lower rf-field. For higher spin values, this variant is as efficient as the high-power one but requires rf-fields smaller than 20 kHz and hence, can be employed for low-γ nuclei and large diameter rotors.[2] These sequences have been extended to obtain MQ-HETCOR spectra.

We have also developed efficient pulse sequences to transfer magnetization from protons to quadrupolar nuclei at slow-moderate or fast MAS.[3-5] For nR £ 20 kHz, the most efficient sequence is D-RINEPT using adiabatic pulses. This transfer has been combined with DNP to detect low-g quadrupolar nuclei with natural abundance near surfaces or sub-surfaces, such as 17O, 95Mo, 47,49Ti, 67Zn.[5] This transfer has been combined with MQMAS to detect DNP-enhanced high-resolution spectra of quadrupolar nuclei, such as 17O, near surfaces.[6]

Finally, we have analyzed the T-HMQC technique[7] for the indirect detection without t1 noise of spin-1/2 nuclei subject to large CSA (195Pt), as well as spin-1 (14N) and spin-3/2 (35Cl) quadrupolar nuclei.[8] For spin-3/2 nuclei, this method can provide a resolution enhancement of ca. 4.[9]

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PT013

Evaluation of Simulated RDC, NOE and 3J Data to Determine the Configuration of Flexible Molecules

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The recently developed molecular dynamics – MDOC – driven by tensorial orientational constraints, provides the singular possibility to simulate NMR data on the time scale of NMR interactions. In MDOC, dipolar interaction tensors are used as orientational constraints that drive reorientation of the whole molecule and internal rotations while the constraints from NOE distances and 3J-couplings are used to adjust the populations of conformers. In liquid state, NMR data of flexible molecules are averaged by their conformer interconversion, which can mostly be simulated in MDOC. It is however a demanding task to apply MDOC towards the elucidation of chiral assignments or stereochemical configurations since the MD may produce highly similar trajectories for several candidates. As an extension to the statistical $n/\chi 2$ quality criterion, we introduce a new tool based on Bayes theorem, which provides a sharp criterion to evaluate stereochemical candidates, structures and assignments. It will be demonstrated that even in demanding cases of highly flexible molecules, the new criterion – χ-probability – would select the right candidate with 98% probability from 16 candidate trajectories. For highly mobile molecules exhibiting a multitude of exchanging rotamers, NOE distances proved to be essential to single out the correct configuration.

Automated chemical shift assignment and protein structure determination with the deep learning method ARTINA

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Advances in deep learning have opened new opportunities for tackling long-standing problems in protein NMR spectroscopy with the potential for rapid acceleration of structural biology research. One of the open problems, defined over 30 years ago, is the automated determination of protein structures, using as input only raw NMR spectra and the sequence. Here we address this challenge with our deep learning approach ARTINA (http://arxiv.org/abs/2201.12041). Our method delivers automatically peak lists, chemical shift assignments, distance restraints, and the structure. The process is executed strictly without human intervention, and can be carried out even by non-experts using our online platform (https://nmrtist.org).

ARTINA utilizes deep residual neural networks to identify and deconvolve cross-peaks in NMR spectra. Detected signal coordinates undergo combinatorial optimization with FLYA algorithm, yielding chemical shift assignments. This step is supported with information about thousands of NMR structures solved in the past, using Graph Neural Networks that model dependencies between individual chemical shifts to facilitate the FLYA assignment process. As a final step, our method calculates 9 protein structure proposals with CYANA, which are ranked by their expected quality with Gradient Boosted Trees.

Our method has been trained and evaluated on an extensive benchmark dataset that consists of 1329 2D/3D/4D NMR spectra, and allows 100 protein structures to be reproduced using their original measurements. In a 5-fold cross-validation experiment, ARTINA demonstrated its ability to determine protein structures with a median RMSD of 1.44 Å to PDB reference, assigning correctly 91.36% of the chemical shifts, as compared with corresponding BMRB depositions.

In practice, ARTINA allows NMR practitioners to obtain shift assignments and protein structures within a few hours after completion of the NMR measurements. Essentially, it reduces the previously tedious manual process to sample preparation and spectra measurement.

PT016

Ultralow-field NMR detection of photochemically induced dynamic nuclear polarization

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Photochemically induced dynamic nuclear polarization (photo-CIDNP) is one of the central methods of spin chemistry [1], it is used to hyperpolarize nuclear spins with light and extract information about the structure, dynamics, and magnetic properties of molecules including short-lived radicals. Of interest are processes occurring at low fields where hyperfine couplings of the involved electrons become the dominant interactions. Existing approaches of nuclear magnetic resonance (NMR) enable one to perform indirect detection measurements of CIDNP, when the sample is irradiated in a low field, followed by mechanical transfer of the sample to an NMR spectrometer for detection. An alternative, direct approach for measuring NMR signals at zero- to ultralow-field (ZULF, typically below 0.5 µT) was developed over the last 20 years [2]. Recently we have demonstrated that photo-CIDNP can be combined with ZULF, allowing one to hyperpolarize heteronuclear long-lived states [3].

In this work, we demonstrate that photo-CIDNP occurring at millitesla fields can be detected by ZULF NMR. Without hyperpolarization, low sensitivity of ZULF NMR limits the method to detection of molecules in neat liquids, often requiring isotopic enrichment of ^{13}C and ^{15}N nuclei. In the present work, ZULF signals produced by photo-CIDNP are enhanced by four orders of magnitude (relative to thermal equilibrium at militesla fields), which allows us to obtain ^{1}H free precession signals for 5 mM para-benzoquinone with a signal-to-noise ratio of ~10 per scan. This opens a new spectroscopic modality for spin-chemistry applications.

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PT017

Study of zeolite anti-caking effects for fertilizers by 1H low-field NMR

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Caking is associated with the consolidation of dry powder and granules, leading to losses of function and/or quality. More than 80% of industrial feedstock are particulate materials that are manufactured in various industries such as pharmaceuticals, agrochemicals, food or fine chemicals. For dry powdered materials, that are produced and/or used in these industries, caking, i.e., the compaction and hardening of a powder mass, is a common and serious issue [1]. It has been the object of studies for pharmaceuticals, foods and fertilisers since 1920's because of its significant impact on product quality. Caking has been described as a three-step event consisting of sorption-dissolution-recrystallisation phases. It constitutes a critical factor in powder/granulate losses during storage while also hampering handling. Current methods for the evaluation of water sorption dynamics are expensive, time-consuming and/or inaccurate. The proposed method allows to follow the water uptake in different domains of the mixed fertiliser/zeolite samples. To our knowledge, this dynamic has not been observed and quantified so far in real-time. 1H low-field NMR relaxometry makes it possible to accurately, precisely and unambiguously determine the water content of complex samples, including its partition between the components of a mixture. The proposed processing strategies, i.e., 2D Inverse Laplace transform, allowed to extract water sorption kinetics individually for each component of the mixture in an innovative way, yielding knowledge that allows the development of fertilisers with more appropriate physical and chemical characteristics. Through this study, it is possible to determine the necessary amount of zeolite to be used in fertiliser production to efficiently suppress subsequent caking according to storage conditions, as well to estimate the time scales after which water uptake reaches critical values leading to caking.

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PT018

Zero- and Ultralow-Field NMR Relaxometry

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NMR relaxometry is a method that enables the characterization of the physical and dynamical properties of samples by measuring nuclear-spin relaxation times. The longitudinal and transverse relaxation times, denoted as T_1 and T_2, respectively, provide valuable information. For example, T_2 variation, originating from the change in blood proteome, could be an indicator for inflammation, insulin resistance.[1]

Recently, ultralow-field (ULF) NMR relaxometry became possible. This owns to the application of non-inductive sensors, which are overly sensitive to low-frequency signals (up to 1 kHz). Among the sensors, optical-pumped magnetometers (OPMs) are prominent sensors, as they are characterized by low price, small size, and noncryogenic operation. Their additional advantages are the ever-growing commercial availability, technical simplicity, and near-zero maintenance.[2]

Here, we present the investigation of NMR relaxometry of chemical and biological samples at zero and ultralow fields (ZULF). Initially, the effect of dissolved paramagnetic oxygen on relaxation processes was examined for ^{13}C-formic acid, with long-lived heteronuclear singlet state[3], and trimethyl-phosphate, possessing numerous heteronuclear states. This was done by the analysis of ^{13}C-^{1}H and ^{31}P-^{1}H J-couplings in zero-field NMR spectra. Next, different concentrations of $CuSO_4$ solutions (0.2- 1 mM), which are of particular interest for quantitative imaging, were used to study the influence of paramagnetic agents on water proton relaxation under the ULF regime (10- 500 µT). Finally, T_1 and T_2 relaxation magnetic-field profiles of human whole-blood and blood-plasma at ULFs were obtained. Even though the measured relaxation times of blood are on the order of 300 ms, OPMs allowed the detection of ULF NMR signals from biological samples. Our work demonstrates the applicability of ZULF NMR relaxometry employing OPMs as a portable, robust, inexpensive, and sensitive tool in chemical and biological analysis.

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PT021

Revealing defects in nanoparticles using very-high-field NMR of quadrupolar nuclei

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Functional nanomaterials often derive their specific properties from the presence of defects. As a local characterization technique, solid-state NMR spectroscopy can provide unique element-specific insights into the structure of these defects. Nevertheless, the lack of resolution and sensitivity of this technique can prevent the observation of defects, especially when they are occupied by quadrupolar nuclei.

The advent of ultra-high field NMR magnets opens new avenues for the observation of defects containing quadrupolar isotopes, notably in nanoparticles. For instance, 2D ^{1}H-^{27}Al through-space HMQC experiments on a series-connected hybrid magnet (SCH) at the NHMFL producing a B_0 field of 35.2 T, i.e. ^{1}H Larmor frequency of 1.5 GHz, have provided the first direct experimental evidence of the presence of bridging silanol Brønsted acid sites, SiOHAl, in amorphous silica alumina (ASA) nanoparticles, which are important acidic catalysts widely employed in petrochemical industry and in bio-refinery [1]. The presence of these bridging silanol sites in ASA, besides the known pseudobridging silanol Brønsted acid sites, SiOH···Al, has been hotly debated in the past few decades. The discovery of these bridging silanol sites with stronger acidity than the pseudobridging ones offers promise for the development of ASA with enhanced acidity.

Besides quadrupolar nuclei of high receptivity, such as ^{27}Al, high magnetic fields are also useful for the detection of low-γ quadrupolar nuclei of low natural abundance, such as ^{67}Zn (4.1%) and ^{33}S (0.76%). Recently, we investigated the structure of ZnS quantum dots using ^{67}Zn and ^{33}S experiments at 18.8 T. These experiments combined with DFT calculations demonstrate the presence of S vacancies, which can modify the optical properties of these nanocrystals [2].

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PT022

Methods for exploring non-Fourier dimensions - from small molecules to proteins

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The possibility of adding extra dimensions to spectra made the NMR spectroscopy a powerful analytical tool. Besides commonly used indirect „Fourier dimensions", i.e., those processed with Fourier transform, we often acquire a series of spectra under varying experimental conditions. These can be pulse sequence parameters (e.g. mixing times or diffusion-encoding gradients), environmental conditions (temperature, pH or concentration) or reaction progress.[1]

The resulting stack of n-dimensional spectra may be considered as an n+1-dimensional object. This concept opens the way to new processing methods for exploring non-Fourier dimensions.

Over the last decade, my group has developed several methods based on time-resolved non-uniform sampling (TR-NUS), Radon transform, and non-stationary signal processing. In my presentation, I will recapitulate the theory of these approaches and show their applications in metabolomics[2], reaction monitoring[3], ligand binding[4] and studying protein dynamics[5]. I will also discuss the currently developed concepts of exploring non-Fourier dimensions in protein research, e.g., fast measurements of TOCSY transfer curves.

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PT023

New NMR methods for structural analysis of fluorinated systems

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Fluorine-containing species are used widely in medicinal and biological chemistry. NMR provides key information on molecular structure, but in systems showing both homonuclear and heteronuclear J-couplings, this information is often difficult to extract due to signal overlap. Spectral resolution can be improved via pure shift ^{1}H NMR methods that suppress the effects of homonuclear couplings, reducing signal overlap and facilitating spectral analysis.[1] However, in complex fluorinated systems this may be insufficient, as the effects of heteronuclear couplings remain. In such systems, heteronuclear spectral editing methods, such as FESTA,[2] can be used to alleviate signal overlap. FESTA methods provide ^{1}H subspectra for individual spin systems that contain a selected fluorine, aiding the extraction of key structural information. However, in the most challenging systems, signal multiplicity can still hinder analysis.

Here, we present a set of new NMR experiments that facilitate the structural analysis of complex fluorinated systems. To unambiguously extract chemical shift information, we propose two general pure shift methods for ^{1}H and ^{19}F acquisition. Both provide fully decoupled pure shift spectra, suppressing the effects of homo- and heteronuclear couplings. However, in complex spectra, even pure shift methods suffer from signal overlap. We show how combining the advantages of pure shift and FESTA methods allows further spectral simplification, giving an individual ^{1}H pure shift spectrum for each fluorine-containing spin system. To obtain stereospecific and conformational information, we present a variation on FESTA that efficiently extracts the signs and magnitudes of heteronuclear couplings by acquiring complementary in-phase and anti-phase spectra.[3] The benefits of these new methods will be demonstrated in the analysis of physically inseparable fluorinated mixtures of chemical and biological interest, highlighting how these methods can be used to obtain crucial structural information.

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Activation and allosteric regulation of HtrA proteases revealed by solution NMR spectroscopy

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Bacterial and human serine proteases of the high temperature requirement A (HtrA) class play important roles within the cellular protein quality control network and the human HtrAs are dysregulated in a myriad of diseases such as cancer and neurodegenerative diseases. Despite being extensively studied biochemically and the availability of high-resolution crystal structures the functional details of this class of proteins in terms of activation and regulation remain only poorly understood. Using advanced high resolution NMR spectroscopy in combination with biochemical assays my team as well as the Kay lab could lately reveal important novel insight. I will present details of our work on bacterial DegP studying its substrate recognition and inherent protien dynamics. Our investigation lead to the identification of its underlying temperature activation mechanism leading to an updated functional cycle. In addition, I will also highlight a recent study using a combination of NMR spectorscopy, biophysical characterization and biochemical assays, on the related mitochondrial HtrA2 protease, providing novel insight into its activation and regulation deepening the understanding of its role in the apoptotic signalling, revealing the possible contribution of divalent cations regulating its activation and protease activity.

PT025

Light-coupled NMR spectroscopy: NMRtorch and its applications

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Light is a form of electromagnetic energy that surrounds and affects life on earth, with a myriad of chemical and biological reactions dependent upon light. Nanoswitches, molecular machines, smart materials, photo-chemistry, optogenetic regulators and photoenzymes can all be driven by various colours of light, with behaviour of these systems needing to be analysed and characterised. Due to its versatility and ability to detect multiple nuclei, NMR spectroscopy is the perfect method to monitor molecular changes in real time. In situ illumination of liquid-state NMR samples enables a wide range of light-dependent chemical and biological phenomena to be studied by this powerful analytical technique. However, the position of an NMR sample deep within the bore of the spectrometer magnet renders such illumination challenging, necessitating either use of fiddly optical fibre-based systems, or specialised probeheads.

Here, we present an alternative universal sample illumination setup, called NMRtorch, which avoids the use of optical fibres, and which is compatible with a wide range of standard probeheads. We demonstrate that extremely efficient multi-wavelength sample illumination can be achieved by attaching LEDs directly to the top of a special NMRtorch tube which contains the sample. The wall of the tube itself acts as a light guide, also illuminating the sample from the outside. We demonstrate how this new setup performs in a number of photo-NMR applications, including photo-isomerisation and photo-chemically induced dynamic nuclear polarisation (photo-CIDNP), and demonstrate the potential for ultraviolet (UV) degradation studies with continuous online NMR assessment. We show how uniform light distribution inside the sample can be achieved and quantified, and how light intensity can be conveniently measured with a reversible multi-wavelength actionometer photoreaction. This novel setup enables users of any typical liquid-state spectrometer to easily perform in situ photo-NMR experiments, using a wide range of wavelengths and their combinations.

PT026

Towards applications of β-NMR at CERN

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Radioactive isotopes are placed in the less frequented corner of the vast NMR-catalog for a variety of reasons. Nonetheless, turning the challenges related to these nuclei into an advantage, their unusual perspective can yield unexpected insights into chemistry and biology. A rising technique, liquid β-detected NMR, is utilizing unstable probe nuclei to gain a significant increase in signal intensity - combining laser-induced hyperpolarization and resonance detection by asymmetric β-emission.

Recently, the technique has made its first steps into applications due to a significantly improved setup. This has opened the path into more detailed analyses of solvents, structures and reactions. Using ionic liquids as a starting point, Na-ions can be utilized as a lens to study the structure and behavior of ionic liquids in great detail.

PT028

Adaptive Magnetic Resonance

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Introduction: Conventional approaches to MRI signal acquisition are static in nature, fixing sequence parameters in advance in anticipation of a wide range of expected tissue parameter values; They are therefore sub-optimal for any given subject. We propose an adaptive approach which uses the measured signal to update and fine-tune the sequence parameters in real time. This targets the specific tissue characteristics of the subject while they are being scanned.

Theory: The proposed adaptive framework maintains a Bayesian probability prior $p(x_1, x_2, ...)$ for tissue parameters $x_1, x_2, ...$ (e.g. $x_1 = T_1$, $x_2 = T_2$, etc). The prior is updated at the end of each TR based on the measurement result, which is used to calculate the likelihood function. The prior is updated via Bayes' rule, by multiplying it by the likelihood and normalizing. The prior is then used to select the sequence parameters in the next excitation to maximize the precision of $x_1, x_2, ...$ in a Cramer-Rao sense (i.e. minimize their standard deviation).

Methods: We demonstrate our framework by a multi-echo (MTE) T2 single-voxel MRS acquisition, targeting the T2 of n-acetyl-aspartate (NAA) at 2.01 ppm, by varying the echo time (TE) in each excitation. Seven healthy volunteers were scanned twelve times using the adaptive framework. Each acquisition took two minutes and yielded a single estimate of the T2 of NAA. The adaptive approach was compared to a static approach which lasted the same amount of time (two minutes), but which fixed the TEs in the advance, assuming a possible range of T2 between 50 and 450 ms.

Results: Both the static and adaptive approaches yielded unbiased estimations of the T2 of NAA, but the standard deviation of the adaptive approach was 1.7-fold smaller (i.e. 1.7-fold better precision). Equivalently, the adaptive approach achieved the same precision with only 40% of the samples, accelerating the experiment 2.5-fold.

PT029

Fine optimization of a dissolution-DNP experimental setting for NMR of metabolic samples at natural abundance

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NMR metabolomic studies extract important information on biological systems but mostly rely on 1D 1H experiments for sensitivity reasons. However, strong peak overlap is a limitation for the analysis of inherently complex biological mixtures. Dissolution Dynamic Nuclear Polarization(1) (d-DNP) offers an opportunity to benefit from the wide spectral range and high resolution of 13C NMR, for metabolomics studies with natural-abundance samples. A preliminary d-DNP study(2) showed the possibility to detect 13C signals on plant and cancer cell extracts in a single scan at natural abundance, results that are inaccessible by conventional state-of-the-art high field NMR. Later, we reported a repeatability <4% for 13C signals on such extracts(3), as suitable for analytical metabolomics. We recently demonstrated the successful introduction of d-DNP into a full untargeted metabolomics workflow applied to the study of plant metabolism(4).

Here we describe the systematic optimization of d-DNP experimental settings for experiments at natural 13C abundance, and show how the resolution, sensitivity, and ultimately the number of detectable signals improve as a result. We have systematically optimized the parameters involved (from sample preparation to signal acquisition) in a semi-automated prototype d-DNP system. The optimization allows to extend the scope of natural abundance 13C metabolomics studies with a high repeatability. The optimized conditions make it possible to identify the previously inaccessible protonated-13C signals of metabolites with improved lineshape. Moreover, it also enables the acquisition of DNP enhanced 1H spectra of the metabolites with 3 orders of improved sensitivity compared to the thermal signal. This paves the way to the use of DNP-hyperpolarised NMR as a general approach in metabolomics studies.

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PT030

Isotopological fingerprinting via 1H/D scrambling identifies SABRE hyperpolarization catalysts

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Hyperpolarization using signal amplification by reversible exchange (SABRE) relies on target molecules and parahydrogen coordinating to a transition metal catalyst. Identification of this coordinated state becomes increasingly important, especially since bio-relevant targets such as pyruvate and amino-acids exhibiting multiple binding sites are becoming compatible with SABRE. We present a fingerprinting method to discriminate and identify SABRE ligand binding sites without requiring the presence of a sensitive or isotope labeled hetero-atom. Adding a small concentration of protons to a deuterated medium, spontaneous 1H/D scrambling of exchangeable protons encodes the ligands each with an isotopological fingerprint. Using rapid 2D zero quantum NMR, the binding sites are decoded from the hydrides in less than a minute. The gained insights are especially useful for a more rational approach in characterizing and designing SABRE mixed-ligand catalyst. [1]

In the future, we therefore predict a variety of hyperpolarization applications featuring sensitivity enhancement of (bio-)molecules involving multiple binding sites to benefit from the isotopological fingerprinting approach to clarify ligation mechanisms necessary for hyperpolarization. This includes development of high-field SABRE experiments where spin-locking of the correct hydrides are essential to allow transfer of polarization to occur. Direct hyperpolarization of amino-acids using SABRE is of particular interest in this context which is currently under investigation in our research facility.

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The AsymPol family: a whole set of highly efficient DNP polarizing agents

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Dynamic Nuclear Polarization (DNP) has become a powerful tool to increase the low sensitivity of magic angle spinning (MAS) solid-state NMR. This hyperpolarization method generally uses biradicals as polarizing agents (PAs). Their unpaired electrons serve as polarization reservoir, which is partly transferred to the nuclear spins at low temperature (~100 K) and under suitable microwave irradiation. Considerable effort has been directed towards further improvements of PAs to obtain higher signal enhancements. However, PA performing well in the standardized conditions of strongly deuterated frozen solutions often show reduced enhancement when used with "real" samples.

Understanding how the chemical structure and relaxation properties of the PAs impact the DNP efficiency is still a long-standing and complex issue.[1] Participating to the efforts in improving DNP efficiency, we introduced a new family of PAs, the AsymPol family, whose core chemical structure was guided by advanced simulations of the DNP efficiency.[2,3] These simulations suggested the use of a relatively short linker with the intention to generate a sizable intramolecular electron dipolar / J-exchange interaction, while avoiding parallel nitroxide orientations. Fine-tuning of the chemical structure to match various solubility and electron relaxation properties lead to a family of efficient PA adapted to a wide range of applications from materials to biology. Moreover, these new radicals do not only perform well in the standard frozen-solution matrices, but as well for application systems, containing e.g. a high density of protons. We will present examples of applications in both water-based and organic solvents, as well as in regimes of high magnetic field and fast MAS, conditions known to be particularly difficult for DNP.

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HYPNOESYS: Hyperpolarization in Liquid-State NMR Spectroscopy using Optically Polarized Crystals

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Nuclear spin hyperpolarization provides a promising route to overcome the challenges imposed by the limited sensitivity of NMR spectroscopy. We present a system that exploits the intermolecular nuclear Overhauser effect (NOE) to transfer polarization from dissolved, spin-polarized pentacene-doped naphthalene crystals to target molecules [Eichhorn et al. J. Am. Chem. Soc. 2022, 144, 6, 2511–2519], called HYPNOESYS. Using naphthalene crystals as a hyperpolarization source offers several benefits. First, it can be hyperpolarized via triplet DNP to almost unity ^{1}H polarization, even at liquid nitrogen temperatures [Iinuma et al. Phys. Rev. Lett. 2000, 84, 171–174]. It is also soluble in common organic solvents, enabling ^{1}H concentrations up to 15-20 M. Finally, it possesses ^{1}H relaxation times sufficiently long for storage, which ultimately enables the decoupling of the source polarization process from the transfer of source polarization to target nuclei.

Fast injection of the solution from the HYPNOESYS into an NMR spectrometer allows us to observe the polarization dynamics for target ^{1}H nuclei which can be readily described by standard NOE models. With the entire process occurring on a timescale of one minute, NMR signal enhancements between -200 and -1730 are achieved at 60 MHz for a range of small molecules. We will further report on recent results transferring polarization to a wider range of target molecules, and combining HYPNOESYS with state-of-the-art high field spectrometers.

PT034

Optimizing hairpin coils for metabolomic analyses

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Microcoil technology, as a way to achieve superior mass sensitivity in RF coils, has been frequently implemented in various NMR probehead designs. It has been shown that microcoil-based NMR probes can obtain a mass sensitivity up to 10 times better than a conventional 5mm Helmholtz coil NMR probe. Recently, Walder et al. proposed an elongated U-shaped 'hairpin'-like resonator to study electrochemical coin cells, that achieved reasonable chemical shift resolution and B1-field homogeneity. In this study, we further explore this geometry, and demonstrate several hairpin-like coil variants produced by simple mechanical machining of printed circuit boards (PCBs). These coils are installed in a home-built probe for in-depth analysis the limit of detection (LOD) and B1/B0 homogeneity. These results will be discussed in view of the corresponding FEM simulations of the coils. The aim is to find the best performing coil for chemical analysis of samples (e.g. urine, plasma) for metabolomics.

PT035

Machine learning-based refinement of the metal coordination sphere in paramagnetic metalloproteins by pseudocontact shifts

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Pseudocontact shifts (PCSs) encode extremely accurate information on the structure of paramagnetic proteins [1]. The dependence of the PCSs on the nuclear coordinates passes through the paramagnetic susceptibility anisotropy tensor [2,3], which is very sensitive to the details of the coordination geometry of the paramagnetic metal ion. Therefore, when analyzed in conjunction with quantum chemistry calculations, PCSs measured for nuclei far from the paramagnetic metal can allow for the structural refinement of the coordination cage of the metal [4]. Starting from the crystallographic model of the metal coordination cage, a structural refinement procedure has been implemented to obtain a susceptibility tensor in good agreement with the PCS-derived anisotropy tensor. Accurate predictions of the susceptibility tensors were performed with ORCA using relativistic CASSCF calculations, with second-order perturbation theory corrections. These calculations are very demanding and a best fit analysis performed with standard procedures would require a prohibitive computational power. This limitation can be overcome through the implementation of machine learning-based protocols. This approach offers the possibility to overcome one of the most important drawbacks of paramagnetic NMR, which is the large line broadening often affecting the signals of nuclei in the immediate vicinity of the paramagnetic center. The knowledge of the metal coordination environment at the highest possible resolution is crucial to understand structure–activity relationships for metal ions in proteins and for the successful use of docking strategies for drug discovery.

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PT036

Utilization of solid-state NMR to determine the local magnetic susceptibility

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To get insight of the local magnetic properties of molecule, i.e. the local magnetic susceptibility, is a crucial topic for physicist and chemists as it will lead to a faster developments of advanced technological applications based on a tailored design of local magnetic properties such as information technologies, high density data storage, contrast agents for MRI, spintronics, etc…

State-of-the-art methods for such measurement have been developped, such as Polarized Neutron Diffraction (PND), SQUID-based magnetometry, muon spin rotation (µ-SR), Electron Paramagnetic Resonance (EPR). Each methods have its strengths and weaknesses. We implemented a model that has been tested and proven efficient in predicting paramagnetic SS-NMR spectra of microcrystalline powders.

To determine the local magnetic susceptibility, we propose a semi-empirical approach based on a parametrized point-dipole model for the local magnetism. The model is based on the knowledge of the crystal structure and the empirical point-dipole approximation for each paramagnetic center in the structure. The dipolar hyperfine interaction is calculated for each NMR observable nucleus by summing the effect of each paramagnetic center on the NMR spectrum within the convergence radius of our model. The resulting spectrum is then compared to experimental data and the model's parameters are optimized to get the best fit.

This method was only possible because this calculation is extremely fast. Besides, it allowed us to perform a statistical analysis of each parameter a statistic analyses of each parameter of the local magnetic susceptibility tensor. We compared our result with SQUID measurement which gave us a good agreement for the isotropic parameter of the local magnetic susceptibility. We will present and discuss our results on lanthanum, cerium, praseodymium and neodymium oxalates.

Promoted Lectures

Solution state NMR reveals insights into the gelation mechanism of paramagnetic metal-coordinated hydrogels

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Transition metal coordination complexes are attracting increasing attention as supramolecular cross-linkers. Nonetheless, there is still a limited understanding of how metal-ligand interactions influence the structure, assembly and properties of hydrogels. In this context, NMR has emerged as the primary tool for the elucidation of such complex systems. In this talk, our recent advances in the NMR-based investigation of supramolecular hydrogels coordinated by transition metals with paramagnetic nature will be discussed.

We envisaged that the distance-dependent Paramagnetic Relaxation Enhancement (PRE), induced by paramagnetic Fe^{3+} ions, could be used to explore the proximity and selectivity of the interaction between the Fe^{3+} ions and the hydrogel network. To prove our hypothesis, we carried out a detailed investigation on the nature of chemical interactions occurring at the carboxymethyl cellulose (CMC) – Fe^{3+} interface. Acquisition of HSQC spectra in the presence and in the absence of Fe^{3+} ions, application of the novel Spin Diffusion Transfer Difference (SDTD) NMR protocol, and combination with Molecular Dynamic calculations, unveiled a selective mode of binding in the formation of CMC-Fe^{3+} hydrogels and a structuration of water around the coordinating Fe^{3+} ions.[1] In specific, SDTD NMR, which is a STD NMR based protocol, provides access to parameters such as solvent network minimum distance (r) and spin diffusion rate at the solid-liquid interface (Dsp).[2] Additionally, we are currently studying the interaction between oxidated nanocellulose and paramagnetic Fe^{3+} ions, correlating changes in nanocellulose assembly with rheological properties.

Importantly, we demonstrated the employability of the PRE property of paramagnetic transition metals to achieve atomistic details on the interaction between the metal ions and the gelator network, paving the way for a deeper understanding of metallo-supramolecular gel formation.

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PT038

Formation and evolution of nanoscale calcium phosphate precursors under biomimetic conditions

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Simulated body fluids (SBFs) are metastable solutions with the same ionic concentration of human blood plasma and supersaturated regarding calcium phosphate phases. They are widely employed in fields such as tissue engineering and bio-inspired materials design. Additionally, SBFs are used as model systems for the study of prenucleation species (PNS), and their early onset during hydroxyapatite precipitation. PNS are nanoscopic solutes defined as transient precursors of the final calcium phosphate phase in the recently proposed alternative pathway of crystallization. Potentially, further investigation on SBFs dynamic behavior at the ionic scale could represent an efficient approach for a better understanding of non-classical crystallization. Therefore, we combine real-time ^{31}P NMR, calcium potentiometry (Ca-ISE), x-ray energy dispersive spectra (XEDS), Cryo-TEM, and computer simulations to characterize modified SBF (mSBF, a simulated body fluid with stable pH over time).

Employing real-time ^{31}P NMR, we identified some calcium phosphate nanoscale inhomogeneities forming 5 hours after mSBF preparation and monitored their evolution until stability. The NMR data were supported by Ca-ISE and XEDS experiments, indicating a depletion of free phosphate (Pi) in the solution and, consequently, the inclusion of Pi into bigger structures. Moreover, the combination of DOSY NMR and cryo-TEM quantitatively mapped the evolution of the phosphate species, detected at first as ~2 nm PNS particles, and, after 24 hours, into ~200 nm aggregated structures. Finally, to understand the kinetic leading to PNS formation, we generated a theoretical model simulating the exchange behavior of phosphate in solution.[1]

In conclusion, we show that mSBF becomes a stable or metastable solution only after ~24 hours from preparation. Our study complements the PNS monitoring by DDNP during calcium phosphate (brushite) precipitation in non-physiological conditions performed by Weber et al.[2]

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Revealing Carbon Capture Chemistry by 17O NMR Spectroscopy

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In order to limit warming to 1.5 °C over the next century, the development of efficient carbon capture and storage (CCS) technologies is essential.[1] Solid adsorbent materials, such as metal-organic frameworks (MOF), are promising alternative to traditional aqueous amine systems.[2,3] Amine-functionalised MOFs, where a reactive amine species is appended to a vacant metal site, are a particularly promising class of materials owing to their high CO_2 selectivity and large surface areas. Although these materials have been widely studied, the binding modes in these materials are still poorly understood, hindering the design of improved materials.[4] Previously, these materials have been explored by combining 13C NMR spectroscopy and DFT calculations.[4] However, poor differentiation is seen in the 13C NMR parameters for closely-related adsorption products and therefore there is a great need to develop new methods for studying carbon capture chemistry. In principle, exploration of the O sites in these materials should provide a more detailed picture. Here we show that 17O NMR spectroscopy is a powerful probe for investigating binding modes in a range of diamine-appended MOFs.

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PT041

GEMSTONE: ultra-selective NMR methods for complex spectra

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2D NMR methods provide extensive structural and conformational information on molecules, but such experiments are time-consuming and can provide more information than is actually needed. 1D ^{1}H selective experiments can often extract the key information required for structural and conformational analysis in a much shorter time. Unfortunately, in ^{1}H NMR the narrow range of chemical shifts and the multiplicity present often cause multiplets to overlap, so that traditional selective excitation methods cannot select a single chemical site. The chemical shift selective filter (CSSF)[1] does allow selection of a single chemical shift in an overlapped region, but is time-consuming because multiple increments are required to achieve selectivity.

Here, a new family of 1D ultra-selective experiments, GEMSTONE[2] (gradient-enhanced multiplet-selective targeted-observation NMR experiment), is demonstrated. GEMSTONE allows selective excitation of a single multiplet even in the presence of severe overlap, requiring only a single scan to achieve multiplet selectivity, saving significant time compared to CSSF. GEMSTONE versions of 1D selective NOESY[2] and TOCSY[3] methods will be shown, providing unambiguous through-space and through-bond correlations, respectively, for individual selected signals. A novel ultra-selective GEMSTONE-ROESY adaptation will also be presented, aiding structural and conformational analysis where the NOE provides little or no intelligible data. The benefits of the new method will be demonstrated in the structural analysis of lacto-N-difucohexaose I, a structurally complex oligosaccharide present in breast milk. Additionally, a pure shift[4] adaptation of the published GEMSTONE-TOCSY[3] sequence will be shown, further simplifying complex spectra by collapsing the multiplicities of the signals observed.

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PT042

Broadband effects of radiation damping during homonuclear total correlation mixing

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The inductive coupling between the transverse magnetization and the radio frequency (RF) circuit creates an oscillating magnetic field, known (rather misleadingly) as the "radiation damping" (RD) field. This RF field is usually so weak that it affects significantly only the resonances which are at the origin of the effect or which have frequencies close to these resonances. Pulse trains, designed for homonuclear total correlation spectroscopy, remove efficiently the chemical shift differences from the effective Hamiltonian over a wide range of frequencies. In this work, it will be shown that the RD field, under these circumstances, strongly influences a much wider range of frequencies than would be expected from its amplitude (1). Counterintuitively, the magnetization of remote resonances can precess away from a strong spin-locking RF field. This phenomenon, will be rationalized by simulations and a theoretical analysis. In particular, the importance of the (non-quadrature) orientation of the RD field will be discussed. Several different mixing sequences will be looked at.

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PT043

Long-lived states and coherences for magnetisation transfer via Overhauser and exchange effects in biomolecules

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Liouville states based on singlet configurations of coupled spins[1] are integrated in long-lived avatars of classical pulse sequences designed for the study of magnetisation transfer in peptides and proteins. We describe the progress made in using long-lived coherences[2] (LLC's) for Overhauser effects and long-lived states (LLS) for the study of chemical exchange.

Calculations and experiments show that magnetisation transfer via rotating-frame Overhauser effects (ROE) can be improved using LLC's. LLC-ROE[3] transfer from a pair of J-coupled protons I and S in the LLC configuration, (Ix - Sx), to a third proton, K, was compared to the classical ROE counterpart, (Ix + Sx) -> K. LLC-ROE is shown by theory to overcome classical ROE in terms of proton magnetisation transfer between a coupled pair (I,S) and a neighbour K for correlation times τc >10 ns, when the distance between K and the midpoint of I and S is considered d[K,(I,S)] = 2.3 Å. Experimentally, we measured the LLC-ROE transfer between aliphatic protons in different peptide residues in AlaGly, with (I,S) = Gly-(Hα^1,Hα^2) and K=Ala-Hα. Over the distance d[Ala-Hα, Gly-(Hα^1,Hα^2)] = 2.3 Å, the measured LLC-ROE magnetisation transfer confirmed the theoretical prediction.

Using magnetisation transfer by exchange and Overhauser effects from water to AlaGly, we showed that hyperpolarized-HDO-based-2D-COSY[4] experiments acquired 1'000 times faster than the classical 2D counterparts reveal the peptide structure in different environments. The populations of labile protons at the N- and C-termini of AlaGly dictate the rate of exchange between two main conformations: a closed one (A) featuring the salt bridge between the termini and an open conformation (B) lacking this bridge. New Carr-Purcell-Meiboom-Gill based LLS-CPMG and LLC-CPMG experiments display clear contrast in terms of relaxation rate constants between environments featuring AlaGly majoritarily in (A) or (B) conformations.

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Deuterium metabolic imaging of human gastric emptying and hepatic and renal glucose metabolism at 7T

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Deuterium metabolic imaging (DMI) is a novel non-invasive method to assess metabolism in vivo using deuterated substrates, such as $[6,6'-^2H_2]$-glucose. The liver and kidneys play a center role in whole-body glucose homeostasis and in type 2 diabetes, both hepatic and renal glucose metabolism are dysregulated. Diabetes is also associated with gastric emptying abnormalities, such as slow/delayed and fast gastric emptying. In this study, we developed a 4-channel ^2H transmit/receive body array coil for DMI in the human abdomen at 7 T and we investigated the feasibility of simultaneously measuring gastric emptying, and hepatic and renal glucose uptake and metabolism with dynamic 3D DMI upon administration of deuterated glucose. The variability of the natural abundance deuterated water signal in the liver, measured in 6 healthy volunteers, was 5.2% ± 0.7%. Dynamic 3D DMI scans with oral administration of $[6,6'-^2H_2]$-glucose in a healthy subject showed that the initial rate of glucose uptake was very similar in the kidney and liver. However in late postprandial period, the dynamics of deuterated glucose disappearance as well as the deuterium labeling of water seemed faster in the kidney. The measured gastric emptying half time was 80 ± 10 min, which is in good agreement with scintigraphy measurements. In conclusion, DMI with oral administration of $[6,6'-^2H_2]$-glucose enables the simultaneous assessment of gastric emptying and liver and kidney glucose uptake and metabolism. When applied in patients with type 2 diabetes, this approach will advance our understanding of the interplay between disturbances in liver and kidney glucose uptake and metabolism and gastric emptying, at a detail which cannot be achieved by any other method.

Parahydrogen hyperpolarization in chemical analysis of biological samples

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Recent developments in nuclear hyperpolarization have shown encouraging results in the analytical chemistry domain, allowing measurements that would be unfeasible by regular means of NMR sensitivity. In particular, the parahydrogen hyperpolarized chemosensing technique [1] has developed into an analytical chemistry tool that allows NMR detection of dilute analytes in complex mixtures, including metabolites in biofluids.

We have used this technique in a urinary pharmacokinetics experiment [2], achieving detection of mid-nanomolar concentration metabolites. In a parallel development, we demonstrated the compatibility of parahydrogen hyperpolarized chemosensing with minimally altered urine samples, producing highly information rich hyperpolarized NMR spectra [3] for metabolomics applications, and proved the feasibility of compiling spectral libraries for assignment of hyperpolarized signals [4].

Herein, we will discuss the important steps and considerations for achieving compatibility of parahydrogen hyperpolarization with biological matrices. We will present the known scope of metabolites and biomolecules that can be analyzed by the hyperpolarized chemosensing approach and demonstrate the first proof of concept applications.

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PT046

COVID-19 metabolic progression and disease prognosis as investigated by NMR metabolomics

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COVID-19 caused by SARS-CoV-2, is a systemic infection that significantly alters human metabolome. Here we intend to look at the evolution of the disease at the metabolome level, as well as, how the metabolome behave after recovery. To that end, we present the metabolic and lipoprotein analysis of blood serum for 1500 individuals, analysed by NMR spectroscopy. Samples include acute COVID-19 patients (n=405), post-acute patients (3-6 days, n=132), recovery (1-2 months, n=118), Long COVID (n = 100) and Controls (n=750). Two novel pulse sequences: DIRE and JEDI were specially designed to disentangle the inflammation and atherosclerotic risk biomarkers and are here presented. NMR data was complemented with cytokines and chemokines analysis for a subset of samples. The lipidomic analysis unraveled a pathogenic redistribution of the lipoprotein particle size and composition to increase the atherosclerotic risk, that partially remains in long COVID patients. At the metabolic level, the damage inflicted at hepatic level during the acute phase is still visible even after recovery, as well as a constant activity of the immune system to reduce inflammatory damage. Altogether, this study highlights the potential of NMR-based metabolomics to investigate infectious diseases such as COVID-19.

PT047

Protein- and ligand-observed 19F NMR spectroscopy in human cells

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The cellular environment can affect the structure and function of pharmacological targets, and the interaction with potential drugs. This added complexity contributes to the high attrition rates in the pre-clinical and clinical phases of drug development. In-cell NMR spectroscopy can potentially fill this gap, as it provides structural and functional information on macromolecules directly in living cells, and has been shown to give precious insights on intracellular drug-target interactions. Nowadays, NMR bioreactors can greatly improve the cell sample stability over time and allow monitoring the evolution of intracellular processes, including protein-drug interactions, by time-resolved in-cell solution NMR. With this approach, intracellular ligand binding can be monitored by protein-observed 1D ^{1}H and 2D ^{1}H-^{15}N spectra. However, this approach suffers from interference from background signals from the cell (in 1D ^{1}H) or from loss of signal (in 2D spectra) caused by the interaction of the target protein with cellular components. Here we propose ^{19}F NMR spectroscopy as an additional tool for investigating protein-ligand interactions in living human cells. The high-sensitivity, background-free nature of ^{19}F makes it an ideal probe for intracellular events. For protein-observed in-cell NMR, fluorine incorporation in the target protein is easily achieved by replacing the desired amino acid type with the fluorinated homolog during protein expression. For ligand-observed experiments, fluorinated ligands can be directly observed as they interact with the intracellular targets, whereas binding of non-fluorinated ligands is indirectly observed by competition binding experiments. In both approaches, simple 1D ^{19}F NMR spectra are sufficient for detecting the signals of interest. We show that binding to proteins 'invisible' by classical ^{1}H-^{15}N NMR can be studied by ^{19}F in-cell NMR, thus greatly extending the applicability of in-cell solution NMR.

PT048

Atomic interrogation of proteins within intact nuclei by DNP-supported solid-state NMR

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Investigating proteins at atomic levels of resolution whilst maintaining native conditions constitutes the cutting-edge of structural biology. [1–3] To this end, we detail a methodology coupling recent advances in ultra-high field dynamic nuclear polarization-supported solid-state NMR (DNP ssNMR) with physical enrichment of sub-cellular compartments to study proteins in situ – in our specific case intact nuclei. For a proof of concept, we utilised our organelle-NMR approach to visualize the nuclear landscape of the omnipresent post-translational modifier ubiquitin.[4] We observe that physical enrichment coupled with ultra-high magnetic fields yields significant gains both in terms of sensitivity and resolution, consequently allowing for cellular detection of vital ubiquitination events like iso-peptide bonds. Altogether our method lays the foundation for investigation of nuclear proteins in a broader cellular context and can be expanded on further to investigate other sub-cellular compartments.

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PT049

High sensitivity NMR for structural determination of neurodegenerative disease-associated proteins inside cells

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The misfolded proteins associated with neurodegenerative disease can adopt a variety of different conformations, some of which are toxic. Because these proteins have identical amino acid sequences, the cellular environment clearly influences the final state, yet most structural studies do not include the cellular context and, perhaps because we are not studying the correct conformation, not a single therapeutic strategy for these diseases addresses the underlying protein misfolding pathology. We use DNP enhanced MAS NMR to study protein structure in native environments - inside living cells - to reveal how both healthy and disease-relevant cellular environments influence protein structure. Careful sample handling can support DNP NMR experiments on frozen intact viable cells and altering the distribution of the DNP polarization agent, AMUPol, can spotlight protein conformations that have differential spatial distributions. This is demonstrated for the protein, alpha synuclein, inside mammalian cells.

PT050

Quadratic spacing of effective gradient area for spatially encoded diffusion NMR

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Diffusion NMR experiments are a powerful tool for the analysis of mixtures. Spatially ENcoded (SPEN) DNMR makes it possible to acquire diffusion data in a single scan, by spatial parallelization of the gradient dimension [1]. SPEN DNMR usually relies on linearly swept (chirp) pulses which provides linear spacing of the effective gradient area along the sample length. However, several algorithms for the fast analysis of diffusion data require quadratic gradient spacing [2].

Here we describe the design of a frequency-swept pulse that yields quadratic spacing (QS) of the effective gradient area in SPEN DNMR experiments, and show that it makes it possible to use fast univariate and multivariate processing of diffusion NMR data [3]. The QS-pulse is designed with the help of numerical spin simulations, and validated experimentally. We show that, using this approach, SPEN DOSY data can be processed using the Direct Exponential Curve Resolution Algorithm (DECRA), which is useful to separate overlapping peaks [2a]. We also show that the data is compatible with the Matrix Pencil Method (MPM), which has recently been described as an efficient approach to analyze diffusion data [2b]. These methods will be useful for the NMR analyses of time-evolving mixtures.

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PT051

RApid Metabolic Identifier for 1D PM-TOCSY (RaMIT)

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Identification of metabolic signatures in NMR based metabolomics is difficult because of overlapping peaks. Phase Modulated TOCSY helps in resolving overlapping spin systems. Deep neural networks have been used to address various challenging problems in NMR analysis. Here we propose a Convolutional Neural Network to identify the metabolites in a 1D PM-TOCSY spectrum.

Data is synthetically created by randomly combining 24 real spectra of individual metabolites (recorded in our lab) with varying amplitudes and phases, thus simulating the PM spectral data. Since the peak positions do not vary significantly and only the intensity of the peak varies corresponding to the concentration of the metabolites, this process of creating data is agreeable. The intensity of each point in the spectrum is input to the model.

The network begins with two 1D-convolutional layers (kernel size of 5 and 32 filters each), followed by a max-pooling layer (pool size of 5). These are again followed by two 1D-convolutional layers (kernel size of 3 and 64 filters each), followed by a max-pooling layer (pool size of 3). These are then flattened and passed to fully connected layers with 128 and 64 nodes respectively. The output layer has 24 nodes corresponding to the 24 metabolites. All layers are activated by $\tanh(x)$ and only the output layer is activated by $\text{sigmoid}(x)$ function.

The model is trained on more than a million samples in mini batches. The network has 1.3 million parameters. The model was tested on 1000 data points not seen before. The accuracy is 98.29% with an F1-score of 0.9833. The false positives were very few, indicating that the network hardly raises a false alarm when a metabolite does not exist.

This proof-of-concept study is encouraging and shows that such DNNs can be practically used for the fast and accurate identification of metabolites using PM-TOCSY.

PT052

Dynamical Xe NMR modelling in molecular and materials cavities

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NMR spectroscopy of ^{129}Xe guest atom in molecular and materials cavities has redeemed its great potential to provide otherwise unattainable, detailed local information about the atomic and electronic structure of the host material. While ^{129}Xe NMR spectral features are very sensitive to physical conditions-dependent dynamical processes affecting both the host and the guest, to connect them to the processes at the microscopic level requires theoretical first-principles modelling. In this presentation, we will show how combining state-of-the-art relativistic quantum-chemical (QC) modelling of the electronic structure and Xe NMR parameters with statistical Monte Carlo (MC) and molecular dynamics (MD) simulations of the whole host-guest system in solid or solvent environments is an essential part of Xe NMR materials studies. Modelling increases the amount of information obtained from simple spectra as well as resolves and predicts new spectral features. Few examples include studies of novel porous solids, e.g. porous organic cages (POCs) and noncovalent porous materials (NPMs), porous liquids of POCs as well as novel Xe molecular sensor candidates, such as metal organic polyhedral (MOPs) and bridged resorcinarene cages (BRC). In solid POC and NPM materials, MC averaging over Xe motion on energy and NMR shielding hypersurfaces provides temperature and Xe loading dependent Xe chemical shift estimates that are valuable in exchange, relaxation, and diffusion modelling of experimental data. Similar modelling data is obtained for porous POC liquids by Xe NMR shielding averaging of snapshots extracted from semi-empirical MD simulations of Xe atom in explicit POC and neat solvents. First, QM+MC modelling of Xe chemical shift provided evidence of Xe@Fe-MOP complex. It was also crucial when hidden Fe-MOP diastereomers were revealed by Hyper-CEST NMR. Finally, the dynamical Xe NMR modelling not only confirms but also predicts unexpected spectral signals in new shift regions for BRC sensor candidates.

PT053

In-vivo Diffusion Tensor Imaging of the mouse abdomen using a driven-equilibrium approach to spatial encoding

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Spatiotemporal encoding (SPEN) is an alternative to Fourier imaging that is robust against distortions and motion [1, 2]. We show that our recently introduced method for DWI, based on a driven-equilibrium approach to spatial encoding [3], termed DP-SPEN, can map in-vivo diffusion properties in the abdomen of the mouse, including the pancreas, in under 8 minutes.

Experiments with N=3 female mice (C57BL/6, 21.3±0.9 g), aged 8-12 weeks, on a 9.4 T horizontal bore scanner. Brain DTI acquired on N=1 animal with DP(W)-SPEN(EPI): TE/TR=37.1(41.4)/2000 ms, 125×125 µm2, 1 axial slice 0.8 mm thick; 2 b-values (500, 1000), 15 directions, 15 b0 images, 1 average, 2 repetitions, total scan time = 15 mins. DP-SPEN abdominal DTI acquired in N=3 animals: TE/TR=23.5/2000 ms, 182×182 µm2, 6 axial slices 1 mm thick; 2 b-values (500, 1000), 12 directions, 15 b0 images, 1 average, 2 repetitions, respiratory triggering, total scan time ≃7min 48s.

DP-SPEN brain DTI yields similar results to DW-EPI while being more robust to distortions in areas of poor B0 homogeneity. DP-SPEN successfully displays abdominal structures dominated by different types of motion. Mean (over animals) MD is (0.83±0.79)×10-3 mm2/s for liver and (0.95±0.15)×10-3 mm2/s for pancreas. Mean FA values are 0.32±0.04 and 0.28±0.03 for liver and pancreas respectively.

Mouse abdomen diffusion properties could be mapped with DP-SPEN without using any pharmacological preparation or additional sequence elements for motion correction. MD values are within expected ranges for liver [4] and FA is relatively low for healthy liver and pancreas. To our knowledge, this is one of the first studies to focus on in-vivo diffusion properties of the mouse pancreas, dominated by very short T2s and motion.

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PT054

Massively multidimensional diffusion MRI: from concepts to restriction sensitive and sparsely-sampled acquisition

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Diffusion MRI indirectly depicts the tissue's microstructure at a few micrometers scale through the water diffusion and characterizes it with quantitative metrics such as fractional anisotropy, mean diffusivity, or diffusion tensor. However, its limited spatial resolution, superior by 1 to 3 orders of magnitude to the diffusion extent, leads to voxel averaged parameters and ambiguities in their interpretations. This length scale gap can be bridged by using non-parametric diffusion tensor distributions (DTDs) to describe the heterogeneity of the microstructure at a sub-voxel level. The specificity required to disentangle the DTDs is obtained by the acquisition of numerous images, weighted by amplitude-modulated multidirectional gradients, and leading to specific signal attenuation for different microstructures. This approach can also be combined with variable TR and TE acquisition providing a better separation and quantification of the DTDs via T1 and T2 correlations. Recently, our group also introduce the use of high-frequency isotropic diffusion encoding to separate the effect of restriction and diffusion enabling depiction of the cellular density of biological tissues[1,2]. In this presentation, we intend to exemplify the possibilities offered by this new multidimensional diffusion framework to provide a more specific description of the contribution of cell types, local chemical composition, axonal density, restriction, and orientations at a sub-voxel level in ex vivo brain or tumor tissues and allows MRI to compete with histology. We will also discuss the various strategies for rapid and sparsely sampled acquisitions of such a massively multidimensional dataset. We believe that these latest developments will pave the way to microstructure studies using MRI in heterogeneous biological tissues both ex vivo and in vivo.

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Clinical applications of sodium TQ/TPPI spectroscopy and microimaging: The case of Type 2 Diabetes Mellitus

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Recent data reveal a mismatch between dietary sodium intake and urinary sodium excretion over prolonged periods, and sodium retention without attendant (osmotically induced) weight-gain. Skin, as the largest organ in the body, has been proposed to serve as a reservoir for sodium by many researchers. Our aim was to identify sodium compartment within the skin using sodium MRI and to quantify sodium stored within that compartment using sodium Multiple quantum filtered spectroscopy in skin biopsies from patients with T2D (n=9) and euglycemic patients with no history of Diabetes Mellitus (n=8). NMR and MRI were performed using 9.4T Bruker Avance III Microimaging system (Bruker, Germany) using 25mm dual tuned 1H/23Na microimaging coil (Bruker, Germany). 1H multi-slice T1/T2* weighted gradient echo MRI protocol was used to visualise anatomical skin regions in intra-operative skin biopsies at histological lenghtscales. 23Na MRI was performed using non-slice selective gradient echo with spectroscopically optimised triple quantum filter (TQF) to visualize stored sodium. 2D triple quantum-filtered with time proportional phase incrementation (TQ-TPPI) spectroscopy was used to determine sodium storage skin capacity in all studied skin biopsies.

The co-localisation of free and stored sodium in skin biopsies vizualised by 23Na MRI iwas found in the dermis layer of the skin. 23Na TQ-TPPI were spectra used to characterize skin sodium storage capacity in controls and T2D patients biopsies.

We demonstrate that (>90%) of both free and stored sodium are located within the dermis compartment of the skin that consists mostly of the extracellular matrix enriched with glycosaminoglycans (GAGs) and collagen. Spectroscopic outcomes can be translated into dermal sodium skin storage capacity that diminishes in case of TD2.

We provide the first evidence for stored sodium within the human dermis, co-locating to the GAG scaffold. We also provide evidence that T2D associates with diminishment of the dermal binding capacity for sodium.

DNP on membrane proteins: Channelrhodopsin-2, the Cannabinoid Receptor 2 and application of AsymPolPOK

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Membrane proteins are often difficult to study due to the requirement of a membrane mimetics and often low expression yields. DNP-enhanced solid-state NMR enables the study of small amounts of protein independent of the molecular weight of the protein and its membrane mimetic. However, the low temperature requirements, usually 100 K, of such experiments result in spectra of low resolution. To overcome this obstacle, highly selective labelling schemes based on specific hypothesis need to be applied.

Here, by using DNP-enhanced solid-state NMR, we show that detailed questions regarding the photocycle of Channelrhosopsin-2 can be answered. This is of special interest as Channelrhodospin-2 is a light-gated ion channel widely used in optogenetic applications. Due to the ability to trap and measure photointermediates at low temperatures, we could determine the chromophore structure of the desensitized photo intermediate. By quantitatively measuring long ^{13}C-^{13}C distances, we could proof a 13-cis,15-syn retinal configuration. In addition, we carried out different trapping protocols to elucidate the positioning of this intermediate in the photocycle (J. Becker-Baldus et al., Angewandte Chemie International Edition 2021,69,2).

Intrigued by the work of Mentink-Vigier et al. (JACS 2018,140,11013), we aimed to improve the sensitivities of membrane proteins under DNP conditions which often suffer from depolarization not accounted for by simply optimizing the microwave on/off enhancements. We therefore carried out a systematic study on membrane protein micelles comparing the gold standard AMUPol with the new radical AsymPolPOK and optimizing the radical concentration. We also investigated the influence of the different radials on the coherence life times. Based on these results we obtain spectra of the cannabinoid receptor 2, a G-protein coupled receptor. We were able to achieve a good sensitivity on this protein and we could study specific sites in the presence of different ligands using the unique pair approach (unpublished data).

PT057

Diamond Rotors for DNP MAS NMR

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Single crystal diamond rotors can enable unprecedented advances in both the sensitivity and resolution of magic angle spinning (MAS) NMR under ambient and Dynamic Nuclear Polarization (DNP) conditions. Diamond has extremely high tensile and elastic moduli, is nearly transparent at THz frequencies, and has exceptional thermal conductivity. While diamond is an optimal material for DNP MAS rotors, significant fabrication challenges have prevented the realization of diamond rotors. Accordingly, we developed a laser micromachining process to successfully fabricate 0.7 mm diamond rotors. We demonstrate MAS results of up to 123 kHz using nitrogen spinning gas at room temperature without rotor damage, and stability of two separate rotors at 111 kHz with a standard deviation of <4 Hz.

PT058

Fast-MAS NMR structure elucidation of fully protonated proteins via innovations for assignment and distance information.

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Fast-MAS solid-state NMR with rotation frequencies > 100 kHz has been enabling access to solid proteins that are available only in minute quantities and without the necessity for deuteration or specific labeling. The elucidation of protein structure in this methodological context, in addition, can benefit from a large number of potential internuclear distance restraints. Exploiting these fundamental advantages of the approach has, however, been suffering from i) difficulties in assigning the protons in the side chains with sufficient confidence and ii) the presence of both, dipolar truncation and spin diffusion contributions to any internuclear magnetization transfers.

Here we will demonstrate the benefits of higher-dimensionality spectral assignment approaches on the one hand and the use of corrected, time-dependent analysis of cross peak buildup on the other hand to permit structure calculation of highest accuracy from minimal amounts of sample.

The C-terminal domains of yeast Hsp90 in vitro and in cells

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Hsp90 is a molecular chaperone responsible for the homeostasis of the cells by acting on a large number of client proteins facilitating their folding. Hsp90 is known to undergo large conformational changes during function coupled to ATP binding and hydrolysis (1). Additionally co-chaperones fine-regulate its structure and therefore function (1). Hsp90 is homodimeric and each monomer consists of three domains: the C-terminal domain (CTD), which is the dimerization site that holds the two monomers together at all ATP states, the M-domain (MD) which is binding site for clients and co-chaperones and the N-terminal domain (NTD) which is the ATP and co-chaperone binding site (1-3). Here, we studied the dimerization of full length Hsp90 and CTD Hsp90 with double electron-electron (DEER) measurements on Gd(III)-labeled CTD mutants. We found differences in the dissociation constant, KD, between the mutants, as well as lower KD for full length compared to CTD-only Hsp90. This suggests the presence of inter-domain communication and stabilization of the CTD dimerization via the MDs and NTDs. We also exploited the same mutants to study the conformation of the CTDs in Hela cells and cell lysates. One of the mutants revealed a narrower CTD conformational space in the cell and lysate, compared to in vitro conditions, which we attribute to interaction with CTD co-chaperones.

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PT060

The predicted structure of a pathogen surface protein validated by pulse dipolar EPR

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The remarkable accuracy protein structure prediction has reached heralds an age where the routine production of experimental high resolution structures becomes obsolete. Often the global fold and backbone conformations are more crucial than small molecule ligands and catalytic residues. In these cases, computational predictions (e.g., AlphaFold2) and tests of their reliability could transform the way we investigate structural assemblies.

Pulse dipolar EPR spectroscopy (PDS) is ideally suited to validate structures and structural heterogeneity by measuring distance distributions between selected sites within the predicted fold. Crucially, the main limitations of complexity and size in NMR, resolution in crystallography and cryo-electron microscopy do not apply to this approach. Furthermore, recent deep learning-based processing approaches are remarkably successful while eliminating intuitive decision making in processing that could lead to confirmation bias.

In this contribution we will show how a few well-chosen spin labelling sites allow validating the predicted fold of a dimeric pathogen surface protein of unknown high-resolution structure and its further refinement based on the experimental distance data. We further explore how the combination of orthogonal labelling approaches and complementary PDS experiments can increase the information content retrievable from a limited number of constructs.

Finally we investigate how the superb sensitivity of PDS can be used for potential cell-based studies of the host-pathogen interaction.

Utilizing EPR spectroscopy to resolve metal-sensitive transcription mechanisms.

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Bacteria are skilled survivors capable of living in environments with high concentrations of different metal ions. Some of these metals are essential, but even essential metals can become detrimental if above a certain concentration within the cytosol. Therefore, bacteria have evolved highly sophisticated and complex biological processes to regulate and maintain intracellular metal homeostasis. One of these processes is controlled by metalloregulators, which respond to metal ions and regulate the transcription of genes that protect the bacteria from metal-induced stress. Upon metal binding, the metalloregulator protein subsequently induces conformational changes in the DNA promoter sequence, leading to transcription initiation and subsequent transcription of genes that mediate adaptive responses to the toxicity of the targeted metal. In our lab, we have been studying three copper-sensitive transcription factors, E. coli and P. aeruginosa CueR, M. tuberculosis CsoR, and S. pneumoniae CopY using pulsed and continuous-wave EPR spectroscopy. Using variety of spin-labeling methodology, on the protein and on the DNA, we show how the copper ion is critical to initiate a series of conformational changes that regulate and initiate gene transcription. We also show how homologues from different bacteria behave differently, and we present the different mechanisms between repressors (ie CsoR and CopY) and activators (CueR).

PT062

Enabling high throughput fragment screening with hyperpolarized NMR

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The capacity of NMR to detect and characterize weak target-ligand interactions makes it ideal for fragment-based drug discovery. Nevertheless, the poor sensitivity of NMR is at the origin of severe drawbacks. Indeed, NMR-based fragment screening is low throughput (50 samples per day), it requires high concentrations (100 µM), and it needs to perform on a high field spectrometer (>600 MHz).

We developed a set of photo-CIDNP NMR methods improving the screening rate to up to several thousand samples per day (up to 10'000). In addition, our technique is operational at low micromolar concentrations (5 µM). This new method performs using a medium field spectrometer 200-600 MHz. We will present the theoretical background and the experimental setup that combines flowthrough NMR and hyperpolarization.

We will also present the results obtained for different screening campaigns on several targets, including challenging ones such as membrane proteins.

Importantly, this new method empowers NMR for high throughput fragment screening and places it as a competitor of state-of-the-art techniques such as X-ray and surface plasmon resonance. Finally, we will emphasize how to use this method to address challenging and orphan targets and impact the drug discovery field.

PT064

Binding of the clinical drug candidate anle138b to lipid-induced α-synuclein fibrils

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The presence of α-synuclein fibrils in inclusion bodies is a hallmark of neurodegenerative diseases such as Parkinson's Disease, Multiple System Atrophy and Dementia with Lewy bodies. α-synuclein aggregation is thought to be involved in cellular pathology and spreading between cells. To date, no disease modifying treatment is available. Hence, atomic resolution structures of small molecule binding to such pathological protein aggregates are of interest for the development of therapeutics and diagnostics. Here we show the interaction between α-synuclein fibrils and anle138b, a clinical drug candidate for disease modifying therapy in neurodegeneration.

Magic angle spinning (MAS) NMR fingerprinting was employed to guide preparation of α-synuclein fibrils grown in the presence of phospholipids and their structure was determined using cryogenic electron microscopy. We used selective isotope amino-acid labelling and cryogenic dynamic nuclear polarization (DNP)-enhanced MAS NMR to locate anle138b in a novel binding site in these fibrils. Multiple binding modes of the compound at this site were explored and quantified in detail using molecular dynamics simulations, revealing stable polar interactions between anle138b and backbone moieties of fibrillar α-synuclein. Our results emphasize that expanding radiotracer target identification as well as structure-based refinement of fibril binding molecules, such as anle138b, could prove to be beneficial.

Posters

PO001

Combining Solution- and Solid-State NMR provides insights into the binding of Microtubule-Associated Proteins to Microtubules

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Microtubules (MT) play an essential role in cell migration, mitosis and polarization. Many of these functions critically rely on Microtubule-associated proteins (MAPs) and their association with MT. So far, little is known about the interaction of MAPs and their intrinsically disordered regions with the dynamic MT surface [1]. In particular, the role of the unstructured C-terminal tails (CTTs) of tubulin, critical for many MAPs interactions, has remained elusive [2]. Considering the importance of dynamics for MT function in cellular processes and human disorders, we have employed nuclear magnetic resonance (NMR) to obtain structural and dynamical information about these interactions [3].

Using a combination of solution- and solid-state NMR spectroscopy, we have examined the interaction of MT with three different MAPs. Namely MAP7, MAP Tau and the CKK domain of the calmodulin-regulated spectrin-associated protein. In our studies, we made use of MT protofilaments, Tubulin-dimers and peptides including those representing the tubulin CTTs. Specific experiments were designed to help probe interactions between the MAPs and the tubulin CTTs. The binding of the MAPs to the whole MT protofilament was studied with solid-state NMR what allowed us to identify interacting and non-interacting residues.

In our contribution, we report on progress on combining our NMR results with information obtained by cryo-EM, biochemical and biophysical methods to gain deeper insight into the binding behaviour of these MAPs to MT. Our results give new information about the binding mechanism of different MAPs to MT and highlight the importance of the tubulin CTTs for these interactions.

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PO002

Site-specific recognition of SARS-CoV-2 nsp1 protein with a tailored titanium dioxide nanoparticle

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The ongoing world-wide Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) pandemic shows the need for new potential sensing and therapeutic means against the CoV viruses. The SARS-CoV-2 nsp1 protein is important, both for replication and pathogenesis, making it an attractive target for intervention. In this study we investigated the interaction of this protein with two types of titania nanoparticles by NMR and discovered that while lactate capped particles essentially did not interact with the protein chain, the aminoalcohol-capped ones showed strong complexation with a distinct part of an ordered α-helix fragment. The structure of the forming complex was elucidated based on NMR data and theoretical calculation. To the best of our knowledge, this is the first time that a tailored titanium oxide nanoparticle was shown to interact specifically with a unique site of the full-length SARS-CoV-2 nsp1 protein, possibly interfering with its functionality.

PO003

Novel NMR Assignment strategy for IDPs and large proteins containing disordered domains

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In recent years, intrinsically disordered proteins (IDPs) and disordered domains in large proteins have attracted great attention. Many of them contain linear motifs that mediate interactions with other factors during formation of multicomponent protein complexes. NMR spectrometry is a valuable tool for characterizing this type of interactions. We propose a robust and reasonably quick protocol to assign the backbone and sidechain resonances in NMR spectra of large IDPs in free or in complex with structured domains. It assumes a twostep process: first, assignment of the unfolded protein in the presence of denaturant, for example: GdmCl. The significantly improved quality of the NMR spectra allows the use of a combination of experiments defining the type of amin acid and accelerated data acquisition using NUS H(N)-type 3D Target Acquisition (TA) experimental approach. To make MUSIC type experiments more efficient, we made modifications to the available pulse sequences with (a) semiconstant time functions to boost resolution in the 15N dimension (b) TROSY and (c) sensitivity improvement options. Second, back titration from the unfolded protein to its native form allows the transfers of amide resonances assignments. This protocol was applied to the structural study of fully disordered nsP3 HVDs of Venezuelan equine encephalitis virus (VEEV) and the SARS-CoV-2 full length nsp1 protein including the folded domain together with the flaking N- and C- terminal intrinsically disordered fragments.

PO004

Structural insights into Biofilm Forming Functional Amyloids

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Solid-state MAS NMR spectroscopy is a powerful technique for structure determination of biological solids at high-resolution. Many examples have been shown to demonstrate a nowadays routine-like application of this technique on insoluble protein systems. We apply MAS NMR on functional amyloids (FA) forming skeleton of bacterial biofilms. Biofilms are produced by a variety of bacteria to protect themselves under stress-inducing conditions. They are considered as a major limitation for antibiotic effectiveness, so a target to fight antimicrobial-resistance. They are composed of bacteria, proteins, carbohydrates, and sometimes also nucleic acids. Amyloids constitute the most important part and deletion of them results in disassembly of the whole biofilm structure.

Here, we present structural insights on FA from different bacteria. MAS NMR data supplying deeper understanding will be presented along with the biophysical results.

Posters

Investigating the Dysfunction of DNAJB 6 Co-chaperon Caused by Myopathy - LGMD1D Disease Mutations

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Myopathy mutations in DNAJB6 chaperone cause a late onset muscle disease called Limb-Girdle Muscular Dystrophy type 1D. LGMD1D is an autosomal disorder identify by muscle weakness, impaired movement control ability. Muscle pathology in LGMD1D includes protein accumulations of aggregated protein and vacuolar myopathology. The molecular mechanisms which lead to the dysfunction of DNAJB6 are not well understood. In this study we investigate the mechanistic relationship between the mutations phenotypes to the disease myopathy.

DnaJB6 is composed of a conserved N-terminal J-domain (JD), glycine and phenylalanine rich region (GF) following serine and threonine rich region, and a C-terminal domain. An NMR structure of DnaJB6 was reported lately, revealing the structure of the J-domain-GF regions and the CTD. This structure reveals a new helix in the GF region as was also discovered by us on DnaJB1, a co-chaperone member of DnaJB6. Our study on DnaJB1 revealed an important role of this new fifth helix in regulating the HSP70 binding.

All LGMD1D-associated mutations localize to the conserved GF region as well as in the JD region of DNAJB6. DnaJB6 is a potent suppressor of poly-Q aggregation. Not like other class B J-domain proteins that interacts with HSP70 via there JD region in order to prevent aggregation of misfolded proteins; DnaJB6 functions as aggregation preventer of misfolded proteins without the need of HSP70. In our study we investigated by NMR the structural changes in DnaJB6 upon the LGMD1D related mutations, and their contributions on the aggregation suppression ability of DnaJB6 with their changes in HSP70 binding. We found that each of the mutations induced a structural change which influence the regulatory role of the fifth helix. These changes are correlated to the mutation proximity toward the fifth helix. We hope these findings will contribute for further understanding of the mechanism evolve the LGMD1 disease.

PO006

Atomic resolution insights into pH change induced deprotonation events in Aβ(1-42) amyloid fibrils

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Alzheimer's disease (AD) is associated with deposition of misfolded aggregates of Amyloid-β (Aβ). The Aβ(1-42) peptide is more aggregation prone and more neurotoxic than the two amino acids shorter peptide Aβ(1-40). High-resolution 3D structures of different polymorphic Aβ(1-42) fibril types have been determined by solid-state NMR spectroscopy as well as cryogenic electron microscopy (cryo-EM) in recent years.

The structure of Aβ(1-42) fibrils grown at acidic pH (1) differs remarkably from fibrils grown at neutral pH, in particular, the full N-terminus is part of the rigid fibril core and forms two extended β-strands (1). The aim of this study was to investigate the effect of a pH shift to neutral pH on the structure of these fibrils using solid-state NMR spectroscopy and molecular dynamics simulations.

We found that a pH shift up to pH 7 does not influence the global fold of the Aβ(1-42) fibrils, but some local changes, mainly for N-terminal residues as well as for the extreme C-terminus, are observed starting from pH 5 on a residue-specific level. In addition, the local conformations of some residues in salt bridges are affected by deprotonation at higher pH values, especially the salt bridge between Asp1-Lys28 and the adjacent Ala42. The change of the protonation state of some residues was observed on different timescales: fast exchange for Asp residues and slow exchange for the C-terminal carboxyl group of Ala42.

Literature:

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Posters

Solid-state NMR studies of YidC – a membrane insertase and chaperone

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E.coli YidC is an inner membrane protein that functions as a membrane insertase by binding the ribosomal nascent chains (RNCs) and facilitating their incorporation into the lipid bilayer. Also functions as a membrane chaperone by assisting in co-translational membrane folding and therefore plays a significant role in cell viability1. The conserved membrane domain has a water-accessible region which allows the water from the cytoplasmic region to the center of the core membrane domain and moreover the penetration of water through the whole protein up to the periplasmic region2. Therefore, this water-accessible pathway may play an important role in the substrate insertion process. Apart from this, the insertion process of the substrates into the lipid bilayer remains unknown. To elucidate the insertion mechanism, we employed solid-state NMR (ssNMR) to obtain a deeper understanding of the structure and dynamics of YidC, as well as its interaction with substrates in its native environment.

In our contribution, we have purified E.coli YidC and reconstituted it into proteoliposomes. Moreover, we measured two-dimensional 13C- 13C correlations and 1H proton-detected experiments to investigate the structure of the free state of YidC using ShiftX and FANDAS-based predictions using a crystal structure as a reference3,4. Currently, we are preparing cellular envelope and proteoliposome samples (perdeuterated) to utilize 1H ssNMR at ultra-high magnetic fields where spectral resolution and sensitivity are maximized to shed light on the structure of YidC unbound form, especially in the water-accessible region.

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Posters

Studying GPCRs in native environments by combining specific pair labeling and solid-state NMR

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G-protein coupled receptors (GPCRs) are transmembrane proteins that transduce extracellular signals into physiological responses. Their large conformational diversity result in complex activation mechanisms and bias signaling pathways. Structure-based drug discovery has been used to identify new potential drug candidates for several GPCRs. However, existing high-resolution structural studies of GPCRs have them reconstituted in detergents and other membrane mimetics. It has been shown that the environment and post-translational modifications of GPCRs play a significant role in signal transduction.[1] Obtaining structural and dynamic information of GPCRs in their native state and environment will be critical in understanding the activation and conformational changes these receptors undergo.

Here we present the protocol for the overexpression and isolation of GPCRs from HEK293T cells, where up to 2 million copies of the receptor per cell can be recovered in the total membrane fraction. Isotopically labeled samples are generated by growing the cells in specific amino acid labeled media. To minimize ambiguity due to spectral crowding, we identified unique and sequential amino acid pairs to be selectively labeled with ^{13}C and ^{15}N isotopes so that heteronuclear signals arise solely from backbone ^{15}N^{13}CO correlations.[2] Background subtraction is completed by acquiring a spectrum of a sample which does not overexpress the GPCR of interest. From this we obtain residue specific dynamic data in the presence and absence of different ligands in a native membrane environment.

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Posters

Proline cis/trans Isomerization in Intrinsically Disordered Proteins

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Intrinsically disordered proteins and protein regions (IDPs/IDRs) - despite the lack of a stable secondary structure - play important roles in diverse biological processes. One of their characteristics is the abundance in proline residues. In this case both cis and trans proline isomers can be present in solution, and this represents a contributing factor to the conformational heterogeneity. Moreover, the different isomers may have different biological roles.

Atomic level characterization of these isomer forms can be challenging under physiological conditions and/or in case of many prolines, or polyproline motifs.

Using as a model the disordered transactivation domain of tumorsuppressor p53 (1-60) we developed novel and improved 1Hα-detected methods that help the spectral assignment [1,2, 3]. We introduced two 1Hα-detected, proline selective, real-time homodecoupled NMR experiments that unambiguously show the isomer form. Based on this we analyzed all detectable proline major and minor forms of p53 (1-60). The measurements are sensitive enough to identify minor conformers present in 4-15% amounts.

Further on, we tested the influence of PTMs, and we showed the consequences of CK2 phosphorylation on the cis/trans-proline equilibrium.

Based on the available experimental data from the literature we performed a statistical analysis on how the amino acid type effects the cis/trans-proline distribution. [4]

The presented methods are applicable under physiological conditions, and they can contribute to find key proline isomers in proteins and statistical analysis results may help in amino acid sequence optimization for biotechnological purposes.

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PO010

Understanding the Structural Basis of Protein Splicing Mechanism using Solution NMR Spectroscopy and MD Simulations

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Inteins (intervening proteins) are enzymes that catalyse peptide bond formation between external proteins (exteins) and their own excision from a precursor protein. More than 600 intein genes have been reported, but only a few have been thoroughly characterized. Understanding the structural basis of their catalysis is important to engineer intein enzymes with novel applications.

We solved the solution NMR structure (PDB code: 7CFV) of a 136-residue DnaX mini-intein, derived from the cyanobacterium Spirulina platensis. In the structure 13 β-strands, one 310-helix, and one α-helix are arranged in a symmetric horseshoe-shape, typically found in the HINT (Hedgehog/INTein) domain superfamily. The NMR structural ensemble has a backbone RMSD of 0.27 Å and heavy atom RMSD of 0.52 Å for all ordered residues. The Spl DnaX mini-intein has a very stable core as determined by NMR-based hydrogen exchange experiments. Backbone 15N-dynamics showed the presence of conserved motions in symmetric positions in the intein structure, which is most likely a result of the symmetrical structure of the protein. Western blot based in vivo enzymatic assay showed Spl DnaX mini-intein to be one of the fastest inteins (Boral, S. et al, Biochemistry, 2020, https://pubs.acs.org/doi/10.1021/acs.biochem.0c00828).

To understand the structural basis of catalysis, we have performed molecular dynamics simulation for a duration of 1.5μs. Networks of residues, which most likely facilitate catalysis, were identified from the MD simulation. The functional roles of these residues have been deciphered by site-directed mutagenesis followed by enzymatic assay of the mutants. Overall, this study showed that the Spl DnaX mini-intein is a robust, highly stable protein and one of the fastest intein enzymes characterized till date and identification of residues facilitating catalysis will enable us to engineer this enzyme for novel applications.

PO011

Heterologous interaction characterization of Hepatitis B Virus core protein by NMR

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Chronic Hepatitis B Virus (HBV) is still a major global health issue. HBV is an enveloped virus enclosing a partially double-stranded DNA, and the core protein forming the capsid is one of the main focus of research to understand the interaction process of the virus. This protein is composed of 183 amino acids and assembles into dimers, which then autoassemble into an icosahedral capsid formed by 120 dimers.

Multiple binding sites have been highlighted in the Cp structure, including the interdimer interface and the spike tip. In addition, we have recently identified using solid-state NMR that Triton X-100, a detergent used in Cp purification, binds in the hydrophobic pocket and induces a conformational switch of the capsid [1]. We now here report details on the binding mode of Triton-X-100 and homologs thereof. To investigate the chemical moieties which are central to the binding event, we have tested interactions of different modified versions of Triton X-100, by means of commercial and specially designed chemical components. The screening analysis was performed using solution NMR of the truncated Cp dimer (Cp149) which allowed to speed up analysis of the large number of compounds compared to solid-state NMR of reassembled capsids. We selected peaks from eight amino acids located near the entry of the pocket to establish a measure for the binding affinity, using Chemical Shift Perturbations (CSPs).

Thanks to NMR screening, the obtained information on the binding modes of the molecules to the pocket can guide the design of antivirals which target hydrophobic pocket interactants.

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NMR studies of Dengue virus capsid protein and its interaction with RNA

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Dengue virus (DENV) is the most prevalent mosquito-borne viral pathogen affecting humans. The structure of dengue viral particles has been determined by cryo-electron microscopy. This revealed in detail the arrangement of the viral surface proteins, E and M, but left the ribonucleoproteins (RNPs), consisting of capsid (DENC) proteins and viral RNA, largely obscure. This suggested an only partially ordered RNP, despite the well-defined dimeric structure of the folded part of DENC previously elucidated. In this context, we decided to investigate this elusive protein-RNA complex using a combination of solution and solid-state NMR. We show that in vitro reconstituted RNPs appear indeed disordered under the electron microscope, but surprisingly solid-state NMR spectra display narrow lines, indicative for high local order. This allowed us to investigate the conformational changes on transition from DENC dimer to RNP. Solution-state NMR titration of the DENV C dimer with RNA allowed us to monitor chemical shift perturbations as a function of RNA quantity. In addition, we compared, by solid-state NMR, the RNPs formed with the viral RNA to RNPs formed with a short DNA used as a vaccine candidate[1]. Taken together, these data surprisingly suggest a novel interaction site of DENC with nucleic acids, different from the site which had been predicted. Our work is the first structural assessment of the DENV ribonucleoprotein complex, and shall allow to further evaluate vaccine candidates based on the DENC protein.

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PO013

Mechanism of tau R3 aggregation and inhibition revealed by NMR-based chemical kinetics

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Protein misfolding diseases are becoming increasingly prevalent with the increase in life expectancy and lifestyle changes over the last century, and constitute a health challenge at a global level. Macroscopic protein deposits of amyloids are associated with diseases such as Alzheimer's (tau and β-amyloid) and Parkinson's (α-synuclein). The mechanisms of protein aggregation are poorly understood, and such knowledge would enable the rational design of therapeutics for these diseases. Several key chemical processes have been proposed; chemical kinetics approaches can be applied to test such models, but are limited by the information content and reproducibility of the data. A recently developed nuclear magnetic resonance spectroscopy (NMR) assay is the basis for a numerical integration method to quantify the kinetics of aggregation [1]. This is a label-free method that can account explicitly for the effects of small molecules and molecular chaperones. The work presented here extends this method by automating the model building, selection and testing routine, and provides a mechanistic model for the aggregation of the biomedically relevant R3 fragment of tau and its inhibition by a non-natural amino-acid compound [2].

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A litmus test for classification of recognition mechanisms of transiently binding proteins

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Partner recognition in protein binding is critical for all biological functions, and yet, delineating its mechanism is challenging, especially when recognition happens within microseconds [1]. We present a novel theoretical and experimental framework to investigate protein binding mechanisms on sub-millisecond timescales beyond the reach of standard rapid-mixing experiments [2]. We base the novel experimental framework on straight-forward kinetic measurements using NMR relaxation dispersion in the fast-exchange regime of conformational transitions on microsecond timescales. Recent technological advances have expanded the kinetic range of these experiments from approximately 50 microseconds to single-digit microseconds [3]. We chose ubiquitin and SH3c from an adapter protein CIN85 [4] since the structure of the ubiquitin/SH3c complex [5] and the free ensemble of ubiquitin had been thoroughly characterized [6]. The novel framework predicts that conformational selection prevails kinetically on ubiquitin's paradigmatic interaction with this SH3c domain. By contrast, the SH3c domain recognizes ubiquitin in a two-state binding process. We then reveal with molecular dynamics simulations and Markov state modeling [7, 8] that the residues of the C-terminus of ubiquitin engage in the conformational selection mechanism by converting from a mixture of extended and closed conformations in the free form to an extended conformation prior to binding. The novel framework is robust and expandable for implementation in other binding scenarios with the potential to show that conformational selection might be the design principle of the hubs of interaction networks.

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PO015

A NMR look at an engineered PET depolymerase

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Plastic environmental pollution is a major issue that our generation has to face to protect our Planet. Plastic recycling not only has the potential to reduce the pollution but also to limit the fossil fuel-based production of new plastics. Enzymes able to break down plastic thereby might sustain such a circular economy. Poly-ethylene terephthalate (PET) degrading enzymes, have recently attracted considerable interest, and were subjected to enzyme engineering to improve their characteristics. A quadruple mutant of Leaf-branch Compost Cutinase (LCC) was identified as a most efficient and promising enzyme (Tournier et al., Nature 2020). Here, we use Nuclear Magnetic Resonance (NMR) to follow the initial LCC enzyme through its different mutations that lead to its improved performance. After assigning the backbone resonances of the LCC enzyme, we experimentally define the calcium binding sites and show their importance on the all-or-nothing thermal unfolding process. To push forward the characterization of this enzyme we assigned a subset of methyl groups as well as histidine side chain resonances. Using the combination of the various NMR signals, we probe their interaction with mono-(2-hydroxyethyl)terephthalic acid (MHET) as a analogue of PET. The latter experiments show that the higher efficacy of the quadruple mutant LCC-ICCG is related to an increased accessibility of the active site. This is also confirmed by the measurement of ms-ms time scale dynamics of the side chains of histidines in which we observed an increased dynamics of the catalytic histidine within the active site.Overall, our present NMR study provide an in-depth understanding of most efficient PET hydrolase and opens the way to investigate further its molecular interaction with a true plastic surface.

Posters

PO016

Screening novel mammalian expression systems and isotope labeling schemes for in-cell NMR studies.

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In-cell structural biology by NMR is appealing in many regards: It proposes, among others, to investigate conformational equilibria or ligand binding in very relevant conditions, i.e in cells[1].The approach has often been limited in its capacities i) by the many difficulties in sample production, and ii) by important signal losses due to promiscuous, transient interactions with cellular entities, which, in turn, urges to use (too) high concentrations of the studied proteins. Establishing simpler protocols and improved labeling schemes for enhancing S/N would help.

In an attempt to facilitate in-cell NMR studies along these two dimensions, we initiated a careful evaluation of protein production in a handful of mammalian cell lines (suspension and adherent cells) using either transient or permanent transfection and various fluorescent tags. We also quantified S/N for various amino acid specific ^{13}C- or novel ^{2}D^{1}ÐN-labeling schemes, starting with cell-free expression for the cleanest possible evaluation, then upon supplementation in mammalian cell culture medium. Here, we report the early results of this program applied to one disordered and one folded protein (α-synuclein and the kinase p38α).

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PO018

Long-range contacts in biomolecular complexes serially enhanced by cross relaxation and rotational resonance under MAS-DNP

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Probing of long-range homonuclear contacts across subunit interfaces by MAS NMR provides important information about structure and function in large biomolecular complexes. However, these contacts also present a complex challenge not only due to the required isotopic labeling schemes but also due to the NMR methods necessary to detect their signals with sufficient sensitivity. Dynamic nuclear polarization (DNP) can significantly improve the latter, and enhancement factor on the order of 100 may allow for experiments otherwise not feasible. At the same time, the design of methods for guiding the enhanced polarization towards the nuclei of interest open the possibility for highly selective detection of particular contacts in sparsely isotope-labeled biomolecular samples.

In this presentation we will introduce the combined use of specific cross relaxation enhancement by active motions under DNP (SCREAM-DNP) with subsequent rotational resonance (R²) homonuclear recoupling in order to selectively probe contacts between the L7Ae protein subunit and the 26mer guide RNA of an archaeal Box C/D RNA–protein complex. By combining DNP-supporting sample preparation, specific ILV-^{13}C labeling of methyl groups within the protein, nucleotide-specific ^{13}C labeling of RNA, and optimized deuteration schemes we can show that a highly efficient polarization transfer is possible. It starts from the DNP polarizing agent, spreads through the proton network, and then spontaneously propagates to the ^{13}C of sparsely introduced methyl groups of the protein by heteronuclear cross relaxation. From here, homonuclear spin diffusion to ^{13}C nuclei on the RNA can be controlled by specific recoupling due to MAS frequency-dependent rotational resonance conditions. All these steps are extremely robust and require no radio frequency fields for homonuclear recoupling or heteronuclear decoupling and provide 1D spectra of homonuclear contacts in short time. We also compare our findings with multi-dimensional correlation spectroscopy both under DNP and with normal MAS NMR under optimized conditions.

PO019

Circularized MSP nanodiscs show improved biophysical properties that enable NMR studies of challenging membrane proteins

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Choosing an appropriate membrane mimetic is essential for structural and functional studies of membrane proteins. A promising lipid-based membrane mimetic are phospholipid nanodiscs, where two copies of a so-called membrane scaffold protein (MSP) wrap around a patch of lipid bilayer. The size of a nanodisc is determined by the length of the MSP, enabling the design of smaller nanodiscs that are suitable for solution-state NMR. As recently reported, covalent MSP circularization further improves nanodisc stability and homogeneity. However, a detailed biophysical analysis comparing linear and circularized MSP nanodiscs has not been reported so far. Here, we compare the membrane fluidity and temperature-dependent size variability of circularized and linear nanodiscs of different sizes using a diverse set of biophysical methods. We show that the membrane fluidity is altered in nanodiscs compared to liposomes, whereas MSP circularization does not have a marked effect. Importantly, in contrast to larger linear MSP nanodiscs, the size and shape of circularized nanodiscs do not change at elevated temperatures above the lipid phase transition temperature. In line with these findings, we could demonstrate that the use of circularized nanodiscs is an important tool to facilitate high-resolution NMR analyses of membrane proteins. Despite their larger size as compared to linear nanodiscs, 2D and 3D NMR experiments of the voltage-dependent anion channel (VDAC1) in circularized nanodiscs show improved spectral quality at temperatures of up to 50 °C. Taken together, this study provides a better understanding of the biophysical properties of (circularized) lipid nanodiscs at elevated temperatures, which facilitates high-resolution solution-state NMR studies of larger and more challenging membrane protein systems in a native lipid environment.

PO020

The unexpected mode of action of the antibiotic plectasin

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Antibiotic resistance is a global health crisis. To combat this threat, antibiotics that use novel mechanisms of action are urgently needed. The natural product plectasin is one such antibiotics (1,2).

Plectasin and its triple mutant NZ2114 (3) show excellent antibacterial activity, including against clinically relevant strains such as MRSA or M. tuberculosis. Plectasin targets the membrane-embedded precursor Lipid II, thereby inhibiting peptidoglycan synthesis. However, the mode of action of plectasin remains unclear, mainly because studying the structure of the plectasin-Lipid II complex in its native membrane environment is challenging.

Using state-of-the-art solid-state NMR (4,5) including hyperpolarization techniques (DNP), we present the unexpected mode of action of plectasin in membranes at high resolution. We demonstrate that the mode of action in membranes drastically differs from the published one in micelles (2). In particular, we show that plectasin forms micron-sized oligomers upon binding to its target Lipid II, which explains the potent post-antibiotic effect of plectasin. Furthermore, we observe that these clusters severely disrupt and form holes in the membrane using high-speed atomic force microscopy. Together, these findings carve out the path towards the rational improvement of these peptides towards their clinical application.

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PO021

Hsp40s play complementary roles in the prevention of tau amyloid formation

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The microtubule-associated protein tau is the major subunit of neurofibrillary tangles associated with neurodegenerative diseases. Tau is an inert, disordered protein in solution, and upon various factors can aggregate and form toxic fibers. Its aggregation can be prevented by molecular chaperones. While numerous chaperones are known to interact with tau, little is known regarding the mechanisms by which these prevent tau aggregation. Here, we study two ATP-independent HSP40 chaperones in comparison to the characterized HSPB1, we describe their effects of on tau amyloid-fiber formation, and show that the three chaperones play complementary roles. Using HSQC NMR, we could characterize the exact binding sites on tau and on the chaperones. We found that although the binding sites in tau are identical in interaction with each chaperone, each chaperone recognizes different tau species. Whereas HSPB1 binds only tau monomers, DNAJB1 and DNAJA2 recognize aggregation-prone conformers and even mature fibers. Using 13CH3 - ILVM labelling, we saw that both Hsp40s bind fibrillar seeds and mature fibers via their C-terminal domain II (CTDII), and DNAJA2 is further capable of recognizing tau monomers by a second, distinct site in CTDI. We used a disease-derived tau mutant and by measuring RDCs we characterized the extended conformation the aggregation-prone and the disease mutant tau adopt vs the more compact inert tau. This can explain what structural element exactly the Hsp40 chaperones recognize. These results lay out the mechanisms by which the diverse members of the Hsp40 family counteract the formation and propagation of toxic tau aggregates, and highlight the fact that chaperones from different families play distinct, yet complementary roles in preventing pathological protein aggregation.

PO022

Semi-automatic tool for backbone assignment of large proteins using their pdb structure model.

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Assignment of the protein backbone is a prerequisite for structure and dynamics studies. Efficient automatic tools have been developed over the years, such as flya [1] and autoassign [2], but manual inspection of triple resonance experiments is often still required to perform the assignment as completely as possible.

We took advantage of the well-designed python model developed in the ccpnmr version3 software [3] to write a module that simultaneously combines structure-based chemical shift predictions obtained by the shiftx2 program [4] and inter-residue connections which allows most of the spin systems to be directly assigned in the protein sequence.

The application of this approach was successfully performed on several proteins up to a 50 kDa fragment antigen binding protein.

This tool can be applied to solid-state and liquid-state projects.

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Design of a glutamine-based single a-helix scaffold to target globular proteins

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Polyglutamine (polyQ) expansion beyond a pathological threshold is associated with a number of neurodegenerative diseases. The polyQ tract of several proteins, such as androgen receptor (AR)[1], huntingtin[2] and CBP[3], can adopt an α-helix structure, in which the helical propensity is influenced by the flanking regions and the polyQ length.

We have recently reported that the α-helix formed by the polyQ tract of AR is stabilized by unusual bifurcated hydrogen bonds where the side and main chains of glutamine residues simultaneously donate a hydrogen to the backbone carbonyl of residue i-4.[1]

Using a combination of solution NMR and molecular dynamics we have studied in detail how the sequence context influences the helical content of the polyQ tract of AR and expanded the analysis to the tract of the TBP protein. Finally, we have exploited our observations to present rules to design peptides that fold into short single α-helices by concatenating glutamine side chain to main chain hydrogen bonds. The resulting peptides are uncharged, contain only natural amino acids, and their sequences can be optimised to interact with specific targets. An important feature of these peptides is their versatility: several residues can act as efficient H-bond acceptors and the peptides can also incorporate a pH-sensitive switch or salt bridges to further stabilize helicity. As a proof of concept, we have designed two peptides to bind the globular target RAP74-CTD. Remarkably, we show several examples of natural occurring sequences combining such strategies that may represent a new class of uncharged single α-helices (SAH) that may have remained undetected.

PO024

Structural Influence of Pyroglutamylation in an Amyloid β(3-42) Fibril Polymorph probed by solid-state NMR

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Extracellular Amyloid β (Aβ) plaques primarily formed by Aβ(1-40) and Aβ(1-42) fibrils are a hallmark of Alzheimer's disease. Aβ can undergo a high variety of different post-translational modifications including the formation of pyroglutamate at position E3 or E11. Pyroglutamate modified (pE) Aβ(3 42) has been shown to have a higher aggregation rate, an increased hydrophobicity and a higher toxicity in vivo compared to Aβ(1-42).

In this study, we produced [U-^{13}C, ^{1}ÐN]- labeled pE-Aβ(3 42) fibrils at low pH and investigated structural changes between pE-Aβ(3 42) fibrils and Aβ(1-42) fibrils[1] grown under identical conditions using solid-state NMR spectroscopy. We could assign 36 of 40 residues of the pE-Aβ(3 42) fibril polymorph. Chemical shift comparison showed a conserved turn region between Phe19 and Ile31. Rotational resonance based experiments proved a through space contact between a ^{13}C of the aromatic ring of Phe19 and the ^{13}Cδ of Ile31 in both pE-Aβ(3 42) and Aβ(1-42).

Interestingly, not only the N-Terminus but also the C-Terminus of the fibril shows significant differences in the chemical shifts, indicating major structural differences in the C-terminal part of the fibril, which belongs to the hydrophobic core of the homodimer in the Aβ(1-42) fibril polymorph[1]. These findings suggest that the pyroglutamylation at the N-terminus influences the fibril structure notably.

A comparison of the chemical shifts obtained in this study to those of other Aβ(1-42) fibril polymorphs showed no significant similarities. Additionally, we could show that these pE-Aβ(3 42) fibrils are resistant towards a pH shift.

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Posters

PO025

Combining high-field solution and solid-State NMR to study membrane protein aggregation: Application to phospholamban

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Phospholamban (PLN) is a 6 kDa membrane protein that interacts with Sarco/endoplasmic Reticulum Ca2+ ATPase (SERCA) to regulate cardiac muscle contractility by governing the transport of calcium ions in the SER, to and from the cytosol.[1]. PLN is believed to exist in an equilibrium between monomeric and pentameric forms. Previously, both solution- and solid-state NMR have been used to study this equilibrium [2,3] and the binding of PLN to SERCA in lipid bilayers [4,5].

Importantly, a deleterious mutation at the arginine 14 position of PLN (PLNR14del) is known to cause cardiomyopathy. Histological analysis indicates that this mutant forms aggregates which leads to lethal cardiomyopathy. There is little to no insight in the aggregation mechanism of this mutant of PLN. Also, limited information has been obtained on the wild type PLNR14del mutant.

Here, we report on solution state NMR studies on the PLN R14del mutant at 900 MHz showing that the protein is well folded in micellar preparations. We also compare our findings to data obtained on WT PLN and report tentative backbone resonance assignments. Complementary to such studies, 1H detected solid-state NMR under Magic Angle Spinning (MAS) provides increasing possibilities to probe membrane protein structure, dynamics and complex formation in lipid bilayers. In our poster, we give an update on using such techniques to study the structure and dynamics of the PLN R14del mutant and other PLN variants in lipid bilayers. These results will provide the basis to understand the molecular mechanism behind the pathological aggregates of PLNR14del.

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PO026

Structural snapshots into the life cycle of filamentous phage viruses

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Filamentous phage infect bacterial cells that possess the F-pili organelle. They are one-micron long viruses containing a single-stranded DNA wrapped by several thousand copies of a mostly-helical coat protein at ratios of 1-2.5 nucleotides per coat protein. Infection occurs when a virus attaches to the pilus bacterial filament and the DNA is transferred into the cell. During replication, the non-structural gene V protein (gVp) attaches to a ssDNA creating a pre-mature phage and signaling the assembly of a new particle.

In recent years we managed to solve the atomic-resolution structures of two intact viruses, M13 (with solid-state NMR) and IKe (with cryoEM), and detect common structural assembly motifs. Dynamic properties studied by chemical-shift-anisotropy recoupling methods suggest a highly rigid helical coat with a mobile DNA interface.

The pre-mature virus made of ssDNA wrapped by gVp is of equivalent length however with very different properties. The coating protein gVp is composed mostly of beta strands and a high percentage of loop and disordered regimes, it is significantly more dynamic then the structural coat protein, and structure determination by solid-state NMR (to 1Å Cα RMSD) of the entire gVp-ssDNA complex shows that it undergoes a very large conformational change upon binding to the DNA, while losing several additional secondary structure motifs.

PO028

Universal lipid markers for early stage embryos and microtissues

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NMR is often referred as the golden standard for in-vivo studies of large organisms. Thanks to its unique resolving power and non-invasive nature, it is nowadays routinely applied to research and clinical investigations. These same investigations would be highly beneficial at the nanoliter scale (nL), typical of early development of mammalian embryos, organoids, and microtissues. Unfortunately, sensitivity and sample handling issues at such small scale (about 100 micrometers) prevented the adoption of NMR. Recently, our team has overcome these limitations with ultra-compact single-chip probes, where microchip transceivers and 3D micro-printing are combined. In this presentation we show that such probes have sufficient sensitivity to detect and resolve NMR signals from individual mammalian pre-implantation embryos and single 3D human microtissues. The signals are largely originating from lipid droplets. We further discuss how the spectral features can be used to determine biomarkers, posing the basis for a novel non-invasive NMR-based microscopic screening tool.

Posters

PO029

Phosphorylation as a molecular switch that controls measles nucleocapsid assembly initiation

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The genome of non-segmented negative-strand RNA viruses, such as measles, RSV, Rabies, Nipah, mumps, takes the form of helical capsids composed of RNA covered by multiple copies of Nucleoprotein (N) [1]. N can be chaperoned in an RNA-free form by the Phosphoprotein (P) [2]. The N-terminal part of P binds to the RNA binding domain of N and protects the surface which is involved in the interaction between protomers in the capsid [3]. The control of N interaction with RNA and the initiation of nucleocapsid assembly is an important check point in the viral lifecycle.

Using a combination of NMR, fluorescence spectroscopy and electron microscopy we show for the first time that phosphorylation of measles P protein plays a role of a molecular switch: Phosphorylation changes the affinity of chaperoning between measles N and P and triggers RNA encapsidation. The mechanism of how P phosphorylation controls N and RNA interaction was studied from both sides of the interaction. Using 15N-labelled protein we studied the effect of phosphorylation on the dynamics of the disordered region of P. Using 13C Methyl-TROSY we could observe RNA-binding domain of N in complex with P and identify the effect of P phosphorylation.

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PO030

Interaction of a protozoan oxidoreductase with a parasite-specific low molecular weight reductant

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Trypanosomatids are protozoan parasites that cause many severe diseases. The WHO has classified African Sleeping Sickness (caused by T. brucei), Chagas (T. cruzi) and Leishmaniases (Leishmania spp.) as so-called "Neglected Tropical Diseases" that globally affect millions of people and their livestock. To facilitate the fight against these parasites, a detailed understanding of their essential enzymes as potential drug targets is needed. All trypanosomatids have a unique thiol metabolism centered on the oxidoreductase tryparedoxin and the low molecular weight thiol trypanothione, which consists of two glutathionyl-fragments linked by spermidine.

To understand the atomistic details of tryparedoxin redox activity and why tryparedoxin prefers trypanothione over glutathione as an electron donor, we looked at the interaction between T. brucei tryparedoxin with covalent inhibitors and trypanothione as well as trypanothione building blocks using NMR spectroscopy, MD simulations and functional assays. With the complete backbone assignments of the T. brucei enzyme in the oxidized and reduced states, it was possible to map structural changes connected to redox switching and trypanothione binding. Interestingly, the lifetime of the disulfide-linked tryparedoxin-trypanothione complexes extends the expectations based on functional assays.

For biochemical analysis, we reconstituted the entire T. brucei redox cascade composed of trypanothione reductase, trypanothione, tryparedoxin and a peroxidase in a cuvette-based assay. This allowed to screen tryparedoxin point mutations regarding redox activity and to identify residues of importance for trypanothione binding. In the next step, we aim to use the information from our chemical shift perturbation and functional assays to generate a detailed, atomistic in silico model of the complexes using computational methods like Docking, Molecular Dynamics (MD) and combined Quantum Chemistry and Molecular Modeling (QM/MM).

Posters

Order in disorder: AUX/IAA protein and its TIR1-Aux/IAA auxin co-receptor system

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Auxin is a central signalling molecule in plant biology with roles in both the patterning of developmental events and the regulation of cellular growth. This is achieved via the TIR1/AFB-auxin-Aux/IAA co-receptor complex. Within this ternary complex, auxin acts as a molecular glue to promote binding of Aux/IAA transcriptional repressor proteins to SCFTIR1/AFB ubiquitin-ligase complexes, thereby catalysing their ubiquitin-mediated proteolysis. We have used a combination of NMR and Molecular Dynamics simulations to gain insight into the solution structure of the amino-terminal half of the Aux/IAA-protein AXR3/IAA17 and its binding in complex with TIR1 and auxin. We show that while the protein presents as intrinsically disordered by NMR, still the critical degron W-P bond occurs with an unusually high (1:1) ratio of cis to trans isomers. Analysis of RDC's confirm a deviation of random coil structure both in the degron motif and near the N-terminus, where a transient helix is formed. Molecular dynamics simulations give a view of the protein populating fluctuating secondary structure elements that associate into transiently structured motifs and imply a richness to the structural ensemble containing higher order structural elements that vary in nature. We then show that assembly of the co-receptor complex involves both auxin-dependent and -independent interaction events. Our results suggests that the complex regulation of auxin dependent events is mirrored in the complex behaviour of the intrinsically disordered Aux/IAA-proteins that are central to the signalling cascade.

PO032

Allosteric communication in tryptophan synthase studied by ssNMR

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The tryptophan synthase bienzyme complex ($\alpha\beta\beta\alpha$, or TrpS) is an allosteric enzyme catalyzing the last two steps in the biosynthesis of the L-tryptophan in bacteria, plants, and fungi. The α-subunit catalyzes the aldolytic cleavage of indole-3-glycerol-phosphate to glyceraldehyde-3-phosphate and indole. The β-subunit (TrpB) is activated by the α-subunit (TrpA) and then catalyzes the condensation between indole and L-serine to yield L-tryptophan. The two reactions are kept in phase by allosteric interactions between the two subunits. This allosteric communication is mediated by the discrete networks of residues that are involved in signal propagation upon effector binding. Existence of this allosteric network also opens up the opportunities of allosteric drug development.

Due to the size limit for solution NMR, the β-subunit has reminded poorly characterized. Our goal is to study β-subunit dynamics in isolation, and its activation by α-subunit using solid-state NMR. To do so, we have expressed, purified, and recorded 2D-HSQC and 2D-TROSY spectra of a wild type TrpB from Pyrococcus furiosus, as well as standalone TrpB variant TrpB(2B9). TrpB(2B9) contains seven mutations that mimic allosteric activation by TrpA, thus making this enzyme self-sufficient. Here, we aim to assign the backbone chemical shifts of the wild type protein and the standalone mutant, and compare their dynamics on the microsecond timescale via solid-state NMR relaxation dispersion methods. This will inform on the effects of the intermolecular interactions on the site-specific dynamics and hence shed light on the underlying allostery.

PO033

Molecular basis of GOF missense mutations of NSDs

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The nuclear receptor-binding SET domain-containing NSD family comprises selective H3K36 dimethyltransferases. The upregulation and recurrent hyperactive missense mutations of NSD enzymes are implicated in oncogenesis and chromatin regulation. Our studies demonstrated differences in functional dynamics between wild-type NSDs and their oncogenic hyperactive variants. NSD3SET (T1232A) showed the widespread mobility changes in the catalytic domain and regulatory loop. In the case of the E1099K mutation of the NSD2SET, activation of slow segmental motions across the SET domain leads to higher turnover rates of the SAM cofactor, causing increased H3K36me2 mark leading to oncogenesis. Identified structural dynamics landscape sheds light on the missense mutation-driven hyperactivity origins paving the way to target NSDs selectively [1].

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PO034

Investigating the natural conformation of a coiled-coil calcium sensor protein in solution by NMR

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The activation of the calcium released activated calcium (CRAC) channel is mediated by the calcium sensor protein, stromal interaction molecule 1 (STIM1) [1]. When activated, STIM1 spans from the endoplasmic reticulum (ER) to the CRAC channel located in the plasma membrane. Depletion of the calcium stored in the ER activates STIM1, resulting in homo-oligomerization and elongation of its cytosolic part. The transition between the resting (closed) and the active (open) conformation is modulated by changes in intra- and intermolecular interactions of the three cytosolic coiled-coil domains of STIM1 (CC1, CC2 and CC3) [2]. To get a better understanding of this large conformational change, it is necessary to study the protein in its most natural form and to identify the interactions between the coiled-coil domains in the resting state.

During our research, a solution-state NMR 3D structure of the monomeric CC1 in its wildtype form was determined and compared to the R304W Stormorken syndrom mutation, which showed weakened coiled coil interactions [3]. Additionally, we could identify mutations in CC3, that destroy coiled-coil contacts and alter backbone dynamics. A resonance assignment of a selected mutant with potential physiological importance is currently in progress, which is essential for understanding the activation mechanism.

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PO035

Exploring the Photocycle Intermediates of a Cyanobacteriochrome by MAS NMR Spectroscopy at Room Temperature

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Cyanobacteriochromes (CBCRs), which constitute a novel subgroup of phytochrome photoreceptors, are composed of an array of GAF domains bearing a covalently bound, linear tetrapyrrole chromophore. Characteristically, a single CBCR-GAF domain can undergo the complete photocycle, consisting of intermediate states that can cover the entire visible spectral range in absorption.[1] Their lifetimes range from a few ns to ms at room temperature.[2,3] In the past decades, the photocycle intermediate states of various phytochrome representatives have been extensively studied by either time-resolved spectroscopy[2,3] or vibrational[4] spectroscopy by thermal trapping. To date, however, it has been challenging to freeze-trap and characterize the intermediate states, especially those of the forward reaction (dark state→photoproduct), of any phytochrome within the NMR magnet.[5]

In our recent study[6], the complete photocycle of the CBCR Slr1393g3 from Synechocystis 6803, which exhibits a red-absorbing Pr dark state and a green-absorbing Pg photoproduct, has been characterized by UV/Vis and MAS NMR spectroscopy techniques, applying ^{13}C-5-aminolevulinic acid labeling scheme of the in vivo assembled chromophore. By embedding the protein in an amorphous glassy sugar matrix[7] in total four intermediate states could be trapped: Lumi-R and Meta-R of the forward reaction Pr→Pg as well as Lumi-G and Meta-G of the reverse reaction Pg→Pr. Based on a series of ^{13}C-^{13}C DARR and ^{13}C SUPER experiments as well as on quantum chemical calculations a detailed mechanism of the photoconversion from Pr→Pg and vice versa will be presented.

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Posters

Investigating gene transcription modulators inside mitochondrial genes

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A mitochondrion is a semi-autonomous double-membrane organelle found in most eukaryotic organisms, including humans. The mitochondrion is responsible for various functions of a properly operating cell. Numerous connections have been discovered between mitochondria and crippling human diseases, i.e., cardiac dysfunction, heart failure, or autism.

The mitochondrial genome encodes proteins involved in the oxidative phosphorylation system. The transcription of the mtDNA strands results in the single polycistronic transcript, which is subsequently processed by RNases excising the tRNA sequences.[1] However, the process is not fully understood. There are multiple unanswered questions: How do mitochondria manage to translate their protein-coding genes whereas only one set of tRNAs is produced in each transcription cycle? How do mitochondria accumulate enough tRNAs for translation?[2]

One feasible explanation would be that mitochondrial genomes undergo insufficient transcription cycles. They generate tRNAs through a yet-unknown mechanism for the selective transcription of mitochondrial tRNA genes. Regulation of suchlike selective transcription could be controlled by the environmentally promoted stabilization of non-canonical DNA/RNA structures called G-quadruplexes.[3] DNP enhanced solid-state NMR spectroscopy is a unique tool capable of non-invasively probing live mitochondria extracted from cells.[4] We report on recent progress in employing DNP to prove the hypothesis of G-quadruplex structures existing in mitochondrial genomic DNA/RNA, as well as the presence of a direct link between G-quadruplex formation and regulation of (tRNA) genes by possible formation of a G-quadruplex-ligand complex.

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PO037

Assessing the applicability of 19F-TRP incorporation for 19F NMR measurements of protein dynamics

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The favorable properties of the fluorine nucleus make 19F NMR spectroscopy an attractive method to examine the structure, interaction and dynamics of biomolecules. Here, we address if 19F NMR methods to extract information on protein dynamics are of general applicability. First, we examine whether the incorporation of 19F labeled tryptophan residues changes motions in proteins, e.g. due to the differences in electronegativity and van der Waals radii of 1H and 19F nuclei. Second, we investigate whether 19F measurements are well suited to report on global protein motions and whether the obtained results are in agreement with 15N derived exchange parameters.

For our studies we used three different proteins: the Dcp1:Dcp2 mRNA decapping complex, the KIX domain of the transcriptional coactivator CREB binding protein and the DcpS scavenger decapping enzyme. These proteins all contain at least one tryptophan residue in a region that has been shown to undergo dynamics with rates between ~ 20 s-1 to ~ 2800 s-1.

We found that fluor-tryptophan incorporation into these proteins was efficient in all cases. Importantly, the CPMG relaxation dispersion (RD) derived protein dynamics were not affected by the 19F atom, as we could show through 15N relaxation measurements. However, for the Dcp1:Dcp2 complex and the KIX domain the exchange rates obtained from 19F RD measurements are much faster than those obtained from 15N RD experiments. The fluor-tryptophan ring thus does not only report on the global conformational changes of these proteins, but also on fast motions of the indole ring. For the DcpS decapping enzyme, in contrast, we could extract dynamics from 19F longitudinal exchange experiments which are in excellent agreement with data that was obtained from methyl TROSY relaxation experiments.

Our results thus highlight potential pitfalls in drawing conclusions regarding global protein dynamics based on 19F relaxation data only.

Posters

PO038

Modulation of c-Src intramolecular fuzzy complex by phosphorylation. A multinuclear NMR approach

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Since its discovery as the first proto-oncogene, Src kinase attracted much attention not only in cancer research but also in structural biology. Early studies focused on the globular SH1, SH2 and SH3 domains but overlooked the long disordered N-terminal regulatory domain containing the SH4 and the Unique domains. Our group demonstrated the functional importance of the Unique domain (1) and characterized an intramolecular fuzzy complex involving the disordered region and the SH3 domain (2).

As disordered regions are prone to post-translational modifications and hence these may acquire novel functions, we have focused on phosphorylation of a human Src construct (SH4, unique and SH3 regions, USH3 for short) by Erk2. Proteomic analysis identified unexpected phosphorylation sites that do not follow the known sequence specificity of Erk2. 31P-NMR has been used to study the kinetics of the various sites in the entire construct as well as in shorter peptides including the target sequence. This analysis reveals that the non-standard sites are only phosphorylated in the entire protein, thus indicating allosteric recognition.

The complete assignment of the NMR spectra of phosphorylated and non-phosphorylated USH3 reveals widespread changes suggesting a significant modification of the fuzzy complex. In-depth analysis has revealed the presence of a number of cis-proline bonds. We have also focused on the participation of arginine side chains using HISQC (3). Structural and functional consequences of changes related to these phosphorylation events are underway.

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PO039

Towards the design of inhibitors against macrolide resistance: Solution and solid-state NMR studies of the ErmB-RNA complex

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Macrolide antibiotics (such as erythromycin) kill bacteria by targeting the peptide exit tunnel of the bacterial ribosome, thereby blocking protein translation.

Macrolide resistance is mainly conferred by the expression of a conserved family of enzymes named Erm (erythromycin resistance rRNA methyltransferases), which are common to a variety of pathogenic bacteria. Binding of macrolides is prevented by the methylation of a specific adenine base in the 23S rRNA (upon methyl transfer from SAM co-factors), during the prokaryotic ribosome assembly.

Structure-based drug design could be used for the rationale design of specific erm enzyme inhibitors, that would be able to combat macrolide resistance. However, to date, due to technical challenges that apparently prevent characterization by X-ray, solution NMR or cryo-EM, no structural data is available for the erm-RNA complex.

Here, we describe the characterization of the complex between a prevalent member of the erm family, ermB, in complex with the 32-mer RNA substrate, which represents the minimal motif capable to mimic the ribosomal RNA binding. Structural studies on this complex are severely complicated by the fast precipitation of the erm-RNA complex which rules out the conventional structural biology techniques.

We apply modern solution and solid state NMR techniques with the goal of characterize the apo-state and determine the erm-RNA complex interface at high-resolution, whose progresses will aid future structure-based drug development.

Posters

Introducing "Stablelabel" cell-free lysates for reduced NMR label conversion

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Cell-free (CF) synthesis is an efficient method to produce labeled proteins for NMR studies. While E. coli lysates are easy and cheap to produce, they also offer the advantage that many amino acid pathways are disrupted during extract processing. This leads to reduced label scrambling and better amino acid stability. Nevertheless, especially the 15N labels of L-aspartate, L-asparagine, L-glutamine, L-glutamate and L-alanine are converted at noticeable rates during protein synthesis. This impairs peak assignment and structural studies of proteins. Furthermore, this leads to dilution of the labels which can become a problem when working with challenging targets such as large complexes or integral membrane proteins. While the use of specific metabolic inhibitors to suppress these unwanted reactions could be an option, their availability and side effects on cell-free production efficiency may restrict their applications.

We therefore generated an E. coli A19 based derivative strain containing a tailored metabolic background that reduces or eliminates most prevalent conversions of NMR labels. Using the lambda phage recombination system for systematic and successive deletion of identified genes responsible for metabolic label conversions, we designed the E. coli A19 derivative "Stablelabel". Despite containing numerous knockout chromosomal mutations, cell-free lysates of Stablelabel are still efficient for protein production in two-compartment cell-free reactions. We provide a detailed protocol for cell-free lysate preparation and their use in cell-free reactions. We further present a comprehensive characterization of amino acid label stability in Stablelabel lysates documented by representative NMR spectra. In combination with cell-free expression and combinatorial labelling strategies, Stablelabel may become a valuable contribution to the NMR community as it allows selective labeling of proteins with reduced spectral complexity that is especially suitable to study protein dynamics or assignments.

PO041

Dynamics and interactions in the 410 kDa RNA exosome

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Solution-state NMR is uniquely suited to study interactions and dynamics of biomolecules in solution and at near atomic resolution. However, conventional solution-state NMR is limited to biomolecules smaller than ~40 kDa. A range of methodological advances has made it possible to extend this size-limit substantially. Here, we combine selective [19]F- and methyl labeling schemes with sensitivity-optimized NMR pulse sequences to study the essential eukaryotic RNA exosome. This complex consists of 10 distinct subunits that form a 410 kDa molecular machine that degrades and processes RNA in 3' to 5' direction. We show that for the exosome solution-state NMR methods can be employed

(i) to validate structural models,

(ii) to study weak or transient substrate interactions and

(iii) to investigate motions and localizations of dynamic regions.

Using this approach we demonstrate that large asymmetric complexes are amenable to a wealth of solution-state NMR techniques when recent methodological advances are combined.

Posters

PO042

Kinase mediated desensitization of GPCRs studied at atomic resolution by NMR spectroscopy

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G protein-coupled receptors (GPCRs) represent the largest family of signaling proteins and are targeted by one third of all clinically used drugs. To control the signaling process of GPCRs, cells have developed a regulated system of GPCR desensitization consisting of two major steps: (i) phosphorylation of the C-terminus and/or intracellular loops of the GPCR via specialized kinases (GRKs), (ii) the recruitment and binding of arrestins. The phosphorylation pattern, produced by GRKs, plays a crucial role in determining conformationally distinct active states of arrestins which cause a variety of cellular responses. Despite high-resolution structural data of different GRK family members, their structural features used to discrimi-nate between active and inactive receptor states and how the binding to agonist-bound re-ceptor increases their catalytic activity remain elusive. Also, the functional roles of the vary-ing phosphorylation sites are still unknown.

Within this project, we want to gain a deeper understanding of the GRK's structural regulation upon activation. To this end, selective $^{13}C\varepsilon$-methionine labeling of GRK1 and GRK2 was performed in insect cells utilizing the baculovirus system. By recording characteristic fingerprint spectra, we can determine distinct conformational changes of the kinase upon binding of physiological effectors (ATP, C-terminal peptides) in contrast to allosteric (anionic phospholipids) or competitive modulators (paroxetine, sangivamycin). The interactions are further characterized in detail by determining KD values by NMR titration experiments.

Additionally, phosphorylation events catalyzed by GRK1/2 were successfully observed on the isolated ^{15}N-labeled C-terminal tails of various GPCRs (hsβÐAR, hsβÐAR, bovine rhodopsin), demonstrating activity of the kinases. By using NMR spectroscopy, we can follow the kinase reaction in a time-resolved manner and at atomic resolution to determine varying phosphorylation patterns.

Unraveling details of the process of GPCR desensitization in turn may aid in the discovery of new therapeutic agents targeting specific GRK or arrestin functions.

PO043

Capturing structure and dynamics in pulmonary surfactant

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Membrane proteins, particularly lipid-trafficking proteins, provide unique opportunities for gaining mechanistic insights via traditional ssNMR approaches and MAS-DNP ssNMR spectroscopy. They also provide unique challenges for preserving native environments and structural conformers, capturing relevant dynamic phenomena, optimal radical incorporation, and sustaining nuclear spin coherences under DNP conditions. I will focus on our studies of membrane-active, amphipathic peptides important to lung function derived from pulmonary surfactant protein B that are sensitive to lipid composition, temperature, and pH. Conventional ssNMR approaches enable us to map lipid trafficking and polymorphisms in this parameter space. Optimization of radical incorporation under conditions that preserve sample integrity enables us to use MAS DNP NMR to capture major and minor structural conformers formed by a membrane-active peptide at physiologically relevant peptide concentrations and conditions. This enables us to test models of peptide conformers adapted as a function of pH and lipid composition that we developed based on measures of peptide partitioning and helicity at ambient temperatures using deuterium NMR, EPR, and circular dichroism. I will present strategies to ensure reproducible polarization enhancements and to maintain sample integrity together with ssNMR measurements demonstrating the utility of DNP for making high-resolution structural measurements and providing mechanistic insights.

PO044

Switching off a GPCR: Watching how GPCR kinases phosphorylate GPCRs at atomic resolution by NMR

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G protein-coupled receptor (GPCR) kinases (GRKs) play a key role in the desensitization of GPCRs and are therefore essential for a signaling pathway targeted by one third of all clinically applied drugs. By selective phosphorylation of the C-terminus and/or intracellular loops of activated GPCRs, they initiate the subsequent recruitment of arrestins to GPCRs. The resulting phosphorylation pattern differs significantly between distinct GRKs and is decisive for the arrestin-mediated cellular responses. Several phosphorylation 'barcodes' and their relation to different GRKs and GPCR-states have been identified, but site-specific kinetic data for the phosphorylation reaction and the role of individual phosphorylations remain elusive. The selective binding and activation of GRKs by agonist-bound GPCRs represents another area of further investigation.

Within this project, we want to deepen the understanding of the phosphorylation reaction catalyzed by GRKs, both with spatial and temporal resolution. To this end, we assigned the C-terminal tails of human βÐ- and βÐ-adrenergic receptors (hsβÐAR and hsβÐAR) as well as bovine rhodopsin and monitored their phosphorylation by human GRK2 and bovine GRK1. In our assays, GRKs, recombinantly expressed in insect cells, are active and produce multiple phosphorylations on their substrates. We then further investigated the influence of native and non-native ligands, including inhibitors, allosteric modulators and preparations of activated receptors, on the activity of GRKs in the phosphorylation reaction on different substrate peptides.

Our results on the exact phosphorylation patterns, their sequence and the influence of external factors will increase our understanding on the complex mechanism of GPCR desensitization.

PO045

Understanding the mechanism of overcoming drug resistance in Candida spp. via 'on cell' NMR approach

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Multidrug resistance in pathogenic microbes is one of the world greatest challenges. The pharmacotherapy of systemic and topical infections due to resistant strains involves enormous costs and usually demonstrates low efficacy. We have observed that colloidal drug delivery systems loaded with antifungal fluconazole may overcome resistance in clinical yeasts. Based on the RT-PCR results, the upregulation of the CDR1/CDR2 genes was detected in the examined Candida strains, meaning that the efflux of the drug from the fungal cell through membrane proteins ('efflux pumps') was the main mechanism of the resistance.

The aim of our study was to explain the mechanism of such an increased activity via monitoring of the molecular level changes in fungal wall/membrane via liquid and solid-state NMR techniques in 'on cell' NMR approach. The experiments were carried out on whole cells incubated in the medium enriched with ^{13}C without any further biotechnological modification to avoid structural changes of the wall or membrane of the microorganism. STD NMR measurements under HR-MAS conditions enabled to differentiate the strains according to the overexpression of the efflux pump based on the values of fractional STD response. In ^{1}H-^{13}C CP/MAS, ^{1}H-^{13}C CPSP/MAS, ^{13}C{^{1}H} MAS NMR spectra changes in the shape and chemical shift of the peaks were observed upon incubation of the fungal cells with the drug loaded into the micelles. Those were assigned to the wall components such as chitin, β-1,3-glucan, β-1,6-glucan, protein, and lipids. Changes in lineshape of ^{31}P spectra (^{1}H-^{31}P CP/MAS, and ^{31}P{^{1}H} MAS NMR) indicated modification of membrane phospholipids arrangement (e.g. derivatives of phosphatidylcholine and phosphatidylglycerol). Overall, we observed that colloidal formulations triggered modification of the microbial structures which might explain the increased activity of the formulation. Understanding the mechanism of nanomaterials' antimicrobial activity may contribute to the development of new medicines with improved safety and efficacy.

PO046

Intrinsically Disordered Tardigrade Proteins Self-Assemble into Fibrous Gels in Response to Environmental Stress

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Tardigrades are microscopic animals remarkable for their ability to survive extreme stress conditions as diverse as extreme pressure, temperature and desiccation. Despite centuries of interest, the molecular mechanisms behind this uncommon resistance remains unknown.

Tardigrade-unique intrinsically disordered proteins have been shown to play an essential role in tardigrade's survival to desiccation. We characterized the conformational and physical behaviour of one of those proteins, CAHS-8, from the tardigrade species Hypsibius exemplaris.

NMR spectroscopy reveals that the protein comprises an extended central α-helical domain flanked by disordered termini. Upon concentration, the protein was shown to successively form oligomers, fibrils and finally a reversible hydrogel constituted of the entangled mesh of fibers, in a strongly temperature-dependent manner. The central helical domain forms the core of the fibrillar structure, with the disordered termini remaining highly dynamic within the gel. Other proteins, either folded or intrinsically disordered, were encapsulated within cavities in the gel and were shown to retain their functional form. The ability to reversibly form fibrous gels and entrap protein inside may be associated with the enhanced protective properties of these tardigrade-unique proteins.

PO047

Uncovering dynamics and an allosteric response in an NRPS cyclization domain

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NMR provides a means to study biomolecules wherein dynamics and biochemical transformations are probed at the atomic level. Frequently, crystallography captures critical structural states, but fleeting interactions and lowly populated states may be largely unnoticed thus limiting mechanistic interpretations. Nonribosomal peptide synthetases (NRPSs) are enzymatic systems that fall in this category. NRPSs use multiple domains to assemble complex therapeutics from simple, organic substrates. The engineering of these mega enzymes has been limited due to heterogeneous and dynamic domain-domain and domain-substrate interactions. Amidst NRPS domains, condensation or cyclization domains link together two substrates each tethered to partner carrier protein domains. Determining the molecular underpinnings of tandem domain and substrate recognition may provide new means to engineer NRPSs to incorporate custom substrates.

Despite many structural studies, how condensation and cyclization domains sense the presence of their carrier protein partners and their attached substrates has challenged the field. We determined the solution structure of the 52 kDa cyclization domain Cy1 from yersiniabactin synthetase and identified global dynamics across the domain using relaxation dispersion NMR. These dynamic residues spanned from one partner carrier protein binding site to another suggestive of a communication network. Using a combination of mutagenesis and an in-situ NMR assay, we demonstrated that the dynamics of Cy1 facilitate an allosteric response towards one of its carrier protein partners only when loaded with a substrate. The results point at a dynamically gated substrate recognition mechanism paired with an allosteric response, and they indicate that dynamics within these domains must be retained in artificial systems to preserve functional tandem domain and substrate communication.

Posters

PO048

Exploring the pH-sensing mechanism of the light-stress regulator protein PsbS and interaction with partner proteins

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During photosynthesis light energy is converted to chemical energy. However, at excess light, photo-oxidative damage can occur in the plant. To prevent damage the plant dissipates excess energy as heat in a process called non-photochemical quenching (NPQ). In higher plants the pH-sensing protein Photosystem II subunit S (PsbS) protein is a key player in sensing light stress by changes in luminal pH. This change is sensed by two glutamates (E69 and E173) that induce conformational changes upon protonation. Understanding of the molecular mechanism of PsbS will create new possibilities for optimization of biomass production via adjustment of NPQ.

The plan for this project is to look at the conformational changes of PsbS in response to pH and its interaction with partner proteins in a membrane. Measurements will be executed with help of 13C, 15N MAS NMR in combination with isotopic labeling of the key residues. In addition interaction of PsbS with partner proteins in a membrane will be studied via co-reconstitution in liposomes.

PO049

Structural Characterization of Membrane-driven Aggregation of human islet amyloid polypeptide (hIAPP)

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Type II diabetes mellitus (T2DM) is characterized by insulin resistance, progressive islet β cell failure, and the presence of extracellular aggregates (islet amyloid) of a 37 amino acid long peptide hormone 'human islet amyloid polypeptide' (hIAPP) in the pancreatic islets of Langerhans. Evidence suggests that the interaction between hIAPP and phospholipid membrane plays a pivotal role in causing β cell failure. A mechanistic understanding of this interaction is essential for a rational design of therapeutic interventions, and requires structural characterization of membrane mediated hIAPP aggregates.

Here we report the aggregation behavior of recombinantly expressed full-length hIAPP in the presence of small unilamellar vesicles (SUVs) composed of zwitterionic POPC and negatively charged POPS phospholipids. Unlike well-dispersed hIAPP fibrils observed in the absence of lipids, bundles of fibrils interspersed with misshaped lipid vesicles are observed in the presence of SUVs. Solution-state NMR and Thioflavin-T (ThT)-based fluorescence assays were used to characterize the impact of peptide:lipid ratio, POPC:POPS ratio, and temperature on the aggregation kinetics of hIAPP. Fibrils prepared using isotopically enriched hIAPP (U-15N-13C) under ThT-screened conditions were packed into 1.3mm magic-angle-spinning (MAS) rotors for solid-state NMR (ssNMR) based structural analysis. A set of 1H-detected correlation spectra (hCANH, hcoCAcoNH, hcaCBcaNH, hcaCBcacoNH, hcaCONH, and hCONH) recorded at 55 kHz MAS allowed the sequence-specific assignment of hIAPP fibrils. Further, distance constraints obtained from ssNMR experiments will be used in conjunction with molecular dynamics simulations to build a structural model for hIAPP fibrils grown in the presence of membranes.

PO050

NMR structure determination of γ-Secretase Substrates

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γ-Secretase is one of the most intensely investigated intramembrane proteases. Its substrates, the number of which is constantly increasing, are involved in a variety of severe and widespread diseases such as Alzheimer's and cardiac diseases. However, the criteria that define a substrate remain elusive, apart from requiring single-span type I transmembrane helix. A hypothesis that γ-secretase cleaves nonspecifically any transmembrane domain (TMD) in its vicinity is, however, contradicted by the fact that single point mutants within TMDs of known substrates can impair or alter cleavage sites.

We compared a panel of γ-secretase substrates by NMR spectroscopy in 80% TFE/water, a mimic of the water-filled enzymatic cavity. Structure calculations revealed that the TMD of the amyloid-precursor protein (APP), one of the most widely studied γ-secretase substrate, did not form a straight α-helix but had a kinked conformation at the central GG-motif. Single point mutations within the TMD altered the kink angle and direction. In addition to APP, we investigated other substrates that are processed by γ-secretase in a variety of different cellular contexts. Based on these observations we present a model for enzyme-substrate interaction and structural features that distinguish a good substrate from a bad one.

PO052

Towards elucidation of structure and interactions of the SARS-CoV-2 accessory protein ORF7b

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The SARS-CoV-2 genome encodes structural proteins, but also non-structural and accessory proteins, which contribute to viral fitness and pathogenesis. We studied by fast MAS solid-state NMR (ssNMR) ORF7b, one of the eleven accessory proteins encoded by the SARS-CoV-2, which is identified as a membrane protein by its highly hydrophobic N-terminal region. We experimentally show that ORF7b assemble into stable multimers by using wheat germ cell-free protein synthesis (WG-CFPS). The transmembrane domain of ORF7b shows a leucine heptad motif that leads us to suggest that multimerization proceeds through a Leucine zipper [1].

On one hand, we investigate the potential interaction of ORF7b with human proteins in the context of COVID-19. Two chosen candidates with leucine-zipper motif are phospholamban that regulates heart rhythm through formation of a pentamer in cardiomyocytes and E-cadherin, which dimerizes in epithelial cell-cell adhesion. Using pulldown assay in the WG-CFPS system, we show that ORF7b indeed interacts with the transmembrane domains of E-cadherin and phospholamban. As follows, ORF7b could interfere with cellular processes and be involved in some of the common symptoms of the COVID-19.

To experimentally assess the formed interfaces, we work in a first step towards elucidating the structure and interactions of ORF7b by ssNMR. For this, we have produced ORF7b in milligram quantities using WG-CFPS, and reconstituted the protein in ERGIC (ER-Golgi Intermediate Compartment) liposomes. Fast MAS proton-detected NMR spectra at 100kHz were subsequently recorded on several selectively labelled preparations as easily achieved by WG-CFPS, assignments of which should reveal the exact localization of the N-terminal α-helix as well as the organization of the C-terminal amphipathic region. This forms the basis to further investigate the ORF7b interactions in multimerization, as well as with cellular proteins.

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PO053

CHARACTERIZATION OF SARS-COV-2 ORF6 ACCESSORY PROTEIN

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SARS-CoV-2 is the causing agent of the worldwide known COVID-19. SARS-COV-2's genome encodes for 4 structural proteins (E, S, M, N), 16 non-structural proteins (Nsp1-16) and other non-essential genes coding for the accessory proteins (ORF3a-ORF9c). Accessory proteins are related to various processes such as increased pathogenesis and resistance against immune response.

Among these accessory proteins, ORF6 protein is a small 7.3 kDa membrane-associated protein found to be a potent interferon antagonist1 and probably the most cytotoxic protein of SARS-CoV-22. The use of a cell-free protein expression system allows us to circumvent the cytotoxicity of this protein and to directly express the protein in a membrane-mimetic environment3.

In this work we show that ORF6 has a double nature, on one side it interacts with membranes, likely as a peripheral membrane protein, on the other side it can be found in a soluble form without detergents, likely in a different fold. Moreover, when embedded in detergent micelles, we observed that ORF6 can be found in several oligomeric states from monomers to tetramers.

Using both solution- and solid-state NMR, we deciphered first structural characteristics of ORF6, and found some surprising differences when comparing to the predicted AlphaFold4 structure. We could sequentially assign the flexible parts of the protein, which allowed us to assess and localize interactions with potential partners of ORF6, including the viral protein Nsp8.

This work shows the multiple faces of ORF6 and advances the understanding of the pathogenic mechanism what could in a future allow new medical strategies to fight against COVID-19.

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PO054

Assignment methodology and dynamics study of the pre-let7 miRNA

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Micro-RNAs (miRNAs) are small RNAs which regulate gene expression by targeting messenger RNAs (mRNA) through a mechanism involving at least partial complementary base pairing. The biogenesis of miRNAs involves the cleavage of a long hairpin stem-loop RNA precursor (pre-miRNA) into the mature miRNA. We focus on let-7 a miRNA highly conserved across species and downregulated in various cancer [1]. LIN28 protein inhibits let-7 biogenesis by binding to the pre-miRNA [2]. RNA is highly flexible and its dynamics can often be related to biological function. In this work, we use solution-state NMR spectroscopy to probe the let-7 pre-miRNA dynamics and complement a recent cryo-EM study on this RNA that suggested that it adopts multiple conformations in solution [3].

First, we present the assignment strategy developed for this challenging system: a large RNA, 73-nucleotide long, with a 27-nucleotide nonhelical dynamic loop. The strategy relies on a divide-and-conquer approach, the use of high magnetic field and the production of several samples with selective labeling and deuteration. Then, NMR spectroscopy is used to characterize slow chemical exchange in this RNA and probe alternate conformational states on the timescale of microsecond to second [4,5]. In addition to these experimental data, we run atomistic molecular dynamics simulations to shed light on the conformational changes in the pre-let7 miRNA.

Overall, this work aims at targeting a large and complex RNA and understand the nature of its dynamic structure landscape in relationship with its function.

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PO055

Yin and Yang: The intricate structural relationship of NusA and the translesion DNA-polymerase IV (DinB)

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Cells are constantly exposed to either endogenous or exogenous DNA damaging agents that cause lesions within DNA strands. These lesions whilst occurring on the template DNA-strand block the movement of RNA polymerase within the progressing transcription elongation complex (TEC) during transcription. Depending on the nature of the lesion, cells are able to explore different repair mechanisms. One of the main repair mechanisms is the activation of DNA repair machineries through the transcription-coupled DNA repair (TCR) pathway, a process in which repair enzymes are recruited to the site of the stalled TEC, subsequently resulting in preferential repair of lesions within transcribed strand. NusA, a crucial transcription elongation factor, becomes lately more and more recognized as playing an essential role in transcription-coupled DNA repair by recruiting the needed repair machineries.

Structurally, NusA is a 50 kDa protein comprised of six sub-domains. Previous studies indicated by far Western blotting that NusA can recruit 40 kDa DNA Polymerase IV (DinB) to the DNA lesion enabling subsequent repair. Due to importance of TCR process for cell survival under toxic and/ or stressful conditions, we set out to reveal the structural basis of the interaction using high-resolution solution nuclear magnetic resonance (NMR) spectroscopy as well as other complementary biophysical and biochemical techniques.

I will report on our current efforts determining the structure of this large complex by solution NMR methods, which is mainly facilitated by the respective carboxy-terminal domains. Nevertheless, additional domains of both binding partners contribute significant auxiliary interactions leading to an intertwined NusA:DinB complex.

PO056

Mechanism of B.subtilis biofilm filament formation and proteins in outer membranes of E.coli

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Most microorganisms form sessile multi-cellular biofilms in which they are protected against stress by a gel-like matrix composed of proteinaceous fibrils, various extracellular polysaccharides (EPS) and often DNA. Bacillus subtilis expresses TasA as the major biofilm protein, yet TapA encoded in the same operon is required for proper biofilm formation. Here we present the three-dimensional structure of TapA and uncover the mechanism of TapA-supported growth of TasA filaments. A combination of analytical ultracentrifugation and NMR reveals further its concentration-dependent support of TasA oligomer formation. The N-terminal region of TapA alone is found sufficient and essential for biofilm formation in vivo. Solid-state NMR revealed intercalation of the homologous N-terminal TasA peptide segment into subsequent protomers to form a regular filament formed by a chain of ß-sandwich structures, in agreement with AlphaFold predictions. Surprisingly, the secondary structure around the intercalated N-terminal strand ß0 is conserved between the filamentous structure of TasA and Fim and Pap proteins forming bacterial pili. In analogy to the chaperone-usher pathway in Gram-negative bacteria TapA is a template for TasA oligomerization.

Structure and dynamics of membrane proteins are considered to be affected by the various membrane approximations applied in structural studies. Here, we have expressed the transmembrane domain of Yersinia adhesin A and the ß-barrel protein OmpG into the protein-depleted outer membrane of a specially designed E.coli strain, and investigate structure and protein dynamics directly in asymmetric outer membrane vesicles, using magic-angle spinning solid-state NMR. For YadA, we applied simple model free and new 'informed' extended model free approaches to analyze local motions and overall mobility in the membrane. A 3D-Gaussian axial fluctuations model is applied to analyze anisotropic motions with respect to the membrane plane.

PO057

Mechanistic insight into the conformational ensemble of IDPs upon interaction with globular protein

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Intrinsically disordered proteins (IDPs) lack a defined secondary and tertiary structure and this facilitates interaction with multiple partners. The avidity and promiscuous nature of these IDPs play a crucial role in biological processes such as cell division and differentiation, signaling and regulation, and recognition pathways. Interaction of IDPs with its specific binding partner either induces folding upon binding i.e., disorder-to-order transition or retains their disordered nature forming fuzzy complexes. Here, we demonstrate the effect of the interaction of globular protein, Small Ubiquitin-like MOdifiers (SUMO1) on the conformational ensemble of natively disordered protein, α-Synuclein. α-Synuclein is a neuronal protein that upon misfolding, undergoes a series of conformational transitions to form β-sheet rich fibrils characteristic of neurodegenerative disorders called α-Synucleopathies. SUMO1 is a post-translational modifier that modifies the proteins via the formation of reversible covalent bonds. In addition, SUMO1 also regulates the function of various proteins via non-covalent interactions involving the hydrophobic patch in the target protein identified as SUMO Binding or Interacting Motif (SBM/SIM). Here, we reveal the non-covalent interaction of hydrophobic nature between α-Synuclein and SUMO1 by using a combinatorial approach involving NMR spectroscopy, analytical ultracentrifugation, and small-angle X-ray scattering, and molecular dynamics simulations. We also discovered that this transient interaction induces conformational compaction in α-Synuclein which could be correlated to the alteration in the amyloidogenic propensity of α-Synuclein in presence of SUMO1.

Posters

Impact of post-translational modifications and disease-related mutations on the structural dynamic properties of cytochrome c

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Cytochrome c is emerging as a moonlighting protein that participates in oxidative phosphorylation as well as DNA damage response and apoptosis. Oxidation of cardiolipin (1,3-bis(sn-3'-phosphatidyl)-sn-glycerol) by cytochrome c is a primal event of apoptosis initiation, enabling the own release of this protein to the cytosol. Such a functional diversity demands tight control of the protein by posttranslational modifications, such as phosphorylation and acetylation. In this work, we aim to decipher how post-translational modifications and disease-related mutation affect the structural dynamic properties of cytochrome c and how the dynamic changes modulate cytochrome c function. The Lys39 acetylation of cytochrome c—assessed by solution NMR—alters dynamic properties and chemical-shifts of those amide backbone signals surroundings the heme group. Tyrosine 48 can be phosphorylated, but also mutated by histidine in thrombocytopenia disease. Then, we have replaced Tyr48 by either His48 to generate the thrombocytopenia-associated variant or by the non-canonical amino acid p-carboxymethyl-L-phenylalanine (pCMF48), nowadays the best stable tyrosine phosphomimetic variant reported. Bidimensional and three-dimensional magic angle spinning solid-state NMR experiments have been recorded with human cytochrome c samples bound to cardiolipin-containing large unilamellar vesicles (LUVs). Dynamics-based spectral editing and solution NMR-like J-mediated INEPT experiments enable us to detect the flexible regions of the lipids and protein moiety of the sample and detect chemical-shift perturbations. Human cytochrome c retains a native-like folding upon interaction with cardiolipin-containing LUVs. However, substitution by pCMF or His residue increases the flexibility of residues located in the protein-membrane interface.

PO059

The Chaperone Trigger Factor's Interactions with Client Proteins

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Molecular chaperones are key players in protein folding, preventing misfolding and protein aggregation, and maintaining cellular protein homeostasis. Understanding the biophysical principles underlying their mechanism of action is a fascinating research topic. NMR is particularly well suited to study the complexes of chaperones with client proteins, which can be highly dynamic [1]. Trigger factor (TF), a bacterial cytosolic ATP-independent chaperone, is the first chaperone to interact with newly translated polypeptide chains emerging from the ribosome [2]. TF also binds client proteins in solution [3]. The biophysical principles of how TF interacts with clients, however, remain unclear. Does it form client complexes at the single conformational limit or rather multi-conformational ensembles? Does it only interact with unfolded extended chains or also partially compacted or folded structures? Using an NMR-based approach, we aim to answer these questions and elucidate the biophysical principles underlying the interactions of TF with client proteins. We build upon previous work describing the structure of TF in complex with segments of unfolded alkaline phosphatase (PhoA) [4]. Our preliminary results have yielded mappings of intensity changes, chemical shift perturbations, and intermolecular NOE contacts that arise when TF interacts with fragments of PhoA, as well as the full-length client. Our outlook is to study the interactions of TF with other clients, for example outer membrane proteins, and make use of paramagnetic NMR approaches to gain further insight into the dynamics of bound clients and the TF-client complex. This will give us a deeper understanding of TF's mechanism of action and its role in protein folding.

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PO060

Linear discriminant analysis reveals hidden patterns in NMR chemical shifts of intrinsically disordered proteins

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Nuclear magnetic resonance spectroscopy (NMR) is the key method to study intrinsically disordered proteins (IDPs). The standard assignment of signals in protein NMR spectra is based on analyzing a set of results from three-dimensional experiments, which provide information on the sequential linkage of chemical shifts of individual nuclei. Yet, even the first step of such analysis, i.e. assignment of observed resonances to particular nuclei, is often very difficult due to low peak dispersion in the spectra of IDPs.

We propose to support the assignment process by finding 'hidden' chemical shift patterns, specific for the amino acid residue types. The patterns are found in the training data from Biological Magnetic Resonance Bank (BMRB) using linear discriminant analysis (LDA), and then used to classify spin systems in the newly studied IDP sample. We show that a procedure can greatly support the analysis of NMR spectra and can be used in many different assignment-related contexts.

PO061

Dbp proteins and GAGs: insights into binding motifs of adhesins from European Borrelia

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Decoring binding proteins (Dbps) of Borrelia spirochetes are vital for initial host infection. DbpA and DbpB bind to decorin via the glycosaminoglycan (GAG) chains and strain-specific variations in their binding affinities to GAGs influence tissue tropism of spirochetes[1]. Dbps have low sequence similarity, but well conserved 3D structure except the "linker" region which has been determined to be the general GAG binding pocket in North American Dbps[2]. Here, we report the NMR characterization of Dbps from European Borreliae and their functional differences.

Resonance assignments, secondary structure motifs and backbone dynamics of 3 European DbpA and DbpB homologs were determined[3]. Binding epitopes and affinities for hexa- and octasaccharides of heparin and dermatan sulfate were derived via NMR titration (chemical shift perturbations, heteronuclear ^{1}H, ^{15}N NOEs) and hydrogen-deuterium exchange mass spectrometry (HDX-MS). All Dbps were able to bind both ligands, whereas the individual affinities and binding residues are specific for each protein and type/length of GAGs. The affinities of European Dbps differ significantly to the previously determined values for North American homologues[4] despite their general structural similarity. These results support the hypothesis of the very selective Dbp-GAGs interplay underlying the different tissue tropisms of Borrelia.

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Characterizing excited states in the ribosome using relaxation dispersion NMR

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The ribosome is a large RNA-protein complex that synthesizes proteins in all living organisms. It undergoes global conformational rearrangements during translation, where its two subunits move relative each other, motions that are well characterized. However, local dynamics of RNA have not been studied in the same detail. The RNA helix 44 (h44) is located at the interface of the two subunits, containing intersubunit bridges that get rearranged during protein synthesis(1) and is therefore our research interest. In particular, the intersubunit bridge B3 region is the focus of my PhD project.

Biomolecules exist in an ensemble of conformations, an equilibrium that can be altered due to molecular interactions(2). We study the higher energy conformational states, termed excited states (ES), interchanging on a micro-to-millisecond time-scale with the most populated state, the ground state (GS), through base-pair rearrangements (Figure 1). In contrast to using traditional structural biology techniques, which most often observe only the GS, these excited states can be elucidated using $R1\rho$ relaxation dispersion NMR(3). Preliminary results from $R1\rho$ relaxation dispersion data indicate the existence of two excited states of the B3 region.

We investigate what effect the local RNA dynamics have on the global motions. Characterizing the dynamic behaviour of h44 in human and bacterial ribosomal RNA will help increase our understanding of the ribosome function during protein synthesis. The differences and similarities between species can further be exploited for designing new and more specific drug interactions for antibiotics.

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PO063

Modulation of Alzheimer's disease Abeta(1-40) fibril polymorphism by the small heat shock protein alpha-B-crystallin

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Deposition of misfolded proteins and their accumulation in tissues and organs accompanies a number of neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, prion diseases, type II diabetes, etc (Dobson 1999). Aggregation of beta-amyloid peptides (Aβ40 and Aβ42) in brain tissue is a biomarker of Alzheimer's disease (AD) (Tanzi and Bertram 2005). Amyloid fibrils exhibit a certain degree of polymorphism (Lu et al. 2013). It has been suggested that differences in the structure of Aβ fibrils obtained from different AD patients (Lu et al. 2013) is the basis of variations in AD. Therefore, understanding the cause of polymorphism and ways of controlling it is of great importance.

Small heat-shock proteins, including αB-crystallin (αB), occur at high levels in the AD brains and colocalize in the amyloid plagues (Sun and MacRae, 2005). Recently it was shown that αB can inhibit Aβ40 aggregation (Mainz et al. 2015), however, its mechanism of action and the impact on the fibril structure remained unclear.

In the present work, we study the influence of αB on aggregation and structure of Aβ40 amyloid fibril. Using various biophysical methods and fluorescent microscopy, we find that αB does only inhibit Aβ40 aggregation but also changes the aggregation pathway at a certain concentration and leads to the formation of a new polymorph. We structurally characterized the two obtained polymorphs using solid-state NMR. We find that both polymorphs have a similar core structure while the N-terminus in the polymorph formed in presence of the chaperone is more flexible. We propose a mechanism that allows to explain the modulation of the amyloid fibril structure and the inhibition of aggregation.

NMR studies of mini-G proteins and their interaction with Ð₁ adrenergic receptors

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G protein-coupled receptors (GPCRs) play a crucial role in human signal transduction and are the target proteins for more than a third of all approved drugs, hence improving the quality of life of hundreds of millions of people globally.

Agonist binding to the βÐ adrenergic receptor (βÐAR) allows G proteins to bind the intracellular cavity of the GPCR. Subsequently, GDP gets exchanged for GTP in the Gα subunit, transducing the signal into the cell. For biophysical and structural studies of receptor complexes, most often surrogate proteins, such as mini-G proteins and thermostabilised (TS) receptors with large truncations of unstructured loops (e.g. in the intracellular loop 3 (ICL3)), need to be used. Therefore, numerous details of the interaction of wild-type GPCRs with their G protein effectors might not be accessible to characterisation. In our work, we set out to deepen our understanding of the binding mechanism of G protein isotypes to βÐAR, including receptor constructs with wild-type ICL3 and different truncations.

XL-ALSOFAST-[^{13}C,^{1}H]-HMQC spectra of βÐAR and mini-G proteins in presence of several small molecule effectors (GDP/GTP as well as receptor agonists and antagonists) were measured to obtain information about the interactions in all states of the classical GPCR activation cycle. Receptors and mini-G protein isotypes were simultaneously observed in the same sample by differential isotope labelling with ^{13}C-methyl-M and ^{13}C-methyl-ILV, respectively. Constructs of the human βÐAR with different lengths of ICL3 truncations were efficiently produced in isotope labelled form in HEK293T cells by transient transfection. These give new insights into the influence of the previously neglected unstructured regions of GPCRs. Understanding GPCR-G protein interactions together with the influence of ICL3 can provide valuable insights into the function of this important class of drug targets.

PO065

High-sensivity ssNMR studies of the Schizophyllum commune cell wall

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Solid-state NMR spectroscopy facilitates non-destructive studies of biomolecules in complex configurations whilst maintaining native interactions.[1] Recently, we have shown how 13C-detected high field magic angle spinning (MAS) solid-state (ss) NMR spectroscopy can be used to elucidate the structural organization at the atomic level of the cell wall of Schizophyllum commune, a mushroom-forming fungus from the Basidiomycota phylum.[2] Our data suggests that the S. commune cell wall contains a rigid core, probed by dipolar (through-space) experiments, that consists of α- and β-(1,3)-glucan, branched mannose, and β-(1,4)-chitin. While the mobile exterior, probed by scalar (through-bond) experiments, mainly consists of β-(1,3)-(1,6)-glucan, mannan and α-glucan residues, as well as amino acids in random coil conformation.[2] Here, we demonstrate the power of ultra-fast 1H-detected MAS ssNMR spectroscopy to further increase sensitivity and spectral resolution. These advancements allowed us to study binding of various substrates to intact fungal cell walls, expanding the use of ssNMR-guided high-resolution studies on fungi in a material science and biomedical context.[3,4]

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PO066

Regulation of Nedd4 family E3 ubiquitin ligases through auto-inhibition

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The post-translational modification of proteins with ubiquitin is involved in nearly all cellular functions in eukaryotes and provides a system for fast and specific regulation by altering activity, localization, interaction surfaces and stability of targeted proteins. Ubiquitination, the attachment of ubiquitin to target proteins, is mediated by the sequential action of three cooperating enzymes, called E1, E2 and E3. In this enzyme cascade, E3 ligases determine the substrate selectivity and are known as key specificity factors in ubiquitin signaling, determining the fate of their substrates. Consequently, E3 ligases themselves are precisely regulated to prevent random ubiquitination reactions. HECT-type E3 ligases of the Nedd4 family, such as Smurf2, Nedd4, Itch and WWP1 are auto-inhibited by intramolecular interaction of their N-terminal C2 and / or WW domains with the catalytic HECT domain. In Smurf2, for example, the C2 domain inhibits HECT domain activity by blocking thioester formation and non-covalent ubiquitin binding, whereas the WW1 domain further enhances the auto-inhibitory interaction. Interestingly, despite the high degree of sequence conservation within the Nedd4 family, the exact mechanisms of auto-inhibition differ between the individual family members. The manipulation of these auto-inhibition mechanisms is an interesting target point for the treatment of diseases associated to ubiquitin signaling. Here, by utilizing methyl TROSY NMR spectroscopy, NMR titration experiments and biochemical assays, we provide new insights on how the auto-inhibition of Nedd4 family ligases is established.

PO067

An Integrated NMR and XL-MS Approach to Improve the Structural Ensemble of Membrane Bound α-Synuclein

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α-synuclein (αS) is an intrinsically disordered protein (IDP) important in several diseases, especially in Parkinson's disease and Lewy body dementia. It can adopt a wide array of structures, forming toxic aggregates in some cases. Both monomeric αS and aggregates can interact with cellular membranes. Whether formed prior to interaction with the membrane or at the membrane interface, αS can lead to membrane disruption and their incorporation in Lewy Bodies. Although regulated interaction with the membrane is crucial for αS functionality, the interaction has been shown to promote aggregation in vitro. High-resolution structural information has been obtained in the SDS-micelle bound state, however, deriving structures from membrane bound αS has been hindered by the large variation in the structural ensemble and the oligomers it forms on membranes. Here, we present the use of NMR derived parameters obtained on SDS-micelle and bicelle bound αS together with chemical crosslink mass spectrometry (XL-MS) on 14N/15N-labelled αS mixtures in order to improve our understanding of the membrane bound state(s) of αS. In contrast to the available micelle bound structure, which focused on the use of nuclear Overhauser effects (NOEs) and residual dipolar couplings (RDCs), our data relies predominantly on paramagnetic relaxation enhancement (PRE) and interference (PRI) measurements for long-range information. Their high sensitivity to compact substates of the ensemble allows us to detect and describe novel conformations of αS. We then proceed to validate our findings by cross-checking the modeled structures with data obtained from XL-MS. In addition to assessing the validity of our NMR derived sturctures, the XL-MS data improves our understanding of oligomers.

PO068

Phospho-dependent BRCA2 recruitment in KIF2C condensates during mitosis

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BReast CAncer protein 2 (BRCA2) is a protein essential for genome integrity. Depletion of BRCA2 causes a wide range of defects in DNA repair and recombination, protection of stalled replication forks, regulation of telomere length, mitosis, meiotic recombination, and fertility. BRCA2 is a very large protein of 3418 amino acids (aa), which exhibits a unique folded domain of about 700 aa and several conserved and disordered regions1,2 which are poorly characterized. Using affinity chromatography and mass spectrometry (Curie Institute), we identified novel PLK1-dependent interactions between BRCA2 and partners3. We focus on the structural characterization of the interaction between two BRCA2 fragments and the KIF2C microtubule depolymerase. To characterize interactions, we used Nuclear Magnetic Resonance (NMR): we monitored phosphorylation of the BRCA2 fragments by the kinase PLK14, and we identified their KIF2C binding motifs. We also identified the KIF2C domain binding to pBRCA2 peptides, and through NMR titration experiments, we mapped the interaction surface on KIF2C showing the importance of phosphorylation. Also we observed that KIF2C is able to phase-separate using Differential Interference Contrast (DIC) microscopy. KIF2C is a protein with ordered and disordered regions. We observed that the N-terminal region of KIF2C, including disordered fragment (86-192 aa), forms droplets under similar conditions as the full-length protein (725 aa). Also, using DIC combined to fluorescence microscopy, we observed that one of the pBRCA2 fragments is recruited to KIF2C condensates. We thus initiated the characterization of the molecular mechanisms responsible for KIF2C binding to BRCA2 and condensate formation in vitro, and we will discuss the putative functions of these events in mitotic cells.

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PO069

Elucidating the Tau-Microtubule interaction by NMR spectroscopy

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Tau is a microtubule-associated protein (MAP) that stabilizes microtubule (MT) bundles [1]. Therefore, tau is vital in the regulation of the dynamic organization of MTs, which play an essential role in cell migration, mitosis and polarization. However, in Alzheimer's disease and other Tauopathies, tau is found to aggregate by a disturbed regulation of its post-translational modifications (PTMs) [2]. Since the aggregation and therefore decreased binding of tau to MT might lead to a destabilization of the latter, understanding this interaction is of significant interest. Despite this great importance and previous progress [3,4], the exact binding mechanism between tau and MT is not yet understood in detail, leaving unclear how each tau residue interacts with the MT surface.

Using a combination of solution- and solid-state NMR spectroscopy, we have examined the interaction of MT with tau. In our studies, we made use of human MT protofilaments, tubulin-dimers and peptides including those representing the tubulin C-terminal tails (CTTs). We designed specific experiments to probe interactions between the MAPs and the tubulin CTTs.

In our contribution, we report on progress on combining our NMR results with information obtained by biochemical and biophysical methods to gain deeper insight into the binding behaviour of tau to MT.

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PO070

Molecular Insights into Canonical Phytochromes by DNP MAS NMR

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Phytochromes (Phys) constitute a superfamily of photosensory proteins that mediate light responses in plants and in many microorganisms.[1] Phys use an open-chain tetrapyrrole (bilin) as the chromophore to perceive light. Light absorption by Phys triggers photoisomerization of the bilin 15,16-double bond between 15Z and 15E configurations. Canonical Phys are photoswitchable between the red-light-absorbing (Pr) dark state (15Z) and far-red-light-absorbing (Pfr) photoproduct (15E).[1] Despite extensive structure/functional studies of this family, the origin of the bathochromic shift associated with the photoproduct formation remains unclear.[2]

The cyanobacterial Phy Cph1 shows particularly close similarities to plant Phys and is tractable for biophysical studies including X-ray crystallography[3] and both conventional solution[4] and solid-state MAS NMR,[5] and more recently, DNP-enhanced MAS NMR.[6] Herein we present the further applications of DNP MAS NMR on this Phy protein to investigate structural changes and charge redistribution of the chromophore and its binding pocket associated with Pr-to-Pfr phototransformation. The results achieved provide a better understanding of the mechanism of Phy molecular action.

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PO071

Solution-state NMR reveal dynamics in the 142 kDa exoribonuclease Xrn1

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Maintaining a balanced level between RNA synthesis and degradation is of critical importance for all cells. One essential component of the eukaryotic RNA decay pathway is the conserved and essential 142 kDa exoribonucleases Xrn1 that processively degrades 5'-monophosphorylated RNA. In addition to the 100 kDa conserved catalytic XRN core domain, Xrn1 contains four C-terminal domains, whose functions are only poorly understood: a Tudor/PAZ domain, a Kyrpides-Ouzounis-Woese (KOW) domain, a winged-helix domain and a SH3-like domain. Static structural information obtained by X-Ray crystallography and cryo-electron microscopy indicate large domain motions upon substrate binding.

Here, we exploit methyl-TROSY NMR methods, fluorescence anisotropy measurements and in vitro RNA degradation assays to study the functional interplay between the catalytic XRN core and the C-terminal domains. We demonstrate that the isolated XRN core is highly dynamic around the active site. In addition, we found that the Tudor/PAZ domain significantly impairs these motions. Interestingly, the reduction in Xrn1 dynamics as caused by the Tudor/PAZ domain is accompanied by a reduced catalytic activity towards multiple RNA substrates.

Our work thus shows that it is possible to obtain detailed and functionally important insights into protein motions for enzyme complexes that well exceed 100 kDa.

PO072

Structural dynamics of the intrinsically disordered SNARE protein SNAP25 in its pre-fusion conformation

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The fusion of synaptic vesicles with the plasma membrane at the neuronal synapse in the process of neuronal exocytosis is a key requirement for the release of neurotransmitters. Here, the assembly of the so-called SNARE (soluble N-ethylmaleimide-sensitive-factor attachment receptor) complex plays a central role by providing the energy necessary for membrane fusion. At the molecular level, important structural transitions of the SNARE proteins and their membrane interactions remain not well understood. The intrinsically disordered protein SNAP25 (synaptosomal-associated protein of 25kDa) is one of the three SNARE proteins composing the SNARE complex. However, the molecular structure and internal dynamics of SNAP25 in its monomeric pre-fusion conformation remain unexplored. Using solution NMR spectroscopy, we have assigned major parts of SNAP25 and studied its secondary structure propensity as well as its structural dynamics by NMR relaxation measurements.

Observing the local anisotropy of protein dynamics using solid-state NMR and Molecular Dynamics

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The local motions occurring within proteins play a fundamental role with regards to how they carry out their biological functions. Quantifying and understanding these motions over a range of timescales can provide useful insight into how proteins behave. Relaxation rates and dipolar order parameters can be measured in a site-specific manner using fast magic-angle spinning solid-state NMR and provide information regarding both the timescales and amplitudes of local motions. Experimentally determined dipolar order parameters measured in the protein GB1 probe different orientations of motion within the rigid peptide plane and provide insight into the significant anisotropy of motion occurring along the protein backbone. By measuring multiple relaxation rates across different temperatures (-13 to 36 °C), fields (600 to 1000 MHz), and nuclei (^{15}N, ^{13}C'), we have built up a picture of the timescales, energetics, and amplitudes of these anisotropic local motions. In this contribution we present one of the largest relaxation rate datasets measured on a single protein and use this to fit a model to allow for the local dynamics of the protein backbone to be probed. Further, we show comparison of these experimentally determined motions to a microsecond long molecular dynamics trajectory on a supercell of GB1, where we find reasonably good agreement between modern molecular dynamics and experimental results.

Posters

ARIAXC Modifying ARIA2 to Use XPLOR-NIH for Structure Calculation

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NMR remains the only high-resolution structure determination method that can determine the structures of proteins, RNA and DNA in solution. Furthermore, over the period 2016-2021, NMR added ~ 1% of the total structures in the PDB and overall accounts for ~5% of structures in the PDB.

ARIA2 is one of the main programs used for the calculation and refinement of structures using NMR based constraints along with CYANA. Here we describe a modification of ARIA2 (ARIAXC) which uses XPLOR-NIH as the underlying molecular dynamics engine providing access to a more modern molecular dynamics implementation enabling access to more experimental forcefields. The experimental forcefields include chemical shifts (including Camshift), X-ray and neutron scattering (including SAX & WAX), paramagnetic relaxation enhancements (PREs and PCS) and other enhancements including radius of gyration potentials and improved water refinement potentials.

As the world changes following the success of alphafold in predicting the structures of proteins, NMR remains a competitive technique especially when considering structures with hidden states and functional dynamics. Furthermore, it may be expected that NMR will also be a good method for rapidly validating and investigating alphafold based structures in solution as it can be extremely quick to access. Use of NMR structure calculation as part of integrated structural biology initiatives in this environment requires access to the most modern techniques and forcefields which ARIAXC helps to provide.

PO075

Looking at dynamic mARN-miARN interactions by 19F-NMR spectroscopy

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Over the last decades, a diversity of non-coding RNA have been revealed such as micro-RNAs (miRNA), that play an essential role in RNA induced gene silencing and target up to 60% of protein-coding genes in humans. As RNA conformational changes can trigger their functional diversity, the investigation of RNA dynamics is a key feature to improve the understanding of their mechanism of action.

Nuclear Magnetic Resonance (NMR) spectroscopy is an efficient tool to investigate dynamic processes. However, obtaining an accurate description of RNA conformational landscape remains long and complex. To overcome this bottleneck, we introduce chemical modifications in the miRNA sequence using solid phase synthesis that enables and facilitates the investigation of miRNAs interactions and dynamics at the molecular level.

Here, we particularly focus on the interaction of let-7, a mi-RNA presents in organisms ranging from nematods to human and shown to be down-regulated in several forms of cancers, with three different relevant mRNA targets (lin28a, HMGA2 and lin41). To probe these interactions at the atomistic scale, a fluorine was site-specifically introduced in let-7, to be used as an NMR probe. Indeed, the natural abundance of 19F nucleus, its sensitivity, as well as its chemical shift dispersion enables a quick and unambiguous analysis of its chemical environment. We show that the introduction of fluorine in let-7 allows for a fast screening of the different complexes and their secondary structures. Moreover, 19F based relaxation dispersion experiments were recorded to investigate the conformational states of these highly dynamical biomolecular complexes.

PO076

Specific lipid interactions in complex membranes at high-resolution

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Specific interactions with lipids are essential for the function of membrane proteins and the properties of biological membranes. Furthermore, many drugs target specific lipids. Yet, interactions with lipids are difficult to probe by structural biology methods. Solid-state NMR could be an ideal tool to directly study specific interactions with lipids, however, this is currently hardly possible due to insufficient sensitivity and the difficulty to distinguish different lipid-types in complex mixed membranes. Here, we present isotopically labelled (^{13}C,^{15}N) phospholipids – which are currently not commercially available – that allow the study of specific lipid interactions in complex membranes at atomic resolution by ssNMR. We demonstrate that, in combination with modern ^{1}H-detected ssNMR, our approach provides unprecedented molecular insights into the mechanisms of drugs that target traces of specific lipids in complex membranes. Taken together, this broadly applicable approach opens novel opportunities to characterize interactions of small molecules, drugs and proteins with membrane phospholipids at high-resolution.

PO077

Conformational dynamics of W71A, E78Q mutant from BCX probed by relaxation dispersion NMR

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Enzyme catalysis is a complex process, where the protein shuttles through multiple minor conformations. GH11 xylanase from Bacillus cirulans (BCX) is an enzyme that plays important role in the degradation of hemicellulose, a renewable biomaterial. In the previous work, Ben Bdira et al. (1) studied the structural dynamics of BCX enzyme mimicking various catalytic states by paramagnetic NMR spectroscopy and Relaxation dispersion NMR (RD-NMR). Overall, they proposed a model in which the protein is highly rigid and exhibits little conformational changes throughout the catalytic cycle. Also, the substrate xylohexaose (X6) can bind to the enzyme in multiple binding modes with different kinetics.

Here, we report a further study conducted on the resting state of BCX enzyme, in a catalytically inactive form with W71A, E78Q mutations. The double mutant of BCX showed the existence of a second conformer in a simple ^{1}H-^{15}N HSQC spectra. That made this particular mutant a suitable candidate for ^{15}N RD-NMR experiments to investigate further into the invisible minor conformation. In the present study, we have used a ^{15}N TROSY-CPMG pulse sequence at the ultra-high field 1.2 GHz and 850 MHz to understand the milliseconds (ms) dynamics. The preliminary results show the presence of a minor state. Especially the residues that are accessible to ms time-scale are located in the active-site cleft. Most of these residues with RD profiles also show a significant chemical shift perturbation upon binding to a short version of the substrate xylotriose (X3).

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Acknowledgments:

PO078

Revealing the role of intrinsically disordered protein regions in the Non-Homologous End-Joining pathway by NMR

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In mammalian cells, DNA double-strand breaks (DSBs) are predominantly repaired by the Non-Homologous End Joining (NHEJ) pathway. XRCC4, DNA ligase IV, XLF and Ku70/80 are core NHEJ effectors that interact and cooperate to tether and re-ligate broken DNA ends. However, when the broken DNA ends cannot be directly ligated, the action of DNA end processing enzymes such as the Artemis nuclease is necessary. While high resolution structures of various complexes of these NHEJ effectors have been obtained, the intrinsically disordered regions (IDRs) of XLF, XRCC4 and Artemis, which range from 70 to 300 residues, have not been characterized.

Here, we use solution-state NMR in combination with EPR/DEER and biochemical assays to characterize the C-terminal domains (CTDs) of XLF, XRCC4, Artemis alone or within full-length protein constructs. We confirm that the CTDs of the three proteins are mostly disordered and engage in a network of multivalent heterotypic and homotypic interactions. In the case of XLFCTD, we identify a short conserve linear motif that interacts with DNA, the folded domain of XLF and the first BRCT domain of DNA ligase IV. For XRCC4CTD, several long-range interactions were detected by PRE and EPR/DEER experiments. In addition, the XRCC4CTD and ArtemisCTD share the same binding site on DNA ligase IV. Importantly, we demonstrate that the interaction network of XLFCTD and XRCC4CTD lead to liquid-liquid phase separation (LLPS) of XLF and the DNA ligase IV/XRCC4 complex in vitro which selectively recruits Artemis and other NHEJ components, increases the rate of DNA end ligation and protects DNA from degradation. Collectively, we propose that these IDRs play a critical role during NHEJ DNA repair by forming multivalent interactions that induce LLPS. Moreover, we highlight the power of NMR in characterizing IDRs in the context of biologically relevant complexes, irrespective of overall protein size.

Posters

MAS NMR structural study of the FAT10 N-domain at 800 MHz

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FAT10[1] plays an important role in the adaptive and innate immune response in mammals.[1] The protein consists of two ubiquitin-like domains (referred to as N- and C-domains), which both show the typical ubiquitin β-grasp fold. FAT10 gets covalently attached to substrate proteins and beside ubiquitin, it is the only ubiquitin-like modifier that targets substrates for degradation by the 26S proteasome. The protein is prone to aggregation and for this reason, only the structure of a stabilized, cysteine-free (Cys-free) mutant could be determined so far.[2] However, it could be shown that the stabilized version of FAT10 and respective conjugates were degraded at slower rates by the 26S proteasome.[2] Thus, it seems that the flexible and loosely folded nature of wild type (WT) FAT10 is important for its biological function.

Here, we report the first MAS NMR structural study of FAT10. We were able to record highly resolved ^{13}C-^{13}C DARR and ^{15}N-^{13}C-^{13}C ZF TEDOR-DARR correlation spectra at 800 MHz for the microcrystalline, uniformly ^{13}C-^{15}N-labelled Cys-free N-domain of FAT10. Site-specific assignment of backbone and sidechain carbon and nitrogen resonances was possible for about 70 % of the residues. Protein backbone torsion angles could be determined from chemical shifts using TALOS-N[3] corresponding to secondary structure elements of the β-grasp fold. With the assignments in hand, we could initiate an investigation of the interaction sites with putative binding partners of the WT FAT10 N-domain.

[1]human leukocyte antigen (HLA)-F adjacent transcript 10

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Disaggregation of amyloid fibres by the human HSP70 chaperone machinery

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Protein aggregation into amyloid fibres is a hallmark of debilitating neurodegenerative diseases including Parkinson's disease (PD). These highly ordered fibrillar aggregates are challenging substrates to the cellular protein quality control machinery due to their high stability and their ability to self-template the amyloid conformation, strongly accelerating aggregation once an initial fibre is formed. It is therefore surprising that the human HSP70 chaperone machinery can not only prevent the aggregation of, but, in specific cooperation with co-chaperones DNAJB1 and HSP110, also dissolves preformed fibres of PD-linked α-synuclein. The underlying mechanism of this unique activity remains poorly understood. Here, we use biochemical tools and solution state NMR spectroscopy to break down key steps of the disaggregation process. We find that the J-domain protein DNAJB1 specifically recognises the oligomeric form of α-synuclein through multivalent interactions and selectively recruits HSP70 to fibres. Surprisingly, DNAJB1 and HSP70 interact with the fibre through opposing C- and N-terminal flexible α-synuclein tails rather than the amyloid core itself. The coordinated binding of DNAJB1 and HSP70 destabilises the fibre, priming the substrate for disassembly. The nucleotide exchange factor (NEF) HSP110 strongly accelerates this process by promoting the loading of multiple HSP70s in a densely packed arrangement at the fibril surface. This crowded arrangement is ideal for the generation of strong "entropic pulling" forces that are proposed to drive chaperone mediated disaggregation. While essential to disaggregation, the unique synergy between the DNAJB1 and HSP110 co-chaperones is not required for other HSP70 activities. These findings thus open the door to the selective targeting of chaperone mediated disaggregation as a therapeutic strategy in neurodegeneration.

PO081

Structure and dynamics of the TRPV1-V4 ion channel N-terminal IDRs as cellular signaling hubs

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Transient receptor potential (TRP) channels are tetrameric non-selective cation channels. They are activated by various physical and chemical stimuli and act as polymodal signal transducers and integrators. While structures of all representative members of mammalian TRP channel subfamilies have been determined by cryo-EM, the distal cytosolic N- and C-termini critical for proper channel function and regulation usually escaped detection due to their inherent flexibility.

In the six human TRP vanilloid channels responsible for heat and pain perception, the general N-terminal domain (NTD) architecture containing an a-helical ankyrin repeat domain (ARD) is conserved. However, while so-called group II TRPV channels, TRPV5 and TRPV6, feature an N-terminal α-helix, the distal cytosolic N-termini of the group I TRPV proteins TRPV1-TRPV4 contain intrinsically disordered regions (IDRs) of variable length (~75 – 150 aa).

We have used a combination of biophysical methods, including NMR spectroscopy, to investigate the disorder content and dynamics of the distal N-termini of the human group I TRPV channels. The computationally predicted and the experimentally obtained extent of disorder were compared to reveal (dis)similarities between the N-terminal regions. Furthermore, the interaction of the N-termini with binding partners (e.g., lipids, $Ca^{2}+$) and their impact on the general structure and dynamics of the overall TRPV-NTDs are investigated to elucidate the formation of transient structural elements and changes in IDR dynamics. Structural rearrangements in combination with disease-related mutations in the N-terminal domain of group I TRPV channels raise the concept that the N-terminal IDRs play a fundamental role in protein function and regulation.

PO082

Determining in-situ membrane protein dynamics using solid-state NMR and MD simulation

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Characterizing dynamics of membrane protein in their native environment is important for understanding their biological activity. The outer membrane (OM) of Gram-negative bacteria consists of lipopolysaccharides in the outer leaflet and mostly phospholipids in the inner leaflet. In particular, the LPS creates a saccharide layer above the membrane that restricts the motion of membrane proteins and allows for interactions between the protein and the 'sugar wall'. For embedded membrane proteins, this asymmetric environment may lead to different structural and dynamic properties compared to proteins in artificial proteoliposomes or a crystal lattice. Here we carried out a ssNMR relaxation study to determine the dynamics of two membrane proteins, Yersinia adhesin A (YadA) and the outer-membrane protein G (OmpG). We compared the dynamics of the proteins in their native OM with those in a more artificial (2D or 3D) microcrystalline environment. For YadA, we supplemented our data with molecular dynamics simulations.

We recorded 15N spin-lattice relaxation rates (R1) and 15N, 13C' spin-lattice relaxation rates in the rotating frame (R1rho) to probe the backbone and peptide-plane motions of YadA and sidechain dynamics of OmpG. The relaxation rates were analyzed with four different motional models, the simple model free, extended model free, collective extended model free and the 3D gaussian axial fluctuations model. For YadA, we observed that the general trend in dynamics over the protein sequence is similar for both environments, though with an overall slightly higher order parameter for the microcrystalline case. From anisotropic parameters we observed that in the lipid environment rocking motions are favoured over rotations. For OmpG our analysis shows that the loops are structured and less dynamic in the native environment whereas they are more dynamic in the artificial environment.

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2 .J. S. Retel et al. Nature Communications, 8, 2073, 2017.

Posters

PO083

Influence of the N-terminal intrinsically disordered region of the SARS-CoV-2 nucleocapsid protein on phase separation

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The nucleocapsid protein of the SARS-CoV-2 (N_SARS-CoV-2) serves various functions. Apart from the structural role, by encapsulating the genomic RNA inside virions, N_SARS-CoV-2 modulates RNA transcription and replication. Modulation of host immune response by interaction with 14-3-3 protein has also been proposed. RNA-induced liquid-liquid phase separation of N_SARS-CoV-2 has been suggested to affect some of N_SARS-CoV-2 functions. Here we focused on the N-terminal intrinsically disordered region (NTE) of N_SARS-CoV-2. This region with low sequence conservation among coronaviruses has a similarity with prions: high asparagine and glutamine content. There is a growing evidence supporting the link between prion-like proteins and neurodegenerative diseases. Interestingly, the mutations of SARS-CoV-2 Omicron variant increased the prion-like nature of the NTE. We showed that deletion of the NTE negatively influences the ability to form liquid-like droplets with polyU RNA and prevents the droplets from forming an immobile species as we observed for a full-length protein(1). Despite lacking a specific RNA binding motive, we detected a direct binding of the NTE with RNA and observed a structural change upon binding. Overall, we characterized the influence of the NTE on RNA-induced liquid-liquid phase separation.

(1)   Savastano A, Ibáñez de Opakua A, Rankovic M, Zweckstetter M (2020) Nucleocapsid protein of SARS-CoV-2 phase separates into RNA-rich polymerase-containing condensates. Nat. Commun. 11:6041.

PO084

Exploring how ligands, G proteins, and arrestins allosterically modulate GPCR conformational dynamics

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GPCRs are the largest family of membrane proteins comprising 3% of the human genome. Unlike many signaling proteins that function as binary switches between 'on and off' states, GPCRs feature basal activity that is increased or decreased upon ligand binding, and then further regulated by allosteric modulators. The intracellular cavity of activated receptors recruit G protein and arrestin transducer molecules equally (balanced signaling) or selectively (biased signaling). A detailed picture of the NTS1 conformational landscape has been established by X-ray crystallography and cryo-EM; yet, the molecular determinants for why a receptor couples to G protein versus arrestin transducers remain poorly defined. Utilizing [13CεH3]-methionine residues located within the transmembrane bundle and near the ligand-binding site, we demonstrate how ligands and PIP2 dynamically prepare the receptor for transducer interaction. In the presence of agonist, NTS1 resonances located throughout the transmembrane region exist as single, broad peaks. Addition of the chimeric Gi/q protein selectively narrows transmembrane helix 5 resonances while simultaneously broadening residues that neighbor the orthosteric pocket. Arrestin-2 induces analogous broadening near the ligand-binding pocket but, in stark contrast to G protein, transmembrane helix resonances appear to double. In the simplest model of two-site conformational exchange, NMR resonances appear as a single peak when interconversion is sub-microsecond or two distinct peaks when it slows beyond the millisecond timescale. Our spectra qualitatively suggest the preexistence of transducer-bound conformations in the agonist-bound state and that ML314, a β-arrestin biased allosteric modulator, fine-tunes exchange between those states. Numerous published NTS1 structures served to develop structural hypotheses such as changes to local shielding or χ3 geometry. Comparison of NMR observables with DFT-calculated chemical shifts and all-atom molecular dynamics simulations indicate methionine-specific conformational dynamics reflect collective motions and global order across the entire receptor. Together, our work demonstrates a thus far unrecognized kinetic component to transducer selection.

Posters

Path-Sum method in comparison to step-wise density Matrix evolution

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NMR theorists have long been working on methods to describe the dynamics of a spin system under the influence of various Hamiltonians. Different elegant spin dynamic theories have evolved over the years with clever algorithm implementations into widely used software like Spinach or SIMPSON.

Currently these algorithms are currently based on the Average Hamiltonian Theory (AHT) approximation. In AHT the Hamiltonian is divided into a series of terms, which each are approximated to be constant over the time of each step and are used to elongate the spin system step-by-step. This is both time consuming, for repeated calculation of each step, as well as a non-exact method.

Our aim was to investigate a new approach of spin dynamic computation: the Path-Sum method. Path-Sum is a recently published analytical method (P.-L. Giscard and C. Bonhomme 2020, Phys. Rev.) to exactly calculate the time ordered exponential of a time dependent Matrix, such as the Hamiltonian in e.g., Schrödinger and Liouville-von-Neumann equations. This mathematics thus provide a promising alternative to AHT in terms of both accuracy and computational time.

Here the open-source high-performance programming environment julia was used to implement both the AHT and the Path-Sum for direct comparison of the methods according to their run-time and computational accuracy.

Posters

PO087

Using deep learning for first-oder shimming

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Shimming in nuclear magnetic resonance (NMR) aims to achieve a uniform magnetic field distribution, as perfect as possible. Currently, shimming precedes most acquisition procedures in the laboratory, and although it is predominantly a semi-automated procedure, it often requires repetition and a considerable amount of experience, which might be cumbersome and time-consuming.

We investigate the feasibility of automating and accelerating the shimming procedure by applying deep learning (DL). We show that DL can relate measured spectral shape to shim current specifications and thus rapidly predict three shim currents simultaneously, given only a batch of four input spectra. Due to the lack of accessible data for developing shimming algorithms, we also introduce a database that served as our DL training set, and allows inference of changes to 1H NMR signals of water depending on shim offsets.

We performed in-situ experiments over 100 random distortions of the first-order shims, and showed that deep regression with ensembles (DRE) improved the spectral quality with high success rates (>90%). We compared the performance of our method with the Nelder-Mead downhill simplex algorithm (a traditional signal-based shimming method), and showed that our algorithm can reduce the necessary NMR acquisitions to reach a certain linewidth (from 21 to 5 acquisitions). Furthermore, the combination of DRE with the simplex method achieved better quality improvements for a fixed number of acquisitions (~3x better after 10 NMR acquisitions).

PO088

NMR Studies of Intermolecular Interactions between Solifenacin and Chemical Derivatizing Agents

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To achieve the differentiation of the chiral active pharmaceutical ingredient (API), solifenacin, we used a method based on the application of derivatizing agents (CDAs) [1−3]. In the study, we used two chiral building blocks of solifenacin succinate, the API used to treat overactive bladder, 1-phenyl-1,2,3,4-tetrahydroisoquinoline (1) and 1-azabicyclo[2.2.2]octan-3-ol (2) and two CDAs: Mosher's acid (3) and 1-(9-anthryl)-2,2,2-trifluoroethanol (4). In particular, we observed in the racemic mixtures of 1, the 50-100 ppb shifts of 13C NMR peaks due to (R)-3 and (R)-4. For (3R)-2, 1H NMR shifts induced by CDAs were approx. 60 ppb. The structures of the complexes between 1, 2 and 3, 4 were further investigated by observing the Overhauser effect and quantum mechanical computations in the Gaussian computer program with the PBE0 functional and aug-cc-pVTZ bases set. Quantum chemical computations were performed for the same pairs of compounds as measured experimentally. We found that the lists of nuclei for which the peaks' shifts induced by CDAs were the most pronounced, the cross-peaks were observed in the 1H NOESY spectra, and those close to each other in the structures of the complexes found by computations are convergent. Therefore, our data shows that using CDAs supported by quantum chemical computations allows us to deduce the enantiomer configuration without using empirical rules for model moderate-size molecules.

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PO089

A SIMULATION FRAMEWORK FOR MAGNETIC SUSCEPTIBILITY INDUCED RELAXATION OF SPINS DIFFUSING IN POROUS MEDIA

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NMR is a versatile technique used extensively to probe and quantify structural features of porous media. The technique is renowned for its applicability in a range of studies from the growth of regenerative tissues to the efficiency of electrodes. In some cases, the method used relies on signal attenuation of T_2 to probe the environment and quantify parameters such as restricted diffusion coefficients, porosity, and tortuosity. This signal attenuation is related to the differences in the magnetic susceptibility between the solid matrix and the surrounding liquid causing localized distortions in the magnetic field. This spatially dependent demagnetization field is known to cause drastic reduction in the lifetime of T_2. In small pores, the magnetic susceptibility induced relaxation mechanism prevents any form of quantitative analysis as the lifetime of T_2 may be shorter than the required delays within the pulse sequence. Although this effect has been experimentally observed, there is currently no complete theory detailing the effect of the demagnetization field on relaxation rates for porous structures of an arbitrary complexity. By taking a joint analytical, computational, and experimental approach, this project aims to derive this theory and apply the findings to the integration of long-lived states (LLS) into diffusion NMR. This contribution outlines the steps taken towards producing a simulation framework capable of calculating the propagator of the spin Hamiltonian responsible for this susceptibility induced relaxation. Details of which include the use of μ-CT for digitizing the porous structure, calculation of the demagnetization field, and Monte-Carlo simulations to simulate Brownian motion. Both the simulation and experiments follow the evolution of nuclear spins diffusing through the demagnetization field during a single echo pulse sequence. The effect on T_2 is studied across varying field strengths, susceptibility differences, and pore sizes to evaluate suitable conditions under which LLS may be utilized.

Posters

PO090

Conformational Selection of Vasopressin upon V1a Receptor Binding

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Vasopressin (VP) is a regulatory neuropeptide that plays multiple essential roles in the body. Its V1a receptor (V1aR) is of high interest in a wide array of drug discovery programs due to its cardiovascular function and roles in the central nervous system. However, significant structural and dynamic details of its receptor interactions remain unclear.

We employed a novel approach to reveal features of conformational selectivity upon VP-V1aR complex formation [1]. We dissected the VP conformational space into three sub-ensembles, each containing distinct structural sets for VP's three-residue C-terminal tail. This was achieved with the help of cross-peak amplitudes found in NOESY spectra. Next, we docked each sub-ensemble in-silico to the V1aR and applied virtual screening strategies to probe VP's conformational space for structures that favor binding. We observed that proline trans-configured extended tail conformations bound to the receptor with three-fold enhanced affinities compared to the compacted trans-configured or both cis- compact and extended conformations.

Our molecular dynamics simulations predicted that removal of the OH group from Tyr^2 turning VP into phenylpressin would remove the Tyr-Arg hydrogen bonding, maintaining high affinity comparable to VP. Indeed, we observed the disappearance of the associated NOE cross-peak by NMR and radioligand displacement assays confirmed potent affinity similar to VP.

The presented "virtual conformational space screening" approach, integrated with NMR spectroscopy, enabled the identification and characterization of a conformational selection-type complex formation mechanism of the VP-V1aR model system, which was confirmed by the prediction of phenylpressin conformation and affinity. This approach confers novel insights and opportunites for targeting the V1aR as well as guidance for ligand design to provide more potent and selective VP analogues.

1. Che, K. et al. Computational and Structural Biotechnology Journal. 19, 5826–5833 (2021).

PO092

RelCalc – A python engine for evaluating relaxation rates symbolically

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Relaxation phenomena play essential roles in all aspects of magnetic resonance experiments ranging from determining an appropriate repetition rate for transients to dissecting the local and global dynamics of molecules. In addition, relaxation theory is instructive in designing experiments that harness slowly relaxing pathways to enable studies of high molecular weight complexes by observing possible relaxation interference effects. Relaxation is driven by rapid fluctuations in local magnetic fields caused by molecular motions. Bloch, Wagness and Redfield (BWR) theory provides a convenient way to calculate relaxation rates, though implementing these calculations is non-trivial for a non-specialist. To facilitate such calculations, RelCalc is presented, which calculates relaxation rates symbolically, yielding both symbolic and/or numerical rates between isolated operators or complete matrices for full Liouvillian simulations. Once the expression rates are calculated, recalculating all relaxation rates for a new set of parameters is very rapid, making the program highly amenable to data analysis. The program takes a spin system, arranges relevant interactions in the molecular frame and then implements a motional model of the user's choice. The motional models available include isotropic and axially symmetric global tumbling, optional rotation about a symmetry axis in the molecular frame, and interactions between rotating spins and 'external' spins that are static in the molecular frame. The efficiency of the software means that large spin systems can be rapidly analysed, removing the need to impose limiting assumptions of motional regime and restricting the choice of relevant interactions. The utility of the software is demonstrated by exploring relaxation interference effects in X2, AX, AX2 and AX3 systems calculated by the program. The results from the program demonstrate that the assumption of the slow tumbling 'macromolecular' limit can be surprisingly poor when studying methyl groups in large proteins.

PO093

A Deep Ensemble Learning Method for Automatic Classification of Multiplets in 1D NMR Spectra

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The identification and characterization of signal peaks in NMR spectra is a crucial yet time-consuming and error-prone stage in the determination of complex chemical compounds. The introduction of automation in the NMR analysis can ease the workflow while increasing the robustness and reproducibility of the results.

Here, we present a novel supervised deep learning method to perform automatic detection and classification of multiplets in one-dimensional proton NMR spectra. The method consists of a probabilistic deep learning approach based on an ensemble of deep convolutional neural networks.

The training set is composed of a large number of synthetic spectra containing classes of basic non-overlapping multiplets only.

All networks in the ensemble produce the same prediction for basic multiplets, while resonances not represented in the training set cause arbitrary errors that differ across the networks. Therefore, high output variance in the ensemble is an indicator of the presence of overlapping multiplets.

Being able to distinguish between basic and overlapping multiplets is a decisive stage. Together with classification within different resonance categories, it helps to perform automated peak picking and coupling constants extraction.

We show that our model can discriminate signal regions effectively and minimize classification errors between different categories of resonances. Most importantly, we demonstrate that the network generalizes remarkably well on real experimental proton NMR spectra. The evaluation is carried out through the implementation of a specific statistical procedure for quantitatively testing the ensemble prediction against experts' annotations.

PO094

Parametric Estimation of NMR data using NMR-EsPy

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Extracting quantitative information from NMR data is of key importance in many areas such as structural elucidation, dynamical studies, and reaction monitoring. The most prevalent means of analysing NMR data is application of Fourier Transformation (FT) on the free induction decay (FID). FT has many attractive features: it is very fast, generates sparse representations, and is far more easily interpretable by humans than time-domain data. Despite this, FT cannot effectively tackle the problem posed by signal overlap, and often does not give access to a parsimonious representation of the information contained in the spectrum.

As an alternative to FT, parametric estimation techniques[1][2] provide access to spectral parameters (amplitudes, frequencies, phases, damping factors), typically by assuming an FID comprises a summation of damped complex sinusoids in the presence of stationary Gaussian noise. While numerous scientific disciplines take advantages of these techniques, they are still not widely used in the NMR community.

Here we present NMR-EsPy[3] (NMR Estimation in Python), a nascent open source Python package, which uses an iterative nonlinear programming technique (Newton's method) in order to acquire estimates of NMR signal parameters. As well as providing a straightforward API, NMR-EsPy has a companion graphical user interface (GUI) which can be run within TopSpin, meaning users are not required to write Python scripts in order to access the functionality of the package.

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Posters

Unpicking the neural networks of DEERNet

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Site-directed spin labelling in EPR spectroscopy yields useful structural information because distance distributions between unpaired electrons may be obtained using DEER, RIDME, and other methods. However, extracting those distributions from experimental data is a mathematically ill-posed problem; Tikhonov regularisation – a popular solution [1] – can be fiddly and susceptible to user bias in the selection of regularisation parameters. A recently established parameter-free alternative is to process DEER data using artificial neural networks [2] – the package that became known as DEERNet matches or exceeds the performance of state-of-the-art DEER and RIDME data processing tools [3].

This communication is about the deep technical side of DEERNet programming: data preprocessing, background signal retrofitting, uncertainty analysis using network ensembles and Jacobian analysis, internal consistency enforcement, detection of corrupted data, dealing with sparsely sampled data, etc. DEERNet is also a rare case of a neural network whose internal functioning is generally understood [4]; we present a behind-the-scenes look at how architectural choices were made to improve the network performance, and at how a Jacobian error analysis was used to generate confidence intervals in the output.

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Posters

Moving magnetic resonance simulations away from piecewise-constant Hamiltonian approximations

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In magnetic resonance simulations, the Liouville - von Neumann equation of motion is commonly integrated using inefficient piecewise-constant exponential propagators. This is because alternatives that support continuously varying generators (e.g. Runge-Kutta methods) do not follow essential conservation laws and yield erroneous long-range dynamics. However, a new class of high-accuracy integrators has recently become available that respects group-theoretical invariants of spin dynamics [1].

In this communication we report technical implementation details and benchmarks of high-accuracy Lie-group quadratures in Spinach [2], which also uses operation reordering to avoid matrix-matrix multiplications in Liouville space - that approach requires less memory and can be more efficient for large spin systems. The improvement in accuracy is dramatic.

The piecewise-constant approximation is a particular nuisance in optimal control of magnetic resonance experiments [3] because instrument response functions rarely allow the pulse to be perfectly piecewise-constant. Piecewise-continuous functions suffer less from instrument response convolution, but the efficient mathematics of the GRAPE method is perturbed because adjacent propagators now depend on the same control coefficient.

In this communication, we use the auxiliary matrix method [4] to calculate the derivatives of exponential propagators yielded by piecewise-linear generators to machine precision. We then use them to improve the GRAPE optimal control algorithm so that the control waveforms can be piecewise-linear; those waveforms place less stringent requirements on hardware compared to piecewise-constant ones. This has important implications throughout magnetic resonance, where complicated shaped pulses are commonplace.

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PO097

Hyperfine chemical shift in host-guest systems of Ru(III) with macrocycles

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Ruthenium(III) compounds have been studied as potential anticancer drugs, though no compound has yet passed clinical trials. One possible way to enhance their effectivity is binding with macrocyclic carriers. Paramagnetism of Ru(III) makes NMR investigations challenging, but also offers an attractive source of structural information about their inclusion complexes. [1,2]

In this account we present relativistic DFT calculations which complement experimental NMR studies of host-guest binding between Ru(III) complexes and macrocycles. Systems based on Ru(III) with an adamantyl anchor and cucurbit[7]uril, which bind in the slow exchange regime, afforded reliable experimental data against which calculations of hyperfine shift were compared. We tested both the point dipole approximation (PDA), whose correct form was recently a subject of controversy [3], and explicit calculation of A-tensors for the whole complex. Since the simple PDA with the equation corrected by Lang et. al. provided a useful picture of hyperfine shifts in these rigid systems, we put it to use in more challenging cases. Cyclodextrins offer two potential binding modes through the narrower or wider portal, and spectra are more complicated due to fast exchange. For Ru(III) with a smaller pyridine ligand, signs of chemical shift induced on the macrocycle differed for α, β, or γ-cyclodextrin. This observation, interpreted with complementary calculations, helped us to shed light on orientation and position of the guest in the cavity.

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PO098

A Simulation Framework to Predict the Relaxation of Nuclear Spins Diffusing in Porous Media

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Nuclear spins diffusing in solution experience a variety of relaxation mechanisms that limit the lifetime of spin orders created during pulse sequences. The very interaction with a magnetic field can induce spin relaxation if the field is inhomogeneous and the spins are free to diffuse in solution. This effect is known as diffusive attenuation and limits, for example, resolution in MRI or NMR experiments that require multiple echoes such as some sequences for singlet NMR. The situation is exacerbated when spin diffusion takes place in the voids of porous media. The differences in magnetic susceptibility between the solid framework and the voids in such systems give rise to local inhomogeneities in the magnetic field. Once a porous medium is placed in a static magnetic field, the spins that move within and in-between pores experience a magnetic field that fluctuates in both magnitude and direction. The theoretical description of relaxation in a porous system is complicated by the fact that the field becomes a function of space, and its local value depends on the intensity of the static magnetic field, the difference between the magnetic susceptibility of the framework and the liquid (or gas) filling the voids, and the actual size and distribution of pores.

With the aim to produce a simulation tool for the quantification of this susceptibility-inhomogeneities-driven relaxation phenomenon in an arbitrarily complicated porous structure, we combined spin dynamics, relaxation theory, Monte Carlo simulations, NMR experiments and micro-computed tomography. In this contribution, I shall describe the various aspects of the simulation framework and demonstrate its validity by comparing simulation results with experimental relaxation measurements. Finally, I shall illustrate our efforts in building a field-cycling-based two-centre NMR spectrometer for diffusion tensor and tortuosity measurements in porous media.

Posters

Deconvolution of Uncorrected High Dynamic Range 1H NMR Spectra: A Physics-Informed Deep Autoencoder Approach

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We introduce a deep learning-based deconvolution approach for 1H NMR spectra, developed by leveraging concepts from the field of physics informed-learning, intelligent labeling, and tailored high dynamic range (HDR) spectral preprocessing.

Since automation and faster workflows are major concerns in NMR spectroscopy, the algorithm handles uncorrected spectra without strict assumptions on phase and baseline correction as well as line shape. Due to the lack of high quality and consistently labeled experimental spectra in quantities needed to train modern deep learning models, we relied on synthetic spectra creation. Moreover, instead of training with synthetic spectra consisting of single lines, we created synthetic multiples that further supported a realistic deconvolution.

We achieved super-human performance on corrected and uncorrected synthetic spectra. Finally, and most importantly, the results on synthetic data translate well to experimental spectra despite the covariate shift. Thus, this tool is a promising candidate for automated expert-level deconvolution of experimental HDR 1H NMR spectra.

PO100

Leave the desktop behind with NMR Online!

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Nuclear Magnetic Resonance (NMR) spectroscopy is used in a multitude of disciplines including Materials Science, Organic Chemistry and Structural Biology. At present, the majority of NMR analysis software packages are bound to desktop computers and operating systems, and limited by age of the software, and even the compiler. These complications can require significant technical understanding to navigate specific hardware requirements and cumbersome installation procedures. Further, a user is restricted to the computational power of the machine onto which the software is installed, thus limiting the size of dataset that can be analysed, and limiting the speed of analysis. In addition to the challenges presented by specific hardware requirements, once a sample has been produced, and the appropriate spectra acquired, the analysis can be complicated by the different analysis software available, and the data transformations required to traverse them.

NMR Online is a community-driven, web-based, cloud-backed, NMR data analysis platform. It runs inside a web browser, removing the need for installation and use on a specific computer. This enables analysis to be done anywhere, on any device! NMR Online removes the complications of data transformations by choosing the correct format for a given software routine, and provides purpose built interfaces between softwares, removing this burden from the end user. Furthermore, all data transformations are invisible to the user, and all data are read in their native formats.

Using a microservice architecture, we have produced a platform that is flexible, scalable and easily extendible – essentially, if we can containerise it, we can host it! By interacting with the NMR community via an ever-growing social media audience, we have crafted a BETA release of NMR Online to suit the wide-ranging needs of this community. The intuitive workflows, and the ability to do NMR anywhere, set NMR Online apart from any existing solution.

PO101

Interplay of fast and slow motion in HET-s(218-289) characterized via NMR relaxation and MD simulation

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Side chain dynamics play important roles for protein structure stabilization and molecular recognition processes. While solid-state NMR can provide timescale-specific parameters from ps to µs, multiple bond reorientations and rotations require more parameters than are accessible via NMR alone. In contrast, molecular dynamics simulations provide the required detail, but accuracy depends on force field quality. Hoffmann et al. recently demonstrated this, showing vast improvement in reproduction of solution-state relaxation by lowering the methyl rotation barrier for side chains in the AMBER ff99SB-ILDN force field [1,2].

We measure solid-state 13C relaxation of specifically-labeled methyl groups (13C, 1xH, 2xD) in HET-s(218-289) fibrils, and quantitatively compare experimental results to several MD simulations with varying force field parameters, confirming that methyl dynamics are more accurately reproduced using methyl barrier corrections from Hoffmann et al. [1,2]. Via our recently developed ROMANCE frame analysis [3], we are furthermore able to separate motion of the H–C bonds into up to seven components and determine the influence of each component on the 13C relaxation. We find, as expected, faster methyl rotation, but other components of the motion are also accelerated, even resulting in better reproduction of backbone dynamics compared to experiment. The result highlights one of the strengths and weaknesses of MD: based on careful tuning of force fields one may obtain powerful insight into the real motion of a protein, but local force-field inaccuracies may propagate to slower and distant motions. Thus, despite rapid development of MD simulation, experiments continue to have a critical role in obtaining accurate dynamics characterization.

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Finite Element Method Modelling of Iron-Oxide Nanoparticle Heat Production Under Low Radio Frequency Field Conditions

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Iron-oxide based magnetic nanoparticles are used as contrast agents in magnetic resonance imaging and as magnetically induced heat generators in magnetic hyperthermia[1] which is a non-invasive cancer treatment method based on delivering nanoparticles to the tumor site. Then, applying alternating magnetic fields (AMF) generated by special coils to heat up nanoparticles, thereby killing and/or sensitizing cancer cells to other forms of therapy. Efficiency is measured by heat production capabilities of nanoparticles under minimum coil power[2]. Therein, measuring temperature rises is more suitable rather than the generated heat. However, nanoparticles' temperature changes are both directly and indirectly influenced by many environmental factors, impeding an accurate performance testing[3]. Therefore, evaluations through generated heat, instead of particle temperature should provide more accurate data on system performance. In this work, COMSOL Multiphysics[4] program is used for developing a model of electromagnetic heating of a single Fe_3O_4 nanoparticle which is electromagnetically heated by a 12.6mT field at 150kHz for 60 minutes. Time average of total heat production by a single nanoparticle was found to be 2.43×10^{-41} Wm^3. Fe_3O_4 is preferred due to its great electromagnetic characteristic and widespread use in drug formula Ferumoxytol[5]. Small size of the nanoparticles create a significant meshing challenge in numerical simulations[6], resulting in unfeasible computation times when using multiple particles. Accordingly, single particle simulation results were used for theoretically calculating heat production capacity of a single injection (1ml) of Ferumoxytol as 7.87×10^{-21} Wm^3. Combining simulations of a single particle and theoretical calculations proved to be effective in creating a basis for future applications towards optimizing input waveforms for AMFs.

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Unraveling a Ligand-Induced Twist of a Homodimeric Enzyme by Pulsed Electron–Electron Double Resonance

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Mechanistic insights into protein–ligand interactions can yield chemical tools for modulating protein function and enable their use for drug design. For the homodimeric enzyme tRNA-guanine transglycosylase (TGT), a putative virulence target of shigellosis, a ligand-induced structural rearrangement of the functional dimer into a non-functional "twisted" homodimer was recently discovered by means of crystallography [1,2]. However, crystallographic observation of both end states does neither verify the ligand-induced transformation of one dimer into the other in solution nor does it shed light on the underlying transformation mechanism. In the present report, we address these questions in an approach that combines site-directed spin labeling with distance measurements based on pulsed electron–electron double resonance (PELDOR or DEER) spectroscopy [3]. The PELDOR experiments on two spin-labeled TGT mutants revealed that the equilibrium state of the ligand-bound TGT corresponds to a mixture of functional and twisted dimers and that the relative amounts of both dimers strongly depend on the type of the added ligand. Additional PELDOR experiments on heterodimeric TGT mixtures showed that the ligand binding favors the dissociation of the functional dimers into monomers which in turn re-associate into the twisted dimers. Based on this, we suggest a dissociation–association mechanism for the formation of the twisted dimer upon ligand binding.

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PO104

EPR as a tool for investigating polyaromatic deposits in zeolite catalysts

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EPR has been a valuable tool for catalysis science since many years. [1] Most of the EPR applications in catalysis focus on the identification and characterization of catalytically active sites, which very often consist in paramagnetic transition metal ion centers. However, other less extensively investigated paramagnetic compounds and centers may form during the catalytic process. One example is a class of polyaromatic molecules, formed as byproducts of many catalytic reactions, commonly called "coke". The heaviest fraction of these polyaromatic compounds can be trapped inside the catalyst, for instance inside the zeolite channels. On a long run this can make the channels and the active sites inaccessible and thus lead to a catalyst deactivation. However, there is some evidence for the fact that, for short reaction times, a small amount of coke could participate to the catalytic reaction and thus be beneficial for the catalytic activity. [2] It is estimated that around 10 % of coke is radicalic, and thus it can be studied by EPR.

The present investigation focuses on methanol-to-hydrocarbons and methyl chloride-to-hydrocarbons reactions, catalyzed by H-ZSM-5 zeolite. CW and pulsed EPR techniques, such as HYSCORE (HYperfine Sub-level COrRElation) and instantaneous diffusion experiments, provide valuable information on different features of the process, such as coke composition and its evolution, coke radicals density, spatial distribution, formation kinetics and mechanism. The EPR results, combined with other experimental techniques and DFT calculations allowed to gain insight into the mechanistic aspects of the two reactions and provides a guideline for future EPR investigations of similar systems.

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PO105

Exploring pulsed Dynamic Nuclear Polarization with Fourier-Synthesized XiX

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Dynamic Nuclear Polarization (DNP) is a powerful technique to increase the sensitivity of nuclear magnetic resonance (NMR) experiments. The drawback of current continuous-wave based DNP techniques (e.g., solid effect and cross effect is their decreasing enhancement with higher static magnetic fields (≥ 7 T) due to inverse scaling of the effective Hamiltonian with respect to BÐ. As such, pulsed DNP experiments such as NOVEL, TOP-DNP and XiX DNP were expected to resolve this issue. However, due to limited microwave power, the NOVEL sequence is hard to realize at higher magnetic fields ($\omega(\mu w) = \omega Ð$). Furthermore, experimental evidence of field-independent enhancements using pulsed DNP schemes (TOP-DNP and XiX-DNP) is still missing. We aim to design new pulsed DNP experiments tailored towards higher magnetic fields.

To gain further insight into pulsed DNP, we designed a DNP sequence called Fourier-Synthesized XiX based on the theoretical description of the DNP process. In this sequence, we use an odd half-range Fourier expansion to describe the amplitude modulation of the original XiX sequence. By selecting a particular Fourier coefficient from the expansion, one obtains only certain resonance conditions (where enhancement of the nuclear signal can be observed) out of all possible resonance conditions contained in the standard XiX experiment. We carried out our experiment with the Fourier-Synthesized XiX using OX063 Trityl radical in glycerol-d_8:D_2O:H_2O (6:3:1 by volume) at X-band (0.35 T) and temperature of 80 K.

Implementation of Fourier-Synthesized XiX at X-band yields promising results, matching our Floquet based simulations. Further developments of pulsed DNP at high magnetic fields is currently being conducted on our 7 T DNP setup.

PO106

Laplace inverted pulsed EPR relaxation to study polymer electrode/Conductive carbon contact in Li-ion battery

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The addition of conductive additives during electrode fabrication is a standard practice to mitigate low intrinsic conductivities of most cathode materials used in Li-ion batteries. To ensure an optimal conduction pathway, these conductive additives (carbon particles) need to be in good contact with the active material. This aspect is crucial for Organic Polymer Radical batteries (ORB) where the insulating polymer backbone could hinder the conductive contact between redox-active groups and the carbon particles.

Herein, we demonstrate the combined use of Pulsed-EPR relaxometry and Inverse Laplace Transform (ILT) to study such electronic contact. The investigated system comprises of PTMA nitroxides, a commonly used redox unit in ORBs, and SuperP carbon black as the conductive additive. Samples with varying PTMA monomer to SuperP ratios (2:1 to 1:30) were prepared by adding nitroxide solutions to SuperP, followed by drying at 60°C. Pulsed-EPR based Inversion recovery experiments (30K) were conducted to obtain T_1 relaxation curves and ILT[1] was used to obtain the corresponding relaxation time distributions. For 1:2, the relaxation distribution consists of three resolved relaxation components corresponding to different grades of contact between the carbon particles and the nitroxide radicals. Upon increasing the SuperP amount in the 1:20 sample, more nitroxide radicals are brought into contact with SuperP, resulting in a decrease of the slower relaxing component and an increase of the faster relaxing components. Exchange interactions between the spins lead to coalescence of spectral features making it difficult to separate these components in the EPR spectrum itself.

Our analysis suggests that the composition of the electrode is a key factor in determining the quality of active material/conductive carbon contact and that pulsed EPR relaxometry in combination with ILT may serve as a robust tool to study these interactions.

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PO107

Mapping the binding orientation of MeCP2 to strand-symmetrically and asymmetrically modified CpG dyads

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The most abundant epigenetic modification of mammalian DNA is 5-methylcytosine (mC) playing key roles in differentiation, development, X-chromosome inactivation, and genomic imprinting. [1] mC modifications are located almost exclusively at CpG dinucleotides and are maintained in a strand-symmetric state. Further iterative oxidation of mC to 5-hydroxymethyl- (hmC), 5-formyl- (fC), and 5-carboxycytosine (caC) occurs resulting in symmetric and asymmetric combinations in the two DNA strands of CpG dyads. Methyl-CpG-binding domain (MBD) proteins, such as human MeCP2, are central readers of methylated CpG dinucleotides. Complete biochemical profiling of MeCP2 binding different combinations of modified cytosine nucleobases at CpGs identified its interaction preferences for each combination. [2] Recently, evolution of MeCP2 using bacterial cell surface display resulted in the first affinity probe for strand-asymmetrically modified hmC/mC CpGs. [3] However, the interaction on a molecular level is still elusive. Here, we used site-directed spin-labeling (SDSL) in combination with double electron-electron resonance (DEER) spectroscopy to map the binding orientation of human MeCP2 to modified CpG dinucleotide-containing DNA. [3] We found MeCP2 binding to strand-symmetrically modified mC/mC CpG dyads oriented in two different ways relative to DNA, whereas one orientation dominated the other. In contrast, evolved MeCP2 T/A/Y/N exhibited only the aforementioned preferred orientation when binding to strand-asymmetrically modified hmC/mC CpGs. Together, our results depict the binding orientation of the DNA duplex reader MeCP2 to modified CpG dyads and reveal the basis for recognition of strand-asymmetrically CpG modifications by evolved MeCP2 T/A/Y/N variant.

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Posters

PO108

Benchtop EPR Spectroscopy of engineered metal oxides enables Integrated Testing Strategy that Reduces Animal Testing

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Products such as skin care, paint, and various fabric treatments rely on the effects of metal oxide structures. Free radicals can be the initiating event of toxicity of these structures, and hence an integrated approach to testing and assessment (IATA) requires the measurement of surface-induced reactions to group similarly reactive nanomaterials and to assess their safe usage. Electron paramagnetic resonance (EPR) has shown to be a reliable and capable instrumental technique for this purpose. It has been recognized and accepted in combination with a defined set of other chemical-physical tools, from our efforts and other collaborators, to use grouping and read-across as an alternative to animal testing.

EPR relies on the presence of unpaired electrons to gain insight into various chemical environments. In this study, the unpaired electrons in question are generated from/by the surface of engineered metal oxide nanomaterials in the form of reactive oxygen species in solution, specifically hydroxyl radicals (•OH). To counter the relatively short lifetime of free radicals in solution at room temperature, the technique of spin trapping/probing was employed. By using the spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and spin probe 1-Hydroxy-3-carboxy-2,2,5,5-tetramethylpyrrolidine (CPH), the lifetime of the generated radicals can be elongated and observed. Both trapped hydroxide radical adducts, DMPO/•OH and CPH/•OH, have characteristic EPR line shapes and multiplicities, where signal area correlates linearly with generated •OH radical. By screening over a wide variety of materials and analyzing the respective adduct signal, the test materials can be ranked and compared against representative test materials with well-known toxicity.

Posters

PELDOR on fully deuterated RNA

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PELDOR (pulsed electron electron double resonance [1]) 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.

The commonly used 4-Pules PELDOR method [2] is dead time free, the zero time of the dipolar evolution is accesable. However the possible distance between the two spin labels depends strongly on the phase memory time in such systems. The phase memory time is manly determined by surrounding spins in the sample and depends on the gyromagnetic ration of such spins. Therfore protons and other electrons are the main contributors. Basic techniques to get rid of such spins is substituting protons by deuterions and reducing the overall concentration of electron spins to a minimum [3].

In this contribution I will show the effects and the maximal possible distances of fully deuterated RNA complexes. These DENV 3'SL RNA (dengue virus [4]) was prepared as fully deuterated complex and some as well as protonate. Due to the size of this complex a maximum spin-spin distance of about 10nm was possible. A second RNA construct (HIV-1 [5]) can have longer distances, however due to the more flexible nature the distance is broader. The used spin label was TPT3(CO) [6].

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PO110

Influence of Spin Label Conformer Ensembles on Pulsed Dipolar EPR Distance Distributions

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Pulsed dipolar EPR spectroscopy (PDS) is a powerful tool to obtain coarse-grained structures of biomolecules like proteins and oligonucleotides [1]. By measuring the dipolar coupling between the spins of unpaired electrons, PDS provides distributions of interspin distances that permit insights into the conformation and the flexibility of the biomolecule. Unpaired electrons may be intrinsically present, e.g., in form of paramagnetic metal ions, or they can be introduced as spin labels that selectively react with, e.g., cysteine residues of a protein. A commonly used spin label for in vitro experiments is MTSSL, while trityls like SLIM [2] and TSL-Mal [3] are advantageous for in cell experiments.

Here, we compare the distance distributions obtained from MTSSL, SLIM, and TSL-Mal on metmyoglobin (MetMb). MetMb contains a spatially fixed low-spin Fe(III)-ion, i.e., only one spin label has to be attached for a PDS experiment measuring the distance distribution label/Fe(III). With one spin site being rigid and using the same labeling site in all three cases, the distance distributions will differ only due to the structure and the flexibility of the three spin labels, ultimately reflecting the differences among the conformational ensembles of the labels.

The experimental findings will be corroborated by simulations using mtsslWizard [4] and CREST/MD [5], which permit a further analysis of the conformer clouds.

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Investigation of manganese doped ferroelectric [NH4][Zn(HCOO)3] formate framework using EPR spectroscopy

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Metal-organic frameworks (MOFs) are extensively studied hybrid materials due to their potential applications in gas storage and separation systems and multiferroic memory devices. These coordination networks are formed from various organic linker molecules and metal centers that constitute porous structures. In the so called dense MOFs, the pore system inherently confines molecules, which are tightly bound to the framework. The most popular class of dense MOFs is metal-formate frameworks, which often exhibit interesting ferromagnetic and ferroelectric properties. Many members of these frameworks exhibit structural phase transitions, related to molecular cation ordering and metal-formate framework deformation. A useful method to study local changes in formate frameworks is electron paramagnetic resonance (EPR) spectroscopy.

In this work we present X-band and Q-band continuous wave (CW), pulse EPR and electron nuclear double resonance (ENDOR) study of manganese doped [NH4][Zn(HCOO)3] (AmZnF) hybrid formate framework, which exhibits ferroelectric phase transition at 190 K. The temperature dependent EPR spectra of AmZn:Mn2+ powder are typical Mn2+ powder patterns of the 3d5 electronic configuration. The obtained non-zero value of the zero-field splitting at low temperature shows the deformation of the MnO6 octahedra.

In order to study the broader environment of the Mn2+ probe ion, we performed pulse EPR experiments. As expected, the low temperature electron spin echo-detected field sweep spectrum corresponds to the powder pattern of the Mn2+ ions, indicating the uniform distribution if Mn2+ centers in crystal lattice. The two and three pulse electron spin echo envelope modulation (ESEEM) measurements data indicates the interaction between Mn2+ center and protons. ENDOR spectrum shows the interactions of different protons with Mn2+ center. Longitudinal relaxation measurements of low temperature phase shows that the longitudinal relaxation is dominated by the direct process of the acoustic lattice phonons at lower temperature and the two-phonon Raman process at higher temperatures.

PO112

Determination of Hyperfine Coupling and Chemical Shielding parameters through Bayesian optimization from 19F-ENDOR spectra

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ENDOR spectroscopy is a magnetic resonance technique with many different applications such as the recently developed intermediate range distance measurements using 19F-nuclear spin labels. [1,2] However, to derive precise structural information from experimental data careful analysis and simulation of the experimental data are crucial.

In this contribution an analysis pipeline for ENDOR spectra at high magnetic fields is presented. The experiments were recorded in batches to account for changes in measurement conditions during long time measurements (> 8h) causing a drift of the data matrix. The previously reported 'drift model' for data processing[3,4] then can be applied to extract the spectrum from the data matrix and to additionally get an uncertainty estimation. Then Bayesian optimaization is used to find simulation parameters that best reproduce the extracted spectrum. Based on the uncertainty of the spectrum (from Drift model) and the deviation of spectrum and simulation with respect to the parameters, the uncertainty of the parameters (from Bayesian optimization) can be estimated.

This approach was applied to 19F-ENDOR spectra at 263 GHz using a previously published nitroxide model system with one fluorine spin label[1,2]. In this case 9 interdependent parameters needed to be optimized: 3 to describe the dipolar hyperfine coupling tensor and 6 for the chemical shielding tensor. For all orientation dependent spectra an improvement in the simulation could be archieved and for all simulation parameters opitimized values with estimated uncertainties were found.

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Characterization of a ground-state triplet vinylidene

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Singlet vinylidenes have been proposed as intermediates in a series of organic reactions, and very few were studied by matrix isolation or gas-phase spectroscopy. Triplet vinylidenes, however, featuring two unpaired electrons at a monosubstituted carbon atom, thus far have only been predicted as electronically excited-state species. In contrast to the rich chemistry of triplet carbenes, triplet vinylidenes represent an unexplored class of carbon-centered diradicals. Here, we report the photochemical generation and low-temperature isotope-sensitive EPR/ENDOR characterization of the first ground-state triplet vinylidene.[1]

One likely reason for the lack of experimental data on ground-state triplet vinylidenes is the missing synthetic access. This was highlighted by Stang in 1978, suggesting diazoalkenes ($R_2C=C=N_2$) to be the best vinylidene precursors.[2] The successful synthesis of stable diazoalkenes in 2021[3,4] enabled the entry into this new fundamental compound class.

Upon irradiation of a diazoalkene precursor, field-swept EPR experiments revealed the formation of a triplet species. Its zero-field splitting parameter D lies in the range typical for well-known triplet carbenes. Key to recognizing the triplet species as a first representative of a novel class was the ^{13}C hyperfine tensor determined from orientation-selective ENDOR experiments. A distinctly small Aiso value demonstrated the compound's electronic structure was significantly different from that of triplet carbenes, in excellent agreement with DFT calculations. Theoretically predicted triplet ground state, with a significant triplet-singlet gap, was experimentally corroborated by temperature-dependent EPR measurements. Additionally, the compound's stability was assessed via changes in the EPR intensity at various temperatures. This work represents a fundamental discovery for organic chemistry, which may lead to applications such as new high-spin organic materials.

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Optimization of Rapid Frequency Scan EPR Experiments at High Magnetic Fields

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Recent developments of the microwave (MW) technique enabled a significant progress of Electron Paramagnetic Resonance (EPR) spectroscopy in the sub-THz frequency range. An important achievement of this progress is the performance of frequency sweep spectral measurements at frequencies above 0.2 THz [1,2]. Even more interesting, in these measurements the irradiating MW frequency can be scanned very rapidly in comparison to relaxation times T1 and T2. This allows us to detect short relaxation times in nanosecond range and to improve the sensitivity of EPR technique. However, High Frequency Rapid Scan (HFRS)-EPR experiments yield more complicated and less studied signals in comparison to spectra which are recorded in usual field sweep CW EPR experiments with very slow scan rates. In this work we perform numerical simulation aiming to optimize conductions of HFRS-EPR experiments. We use computational method based on the quantum statistical Liouville/von Neumann equation for the density matrix to determine spin dynamics of paramagnetic system affected by the microwaves with a rapidly swept frequency[3]. Using nitroxide spin system as a demonstration example, we investigate dependence of rapid scan signal on various experimental parameters such as frequency scan rate, MW power, frequency time dependence, relaxation times, radical concentration and presence of spin exchange interaction to find optimal conditions for HFRS-EPR experiments. Additionally, we optimize procedure of experimental data analysis in order to extract information about molecular dynamics and relaxation processes based on HFRS-EPR measurements.

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An insight in the structural dynamics of UreG in cellular environment: a SDSL-EPR study

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UreG is a GTPase that assists the maturation of nickel-dependent urease. It couples the hydrolysis of GTP with the delivery of Ni(II) ions into the active site of the enzyme cooperating with three additional chaperones. The crystal structure of UreG shows a fully folded protein, while in solution the protein features an intrinsic flexibility1. Because of the combination of this structural flexibility and the hydrolase activity, UreG was classified as the firstly discovered intrinsically disordered (ID)-enzyme2.

We previously demonstrated that Site-Directed Spin Labeling coupled to Electron Paramagnetic Resonance (SDSL-EPR) is a well suited technique to study the local dynamics of UreG in vitro. Using this approach we showed that folded states are coexisting with disordered ones all along the protein backbone3,4. Furthermore, we discovered an allosteric regulation of the two binding sites of the protein, for GTP and Ni(II). The physiological relevance of the balance between these two conformational states is not clear yet.

In this work we firstly investigate the dynamics of UreG by nitroxide-based SDSL-EPR in vitro, in the presence of crowding agents. Subsequently, we developed two delivery methods to internalize spin-labeled variants of UreG in E. coli. We show, for the first time, that the very flexible conformer of UreG exist also in-cell. On the other hand, a more compact folded state appears in the cell, and is probably needed for the native activity of the protein. We demonstrate that the integration of in vitro data with in-cell ones is fundamental for characterizing physiologically relevant rearrangements of conformations and that SDSL-EPR is a valuable approach for studying them.

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PO116

Modelling Conformational Flexibility in a Spectrally Addressable Multi-Spin Molecular Qubit

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Dipolar coupled multi-spin systems have the potential to be used as molecular qubits.[1] Herein we report the synthesis of a g-engineered Cu(II) porphyrin functionalised hybrid [2]-rotaxane molecular multi-spin qubit with three individually addressable, weakly interacting, S = 1/2 centres of differing g-values.[2] We use pulsed Electron Paramagnetic Resonance (EPR) techniques to characterise and to separately address the individual electron spin qubits; Cu(II), Cr_7Ni ring and a nitroxide, to determine the strength of the inter-qubit dipolar interaction. Orientation selective Relaxation-Induced Dipolar Modulation Enhancement (os-RIDME)[3] detecting across the Cu(II) spectrum revealed a strongly correlated Cu(II)-Cr_7Ni ring relationship while os-RIDME detecting on the nitroxide resonance resulted in addressability of either the nitroxide-Cu(II) interaction or the nitroxide-Cr_7Ni ring interaction by varying the experimental mixing block time and temperature. Orientation selective Double Electron-Electron Resonance (os-DEER)[4] measured between the nitroxide and Cu(II) confirm a global fit of accessible orientations of the multi-qubit architecture and a self-consistent model of the complex derived purely from the dipolar data.

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PO117

Peptide-RNA Coacervates as a Cradle for the Evolution of Folded Domains

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Peptide-RNA coacervates can result in the concentration and compartmentalization of simple biopolymers. Given their primordial relevance peptide-RNA coacervates have been proposed to be key site of early protein evolution. To understand how the complex structures of proteins could have emerged from simple polypeptide, we used the helix-hairpin-helix (HhH) motif, an ancient and ubiquitous nucleic acid binding element, which upon duplication and fusion, adopts a rotationally symmetric architecture called the (HhH)2-Fold.(1) Using double electron-electron resonance (DEER) distance measurements on site-specifically nitroxide spin labeled peptides containing one HhH motif we showed that a single, simple HhH motif can dimerize to form the (HhH)2 fold retaining partial helical structure even in the absence of RNA. The peptide can form coacevates at different peptide/RNA ratio and using continuous wave (CW) EPR spectroscopy we could distinguish free and RNA bound form as fast and slow rotational dynamics, respectively. Combining a series DEER and echo decay measurements at different peptide/RNA ratio we also showed that polypeptides within peptide-RNA coacervates also adopted dimers with the dimerization promoted by their association with RNA. Interestingly, RNA bound peptides showed significant loss in echo intensity and DEER modulation depth and background owing to fast phase relaxation from high local spin concentration. We conclude that coacervates are a unique testing ground for peptide oligomerization and that phase separating peptides could have been a resource for the construction of complex protein structures via common evolutionary processes, such as duplication and fusion.

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Posters

Distance measurements reveal dynamics of monomer reshuffling in G-quadruplexes

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G-quadruplexes (GQs) are DNA secondary structures formed by self-assembly of guanine-rich oligonucleotides via Hoogsteen base pairing stabilized by central cations. The in vivo formation of GQs in oncogene regulatory regions and within telomeres makes them an interesting target for cancer research.[1] The ability to assemble into higher-order structures, inherent to various GQs is believed to play a role in their biological activity.[2] Therefore, understanding these structures and their formation is essential.

We recently demonstrated the dimerization of GQs in solution via pulsed-dipolar EPR (PDEPR) spectroscopy in combination with Cu^{2+} labeling of the monomers.[3] The unprecedented accuracy of our data to distinguish GQ dimers with three and four G-tetrads per monomer (six and eight per dimer, respectively) is a testimony to the strength of our approach for elucidating structure of GQs.

Given the dynamic nature of such a system, we expected that the two GQ homodimers of different lengths exchange monomers upon mixing, i.e., to form heterodimers with seven G-tetrads. Here, we present the detection of such heterodimers. We monitored the formation of the mixed-species as a function of mixing time. We resolved three different dimer species. Our ability to monitor the time-dependent monomer exchange enables key insights into important reaction parameters such as binding kinetics. The ability to access these parameters is essential for developing drugs targeting higher-order GQ structures.

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PO119

ESEEM spectroscopy of methyl group quantum tunneling in
Co-doped dimethylammonium zinc formate

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Methyl groups are ubiquitous functional groups in chemistry making them an abundant motif of organic and hybrid materials. At low temperature, the methyl group acts as a quantum rotor and undergoes quantum tunneling. Recently, quantum tunneling of methyl groups was observed using electron spin echo envelope modulation (ESEEM) spectroscopy in Mn-doped $[(CH_3)_2NH_2][Zn(HCOO)_3]$ (DMAZn:Mn) hybrid perovskite. Despite this and subsequent studies, many unsolved problems remain, including the influence of relaxation times on observing the tunnel splittings.

In this work, we use Co(II) as a paramagnetic center to study the methyl group tunneling in $[(CH_3)_2NH_2][Zn(HCOO)_3]$ (DMAZn:Co) hybrid perovskite. The tunneling signal appears as a field-independent line in three-pulse ESEEM spectra. By comparing our results with the Mn(II) case, we investigate how paramagnetic dopants with different spin Hamiltonians and relaxation times affect the rotation barrier and thus the methyl group tunneling.

Posters

PO120

The global conformational equilibrium of the kinase Akt1 monitored by DEER spectroscopy and multilateration

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The serine/threonine kinase Akt1 plays a key role in cell signalling and is part of the PI3K/Akt pathway. A dysregulation due to mutations in the protein sequence has been shown to lead to serious diseases including cancer. Akt consists of two domains, the pleckstrin homology (PH) domain and the kinase domain which are connected by a flexible linker. Protein function and activity seem to depend on the relative conformation of these domains to each other [1].

Site-directed spin labelling in combination with DEER distance measurements and subsequent multilateration of the obtained distance distributions proved to be an ideal tool to track conformational changes of kinases [2]. Here, the global conformation of the kinase Akt1 was visualized in its different states of activation. While in the inactive "PH-in" conformation the two domains are close to each other and restricted in their movement, the "PH-out" conformation seems to be a highly flexible state, where the domains are further separated. The apo state shows an intermediate flexibility and distance between the PH and kinase domain. Additionally, it was demonstrated that the cancer-associated E17K mutation has no significant influence on these global conformations of Akt1.

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Posters

Parahydrogen-based Hyperpolarization of Biomolecules via Chemical Exchange

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The main sensitivity limitation of NMR/MRI can be overcome using hyperpolarization techniques. Parahydrogen-based methods are of particular interest due to their low cost and ability to quickly produce large boluses of hyperpolarized material. Integration of such techniques with portable (benchtop or ultralow-field) NMR spectrometers holds great promise for democratizing NMR equipment and improving sensitivity. A major drawback of parahydrogen-induced polarization (PHIP) is the need for dedicated compound (precursor) which is modified during the reaction to produce a hyperpolarized target molecule. Recently, this shortcoming has been addressed via relay of the hyperpolarization to the target by the chemical exchange (PHIP-X). This approach extends the applicability of PHIP towards all biomolecules possessing exchangeable protons while no brutto alteration occurs with a target molecule[1].

Here, we present an $^{15}N/^1H$ NMR investigation of a PHIP-X-based hyperpolarization of biomolecules with -NH groups: urea, ammonium, and glycine. As a transfer molecule, propargyl alcohol was used with a subsequent polarization transfer via the proton exchange mechanism at low magnetic fields. To maximize the efficiency of the polarization transfer, we investigated catalyst concentration, parahydrogen bubbling time, and polarization transfer field. NMR pulse sequence INEPT (insensitive nuclei enhanced by polarization transfer) was used to enhance ^{15}N NMR signal of target biomolecules. Signal enhancement of ~17,100 was observed for [^{13}C, $^{15}N_2$]-urea and $^{15}N_2$-urea at 1 tesla corresponding to the polarization levels of ~1.2% per molecule. We also showed that higher concentration of the transfer agent enables repolarization of the target molecule yielding acquisition of many NMR transients. In addition to biomolecules such as ^{15}N-ammonium, ^{15}N-urea, and ^{15}N-glycine, ^{15}N-benzamide was hyperpolarized by PHIP-X with the obtained optimum parameters. These results demonstrate the great potential of PHIP-X for hyperpolarization of a wide range of chemicals further improving the applicability of portable spectrometers.

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Improving NMR sensitivity with microcoil-based Photo-CIDNP hyperpolarization

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In order to improve the limit of detection (LOD) in NMR, microcoils have been developed to increase the sample mass sensitivity of the NMR probe. This concept is based on the inverse scaling of the coil diameter with the detection sensitivity of the radiofrequency coil. NMR microcoils are being exploited both commercially and are seeing development in-house at various NMR research groups. Although microcoils improve the LOD for mass-limited samples effectively, the sensitivity for concentration-limited samples can still be improved.

In the past years we have worked on the development and application of various microcoil geometries, namely planar spiral and solenoidal microcoils. The microfluidic NMR chips have good mass sensitivity, with active volumes in the (sub)microliter range. Planar spiral microcoils can be used in non-tuned, broadband mode, which means a single microcoil can be used for NMR experiments on both proton and x-nuclei. Moreover, a single broadband planar spiral coil can perform homo- and heteronuclear multidimensional NMR experiments. The planar spiral microcoils have been applied for on-line reaction monitoring of thermally and photochemically activated reactions. Solenoidal microcoils have the benefit of being easily constructed using our ESCARGOT method, with the coils being stabilized inside a PDMS matrix.

To further improve the sensitivity gain from using these microcoils, we introduce a system for microfluidic on-flow Photo-CIDNP hyperpolarization. With this type of system, the improved detector sensitivity, as well as an increased nuclear spin polarization are combined to improve the mass and concentration LOD in NMR. We have been able to improve the LOD down to the (sub-)picomole range, rivalling state-of-the-art NMR equipment in mass sensitivity. This major sensitivity improvement also concerns the concentration LOD, enabling the detection in the (sub-)mM range within minutes, with μL down to nL sample volumes.

PO123

Deuteron-decoupled singlet NMR in the microtesla regime for the generation of hyperpolarised agents

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Magnetic Resonance techniques provide powerful analytic tools due to broad applicability and versatility. Relatively small polarisation levels of the nuclear spins however result in an inherently low sensitivity. This problem may be resolved by utilising nuclear hyperpolarisation techniques. In the case of hydrogenative PHIP para-enriched hydrogen gas is initially reacted with a precursor molecule, the non-magnetic singlet order of the hydrogen-pair is then converted into observable magnetisation on a target nucleus.

There is increasing interest in performing the polarisation transfer step at low magnetic fields moving away from expensive equipment requirements. Several polarisation transfer techniques at low fields are optimised for H_2X spin systems and suffer in the presence of additional proton spins. A common optimisation strategy of the transfer step involves isotopic substitution of disruptive proton nuclei by deuterium. Deuterium spins simplify the spin dynamics but introduce singlet order polarisation sinks due to their own quadrupolar relaxation. This is particularly devastating during the bubbling period of the reaction. Additional losses are often caused by coherent singlet-triplet-mixing mediated by the target nucleus.

To address these issues, we combine our recently introduced WOLF (Weak Oscillating Low Frequency) and STORM (Singlet-Triplet Oscillations through Rotating Magnetic fields) pulses[1,2]. STORM pulses were designed to be resilient with respect to quadrupolar nuclei during the transfer step but did not address any losses during the reaction. Here we show that simultaneous application of a WOLF and STORM pulse not only decouples the deuterium spins during the transfer and bubbling period, but also suppresses coherent leakage effects. Our two-fold suppression strategy leads to significant improvements in the resulting polarisation levels when working in the microtesla regime. We illustrate some of the concepts by considering isotopically labelled succinic acid-1-^{13}C-2,3-D_2, which displays a four-fold increase in its polarisation levels compared to conventional field sweep methods.

[1] https://doi.org/10.1063/5.0065863

[2] https://doi.org/10.48550/arXiv.2202.04604

The Role of Methyl Dynamics in DNP

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Methyl NMR studies have become very popular during the last decades. The fast three-fold reorientation of the methyl group around its symmetry axis (H_3C-C) yields advantageous relaxation properties and thus well-resolved NMR spectra. [1] Moreover, it was recently shown that the three-fold reorientation is still active under DNP conditions [2], which is exploited in SCREAM-DNP (Specific Cross Relaxation Enhancement by Active Motions under DNP). Here, the polarization transfer from the hyperpolarized ^1H spins to ^{13}C is driven by the cross-relaxation-promoting methyl dynamics. The specifically hyperpolarized methyl-^{13}C can then be used, for example, for investigating the binding of a ligand in a large biomolecular complex. [3]

The aim of this study is to present a more detailed, quantitative understanding of methyl dynamics particularly under DNP conditions, which have been underreported so far. Therefore, selectively deuterated methyl groups of various methyl-bearing molecules, e.g. amino acids, are investigated by ^1H-^2H CPMAS. Our results suggest a distribution of R_1 relaxation rates represented by a stretched exponential function as it was also shown in wetted protein powder. [4] The calculated activation energies of the three-fold hopping mechanism strongly depend on the sterical environment of the methyl group which has been already qualitatively reported by SCREAM-DNP enhancement factors. [5] We will also present perspectives on the measurement of methyl dynamics in proteins under DNP conditions.

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PO125

PHIPNOESYS: A System for Intermolecular Nuclear-Overhauser-Effect-Mediated Transfer of Parahydrogen-Induced Polarization

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The relatively weak coupling of nuclear spins to the environment is both a blessing and a curse for NMR: on one hand, it enables long spin coherence times and therefore high resolution and chemical specificity – on the other hand, it limits sensitivity because the equilibrium nuclear magnetization is so small. The most promising path to increased sensitivity seems to be to move beyond equilibrium polarization with so-called hyperpolarization techniques.

We recently reported a new strategy for polarization enhancement, where naphthalene crystals are optically polarized to ca. 20-25% ^{1}H spin polarization, then dissolved in target solutions. This allows for the transfer of hyperpolarization from the "source" material to analytes of interest via the intermolecular nuclear Overhauser effect, with a system we call HYPNOESYS [1]. Advances in parahydrogen-induced polarization (PHIP) have since led to development of a PHIP-based HYPNOESYS–or PHIPNOESYS–prototype. Rather than naphthalene crystals, the system reacts [1-^{13}C,d$_6$] dimethyl acetylenedicarboxylate with parahydrogen to produce singlet-polarized dimethyl maleate, then converts the singlet order into in-phase ^{1}H magnetization via a field sweep. This hyperpolarized source material is then mixed with a solution of interest and transported into an NMR spectrometer for measurement.

In initial tests, PHIPNOESYS has achieved source ^{1}H polarization >8% at ^{1}H concentrations >1M. When mixed with a target solution containing diethyl [difluoro(trimethylsilyl)methyl]phosphonate, all ^{1}H sites were enhanced by a factor of 2-4. Interestingly, we have made other measurements that suggest that at least another order of magnitude in signal enhancement is achievable. We will discuss our efforts to achieve this improvement, as well as some interesting spin physics relevant at very high levels of nuclear magnetization.

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PO126

Diamond-based hyperpolarization at X-band frequencies

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Hyperpolarization techniques are actively developed, as a route to overcome the low intrisic sensitivity of nuclear magnetic resonance. Established methods have been shown to provide strongly enhanced signals, by up to more than 4 orders of magnitude using e.g., Dynamical Nuclear Polarization [1]. Methods based on optically pumped spin triplets allow to circumvent thermal effects, allowing one to efficiently conduct DNP at room temperature. In particular, a possibility exists to use the Nitrogen-Vacancy (NV-) in diamond as a source of polarization. While a number of experiments have shown that NV- can be used as a resource to efficiently hyperpolarize the 13C spins in diamond in bulk or powder form [2,3,4], achieving sizeable hyperpolarization levels in nanodiamonds, together with the constraint of room temperature, remains challenging. This has until now put off the prospect of using these, for instance, as candidate tracers for Magnetic Resonance Imaging. While some previously described hyperpolarization schemes rely on operation at magnetic fields as low as 10-30 mT [2], switching the magnetic field to higher values of operation could bring the benefit of prolonged nuclear polarization buildup times. We will discuss experiments performed at X-band frequencies and   with a comparatively high magnetic field, of ~300 mT, in single crystal, micro- and nanodiamonds. Benefit of using robust polarization sequences [3], in that context, will be reviewed. Last, we will propose a model for understanding particle size effects, and briefly discuss possible ways to ensure the illumination of large quantities (mg) of nanodiamonds, taking into account their strong light scattering properties.

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PO127

Quantum coherences as origin and source for further optimization of signal amplification by reversible exchange

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Parahydrogen-induced polarization (PHIP) is a quickly developing, cost-efficient hyperpolarization method to enhance MR signals. Here, we study the spin order transfer of parahydrogen (pH2) and a substrate at ultralow-fields (ULF, < 10 mT) using a PHIP variant called signal amplification by reversible exchange (SABRE). PHIP and SABRE already feature high 13C and 15N polarization levels above 20%, however, not for all substrates.

We used a magnetic field cycling SQUID-based NMR spectrometer that operates at $B0 \approx 55$ µT. SQUID-NMR allows several heteronuclei to be measured simultaneously. Two substrates: 3-19F-pyridine and 15N-acetonitrile were polarized by SABRE in methanol using the [IrIMesCODCl] catalyst system.

We observed that high order spin states are generated in the SABRE experiment with a polarization field $Bp = 5.2$ mT. Using correlation spectroscopy (COSY), we were able to measure multiple quantum coherences (MQC) and differentiate them using a 4-step phase cycle technique. Homonuclear (TH or TF) and heteronuclear 1H-19F (THF) coherences of 19F-pyridine from -3 to +3 were detectable experimentally.

Alternating magnetic fields between $Blow \sim 1$ µT and B0 (alt-SABRE-SHEATH) make use of the strong coupling at Blow and the fast coherent evolution at B0. The spin order flow between pH2 and 15N of 15N-acetonitrile were measured with the frequency of $\nu\_low \approx 119.0$ Hz at Blow and $\nu\_high \approx 2541$ Hz at B0. The resulting alt-SABRE-SHEATH 15N polarization was up to 30% higher than in the SABRE-SHEATH experiment with an optimal constant hyperpolarization field.

Both experiments were reproduced using spin dynamics simulations.

The observation of MQCs confirmed the general understanding that at ULF, high order multi-spin states are populated. As a result, polarization is distributed among many spins, resulting in lower polarization for a specific nucleus. Applying new alt-SABRE-SHEATH protocols to other labeled metabolites (e.g. nicotinamide) and drugs (e.g. metronidazole) might further improve this technique towards in vivo applications.

PO128

Temperature-Ramped Batch-Mode Spin-Exchange Optical Pumping of Xenon-129 using a 3rd-generation Automated XeUS Hyperpolarizer

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The clinical-scale batch-mode generation-3 XeUS hyperpolarizer device can perform hyperpolarization of 129Xe via the Spin-Exchange optical pumping (SEOP) method safely at temperature range 60-95 °C. High temperature SEOP associates with faster rate of polarization buildup but yields a lower %PXe. We demonstrate a hybrid temperature-ramp approach of batch-mode SEOP, which takes advantages of both high-temperature fast buildup rates and maximum %PXe at lower polarization temperature. This ramp approach (95-85 °C) yields %PXe = 39.8%, whereas 95 °C polarization process produces %PXe = 35.9% in same period of time of 42 minutes. We have also tested the 129Xe polarization retention after hyperpolarized gas ejection in an external phantom using ex-situ polarimetry of 47.5 mT portable MRI. Additionally, we report on systematic quality assurance (QA) studies of limits of the device operation parameters and their effect of the robustness of SEOP hyperpolarizer operation. Specifically, we studied back-to-back reproducibility, the effects of SEOP temperature, calibrations of in-situ excitation RF pulse length, in-situ polarimetry resonance offset from 40.8 kHz resonance condition. The overall results of this study lead to better quality and more robust production of HP 129Xe gas on a clinical scale for imaging and bio-sensing applications. Moreover, we build a next-generation low-cost pocket-size (10 cm x 10 cm x 2 cm) XeUS NMR spectrometer which is capable of ultra-low field (40 kHz) signal-shot and signal-average detection. This NMR is tested using both in-situ detection of HP 129Xe and HP [1-13C]pyruvate. The polarization level was compared with our conventional way of polarization detection using Magritek NMR spectrometer.

PO129

Source suppression and spin dynamics in hyperpolarized liquid state NMR spectroscopy by optically polarized crystals

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One of the key challenges in solution-state NMR is sensitivity improvement. To tackle this issue, some of us recently introduced a new approach to hyperpolarization of solutions by dissolution of optically polarized, pentacene-doped naphthalene crystals.[a] This novel protocol exploits the intermolecular nuclear Overhauser effect (NOE) to transfer magnetization from the highly polarized naphthalene protons to other species in solution, and its main strength is that no supramolecular forces between naphthalene and the target molecules are in general required for the transfer to occur. Despite the low efficiency of intermolecular cross relaxation, the starting polarization of the optically polarized naphthalene is so high that (negative) proton signal enhancements between several hundreds to 1730 have been reported on small molecules at 61 MHz. Further experiments support the idea that significant enhancements on small molecules can be obtained also at higher fields (400 MHz). Whilst this method paves a new way to hyperpolarization in liquids, it also creates new challenges: first, the huge residual naphthalene magnetization causes undesirable effects such as radiation damping and receiver saturation, and second, strategies for efficient polarization of medium to large-sized targets at high fields in the T_1 minimum range must be foreseen.

Here we demonstrate that signal suppression pulse sequences can be successfully implemented to overcome both radiation damping and receiver saturation. In addition, such suppression techniques can be integrated into more complex experiments to broaden the collection of NMR experiments that one can apply in this context. We also show that polarization of medium and large systems can be improved by tuning the experiment or by exploiting additional interactions between source and target species.

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Posters

A triple resonance (e, 1H, 13C) probehead for DNP experiments in liquids at 9.4 Tesla

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Here we describe the design and performance of a probehead for Overhauser DNP experiments of 1H and 13C nuclear spins in liquid samples with a volume of up to 100 nl. We demonstrate on a 13C-labeled sodium pyruvate sample in water that proton decoupling under DNP conditions is possible with this new triple-resonance DNP probehead [1]. In addition, the heat dissipation from the sample has been greatly improved in comparison with the previous design [2] making it possible to keep the liquid sample at constant temperature almost independent of the irradiation with the 263 GHz microwave gyrotron up to several watt of output power. This improved performance allows disentangling the role of the sample temperature and the applied microwave power for the DNP efficiency in liquids at high magnetic fields and to obtain a quantitative determination of the EPR saturation factor by observing the suppression of the paramagnetic shift as a function of the applied microwave power [3].

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PO131

Real-time monitoring of rapidly signal-enhanced metabolites in Parkinson disease cell models via PHIP

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Nuclear magnetic resonance (NMR) has found a wide range of applications from analytics to biomedicine, but is largely limited due to the inherently low sensitivity. To overcome the sensitivity challenge, we present here a promising methodology to enhance the signals of 13C-labelled molecules via para-hydrogen induced polarization (PHIP) and that is based on the side-arm approach. In particular for pyruvate, an important metabolite in the cellular glycolysis pathway, we achieved an average of 27% 13C polarization (48,000-fold enhancement) of 1-13C pyruvate in aqueous solution which allowed us to monitor cellular energy metabolism in real time. As a proof of principle, our technique showed enough sensitivity to differentiate the pyruvate-to-lactate conversion rates between cells with and without treatment of Mitochondria complex I inhibitor, results which are in accordance with commercial lactate and ATP assay kits. For the first time, we extended our application to explore pyruvate metabolism in cell models with knock-out and overexpression of the gene of the α-synuclein protein. Aggregation of this protein is an important hallmark for Parkinson's disease. Based on our results, we envision the potential of our approach as biomarker for future disease and drug studies as well as bio-medicinal imaging especially in the field of neurodegeneration.

Hyperpolarised 2D 1H-1H NMR for the analysis of mixtures

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NMR spectroscopy is a powerful tool for the analysis of mixtures. An array of 1D and 2D experiments makes it possible to identify compounds in mixtures such as reacting media, biological extracts or biofluids. The sensitivity of NMR is, however, a major limitation for analytical applications. Hyperpolarisation methods have been developed that combine key features for mixture analysis: i/ the possibility to polarise 1H nuclei, and reach more favourable limits of detection for natural-abundance samples, ii/ broadband hyperpolarisation, that is not specific to classes of compounds. These methods include dissolution dynamic nuclear polarisation (D-DNP) [1], and the recently reported HYPNOESYS method [2], based on the dissolution of highly polarised naphthalene crystals followed by intermolecular cross-relaxation. The two methods, however, also share the feature that the enhanced polarisation is short-lived and not generally compatible with the acquisition of 2D spectra by conventional methods.

Here we show that multiple kinds of 2D 1H-1H spectra can be acquired for hyperpolarised mixtures, using ultrafast 2D NMR based on spatial parallelisation. First, we describe the acquisition of 2D TOCSY spectra for mixtures hyperpolarised by D-DNP. We also show that high-quality multiple-quantum/single-quantum spectra, of the kind employed for maximum-quantum analysis of aromatic mixtures, can be obtained. Enhancement of over 100 are observed, thanks to a fast and stable dissolution process [3]. We then describe the first 2D data sets obtained with the HYPNOESYS approach, which has the advantages of simplicity and portability. Using a benchtop NMR spectrometer, 2D COSY spectra are obtained in a single scan, with enhancement of up to 28. These results open complementary routes towards the sensitive and information-rich analysis of mixtures.

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[3] Singh et al., Chem. Commun. 57, 8035 (2021)

PO133

Dissolution DNP of complex mixtures using hyperpolarizing polymer (HYPOP) matrices

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Dissolution Dynamic Nuclear Polarization (dDNP), since its discovery, proved to be a powerful tool for Magnetic Resonance. Furthermore, it can be coupled with both NMR and MRI. Due to the high interest of biological applications, a lot of study focus their work on biomolecules, most of the time separately. [1] A main issue for analyzing complex mixtures using dDNP is due to the formulation limitations of classical sample: radical doping, glass forming agent, solubility of the target….

Recently, our group developed a novel approach based on porous polymer containing radicals covalently attached in the bulk. [2] Such materials can be impregnated with doped solutions containing mixture of target molecules. The whole sample can be polarized (P(1H)>50% and P(13C)~25%) with the advantage that impregnated solutions neither need extra radicals nor glass forming agent, allowing to use a broader range of chemical species usually incompatible with nitroxide radicals (such as pyruvic and ascorbic acid), and concentrated solutions of salts. Furthermore, polymers can be used in dissolution process, only requiring the addition of an in-line filtration step.

This work focuses on the analysis by dDNP of mixture of molecules using this kind of porous polarizing materials and investigate the possibility to precipitate molecules in those materials, crossing the solubility limit barrier commonly impacting in standard formulation.

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PO134

Non-intuitive AC field sequences dramatically improve SABRE efficiency

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Signal Amplification By Reversible Exchange (SABRE) and heteronuclear extended SABRE (X-SABRE) enhance the signal of magnetic resonance techniques in less time and at a fraction of the cost of other hyperpolarization methods (<1% of the cost of dDNP). However, as SABRE is a relatively new technique, the physics governing the dynamics of polarization transfer have not been fully characterized. Here we discuss recent advances in the theoretical understanding, which have led to experimentally demonstrated signal enhancements under newly explored low-field sequences.

SABRE techniques either match a low field to energy level differences or apply RF pulses to shift levels into resonance, ideas that are rooted in conventional magnetic resonance techniques and Level Anti-Crossings (LACs). This traditional framework broadly describes SABRE dynamics, but SABRE demonstrates non-traditional dynamics in a low magnetic field regime which has not been extensively explored. Here we move away from the traditional approach to instead use perturbation theory and average Hamiltonian calculations to assess population transfer efficiency under newly introduced AC field sequences.

We use our physically accurate numerical simulations of SABRE polarization transfer as well as direct experiment to demonstrate that population transfer into target states can be optimized under fields far from the normal resonance conditions by strategically driving a phase shift in the heteronuclear J coupling terms of our effective Hamiltonian with an oscillating leading field. We additionally probe sequences with more general field geometries by applying multiaxial field sequences like a simple CW decoupling pulse along x in combination with an unbalanced square wave along z to prevent loss of singlet spin order to off target coherence transfer pathways. With this work, we improve the utility and generality of SABRE and open the technique up to further optimization in regimes which have otherwise been overlooked.

Revealing Rubber-silica Interaction in Tire Compound by 2D 29Si-29Si Solid-State NMR Enhanced by DNP

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Synthetic styrene-butadiene rubber (SBR) are often reinforced with filler nanoparticles to improve mechanical properties and meet specific requirements that can vary depending on the application. In the tire industry, the use of silica nanoparticles (SiO_2 NPs) as filler and polymers with silanol functional groups has become increasingly important. 29Si solid-state NMR has the potential to probe the interaction between SiO_2 NPs and silanes or polymer functional silanol groups in composite materials. However, due to the low concentration of silanol in the SBR sample, low natural abundance (4.7%) of 29Si, the difficulty and expense of isotopic labeling, 29Si NMR faces huge sensitivity challenge.

In this presentation, we show that by utilizing dynamic nuclear polarization under magic angle spinning (MAS-DNP), the sensitivity of the 29Si NMR can be dramatically increased. The MAS-DNP based on cAsymPol-POK and cAsymPol-TEK biradical and under 100 K resulting in a time saving factor of ~ 900, which allows the acquisition of 29Si-29Si correlation in SBR samples. Both through-space and through-bond 2D 29Si-29Si correlation experiments successfully distinguish the chemical bonding or the physical-adsorption of SiO_2 NP in SBR. This work demonstrates the great potential of MAS-DNP to characterize polymer-nanoparticle interactions for industrial relevant applications.

PO136

Solid state DNP-enhanced 1H NMR signals of γ-irradiated samples

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Dynamic Nuclear Polarization (DNP) has developed into a powerful technique to enhance the Nuclear Magnetic Resonance (NMR) signals in solids and liquids (1,2). DNP begins by transferring spin order from electrons to nuclear spins, by microwave irradiation (1,2). A feature associated to these experiments relates to their need to rely on "polarizing juices", where the sample of interest is dissolved together with stable electrons providing the DNP effect, in a common glassing matrix. While very general this approach may exhibit limitations associated to sample dilution and/or radical-driven relaxation losses, that compromise the maximum NMR sensitivity. This has been dealt with by using photochemically-induced radicals and polarizing solid matrices (3-4); here, we explore the performance of polarizing agents formed in situ, via γ-irradiation (5). Specifically, a series of common organic compounds were taken to a nearby ^{60}Co gamma irradiation facility and irradiated at with doses 50-150 kGy. The ensuing samples were examined solids under static DNP conditions at 3.3 T and 5-7 K range, using Continuous Wave Electron Paramagnetic Resonance (CW-EPR). This allowed us to identify the formation of stable organic radicals and their concentration dependence vs irradiation. DNP measurements were then performed, observing the ^{1}H signals of the compounds bearing the paramagnetic moiety. It was found that both the radical concentration, as well as the hyperfine couplings to nearby nuclei, affect the performance of DNP; the majority of candidates produced were good candidates for solids DNP procedures.

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A Device for the Oxidative Purification of Hyperpolarised Noble Gases after Spin Exchange Optical Pumping

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Hyperpolarized (HP) noble gases are effective contrast agents for pulmonary MRI to investigate lung function and microstructure. Following earlier work with helium-3, HP xenon-129 is approved for clinical MRI use in the UK. Additionally, krypton-83 is under active development in model systems and previous work demonstrated surface sensitive contrast via interactions of its nuclear quadrupole moment.

Most commonly, the HP state is achieved through Spin Exchange Optical Pumping (SEOP). Establishing this non-equilibrium spin state results in an MR signal amplification of 4-5 orders of magnitude compared to that of the thermal equilibrium.

However, SEOP routinely necessitates dilution of the pumped species with buffer gases such as nitrogen and helium. Consequently, typical HP gas concentrations of 3 – 10% lead to reduced signal intensities and the gas requires purification before delivery. Current cryogenic techniques to attain concentrated HP xenon complicate the production process and may cause some spin polarisation losses. Furthermore, cryogenic separation leads to rapid depolarization of HP krypton in the frozen state.

A completely novel approach to purify HP noble gas was introduced previously, that utilizes hydrogen as a buffer gas for SEOP, followed by its removal through catalytic triggered combustion.

Here, we present a prototype 'combustion system' comprising of a 4L reaction vessel and platinum coated catalyst to purify significant quantities of HP gas at low field. The computer-controlled process prepares batches of concentrated HP gas in just 15s with no significant penalty to the xenon or krypton polarisation due to the combustion process. Upon recompression, most of the resulting water vapour condenses out and the little that remains serves to extend the lifetime of the noble gas HP state. Development of this system focuses on more efficient HP gas recompression as well as increasing the purified HP gas volume to facilitate future clinical applications.

PO138

Microwave heating quantified by EPR near Helium-temperature DNP conditions

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In dynamic nuclear polarization (DNP) the very high, but temperature-dependent, electron polarization is used for hyperpolarization of nuclear spins. Microwave irradiation is essential for polarization transfer in DNP, but can also lead to sample heating through the electric part of the microwave field. Sample temperatures have been suspected to exceed the nominal cryostat temperature. Usually heating is only observed indirectly by a decrease in DNP enhancement. We examined microwave heating in a more direct approach via detection of the EPR line. Understanding and minimizing the sample heating effects, results in improved EPR sensitivity. With the development of pulsed DNP techniques it has become possible to excite electron spins and drive the DNP process with lower overall microwave irradiation.

EPR signals can be observed by longitudinal detection (LOD), using a dedicated detection coil. LOD relies on saturation and recovery of the electron Mz magnetization, with, in our case, a sub-millisecond time constant. The magnitude of the signal is proportional to the number of spins and depends on the temperature of the sample through the Boltzmann distribution. Heating effects have been investigated using a sample of 50 mM 4-oxo-TEMPO in 1:1 (v/v) water/glycerin at 7 T, 3.3 K while irradiating the sample with 66 mW power at 197 GHz. Irradiating the sample with microwaves prior to LOD measurements leads to an exponential decay of the EPR signal with a time constant of approximately 20 ms towards a constant value of 61.5 % of the maximum intensity. The signal relaxes back to thermal equilibrium with a similar time constant. We interpret this as an increased sample temperature, corresponding to 7.6 K. Since DNP efficiency in terms of maximum achievable polarization is also strongly dependent on temperature, DNP methods with lower power requirements should improve the efficiency of the DNP process.

PO139

Assisted Co-Ligand SABRE Polarising Keto-Acid Molecules

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Signal Amplification By Reversible Exchange platform reflects a highly desirable simple and low-cost route for hyperpolarisation and our main objective is to dramatically improve the range of biomolecules on which it can be utilised.

The original version of SABRE [1] hyperpolarises the heterocyclic aromatic amines (such as pyridine, nicotinamide, methyl nicotinate) which are weakly bound to the iridium of the catalyst. However, SABRE was unable to hyperpolarise biological substrates such as pyruvate, glucose, lactate, carbonate, etc., as they do not bind the iridium metal centre. As a consequence, SABRE is limited to the molecules that have affinity for the catalyst. A new version of SABRE that is named SABRE-Relay [2] was developed as a new technology to hyperpolarise any molecule having at least one labile proton, thus including the biological substrates. However, SABRE-Relay was not efficient in hyperpolarising keto-acid substrates because of the parasite condensation reaction between keto-acid substrate and an amine compound which is the hyperpolarising agent in SABRE-Relay.

The recent advancement in the form of co-ligands has made SABRE applicable towards keto-acid molecules e.g. pyruvate, a key step towards its potential clinical application. After succeeding in polarising pyruvate, a new SABRE version was introduced to hyperpolarise molecules with O-donor sites such as oxalic acid, maleic acid, acetoacetic acid, etc.[3]

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HypFlow - Inexhaustible Spring of Hyperpolarization

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Hyperpolarization by dissolution dynamic nuclear polarization (dDNP)1 has recently evolved into a robust method providing a decisive solution to this lack of sensitivity as it enhances nuclear spin polarization - and therefore sensitivity - by up to four orders of magnitude. High electron spin polarization is transferred to nuclear spins of interest in the frozen state. It is then followed by a 'single shot' irreversible melt, dilution, and transfer to a room temperature liquid-state NMR apparatus. This strategy is compatible with a few applications in which exhaustibility and pollution can be accommodated.

However, most NMR applications are multi-scan by essence and incompatible with hyperpolarization by dDNP. Indeed, NMR experiments quasi-systematically rely on multi-scan approaches. This situation is untenable, preventing numerous laboratories and industries to greatly benefit from this hyperpolarization strategy

The bottleneck for producing pure and inexhaustible hyperpolarized solutions comes from the very nature of the instrumental and sample formulation design, so far conceptualized and conceived for 'single-shot', contaminated, and diluted experiments.

On this poster, I present some of the most recent work on instrumental design, methodological and chemical developments,2,3 opening the way to a new approach that we are starting to develop, that aims at producing pure and inexhaustible streams of hyperpolarization on arbitrary complex mixtures.

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PO141

Reaction monitoring on organic and biological reactions using parahydrogen based hyperpolarization technique

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NMR (Nuclear Magnetic Resonance) and MRI (Magnetic Resonance Imaging) are the key spectroscopic and medical imaging technology in both science and industry. However, a high cost and effort for installation and maintenance of superconducting magnets and cryogenic conditions are considered as the major obstacle in taking full advantage of NMR and MRI. One of the best ways to overcome the need for superconducting magnets is to exploit hyperpolarization, in which spins are polarized beyond the Boltzmann distribution. Of several potential hyperpolarization techniques, parahydrogen, one of the spin isomers of hydrogen, is in particular a promising tool for obtaining hyperpolarized materials and enhancing reaction monitoring sensitivities. Here, several recent data on hyperpolarized materials will be introduced and reaction monitoring on amide coupling and hydrolysis of Remdesivir using Signal Amplification by Reversible Exchange (SABRE) will be discussed with its meaning and its future applications. Of other known reaction monitoring examples using SABRE system, this reaction monitoring on Remdesivir by esterase is the first report on the biological reaction monitoring using hyperpolarization.

PO142

Synthesis of Parahydrogen Derived Singlet State Molecules

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Singlet states have recently attracted much attention within the magnetic resonance community because of their property to store spin order for a period much longer than thermal relaxation. This is because these states are immune to mechanisms such as intramolecular dipolar relaxation, which is often the predominant relaxation mechanism in liquid-state NMR. The property of storing spin order significantly longer than commonly measured spin states has enabled studies of e.g., slow diffusive processes, in vivo metabolomic imaging/spectroscopy and protein-ligand interactions. A naturally occurring example of a singlet state is parahydrogen, a spin isomer of molecular hydrogen. It is known that parahydrogen can significantly improve the sensitivity of NMR through hyperpolarisation. Therefore, parahydrogen may be used to extend both the timescale and sensitivity of NMR-experiments. Some examples hereof have already appeared in literature. To this end, it is necessary to synthesise molecules, in high yield, with protons derived from parahydrogen, which retain their singlet state character. This can be achieved using hydrogenation catalysts with pairwise transfer mechanisms.

Here, we analyse the effects of processes which hamper the synthesis of singlet state molecules using parahydrogen. Namely, the transient inequivalence of singlet protons during or after the synthesis leads to a 'breaking' of the singlet state. This is typically associated to a hyperpolarised emission signal in the NMR spectrum. This inequivalence can occur in several ways. We discuss the dependence of these processes on the applied reaction conditions and possibilities to circumvent these effects, leading to the generation of singlet state molecules in good yield.

PO143

Low-field 1H Relaxation via Radical Non-Zeeman Reservoir in Solid Pyruvic Acid

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We report on the low-field nuclear spin relaxation in pyruvic acid, doped with trityl, and show that the ¹H relaxation time constants at low fields scale linearly with the applied field. The data are modeled using a thermodynamic approach, in which heat is transferred via triple-spin-flips involving two electron spins and one nuclear spin. The triple-spin-flip rate is calculated from first principles using a formalism developed by Wenckebach. The heat capacity of the electron Non-Zeeman reservoir is determined from the ¹H relaxation data at intermediate fields, which leads to a parameter-free, yet nearly quantitative description of the observed ¹H relaxation rates from 5 mT to 2 T.

Posters

Extending Indirect Cross Effect DNP Model with Broadband Irradiation and T1e Anisotropy

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Dynamic nuclear polarization (DNP) at high-field magnetic fields has become a prominent technique for signal enhancement in Nuclear Magnetic Resonance (NMR). In static samples, the highest DNP enhancement is usually observed for high radical concentrations 15 – 40 mM. Under these conditions, the dominant DNP mechanism for broadline radicals is the electron-electron spectral-diffusion-based indirect cross effect (iCE).[1] To further increase the DNP performance, broadband microwave irradiation is often applied. In our recent work we extended the iCE theory to explicitly include broadband irradiation.[2] We demonstrate that our theory allows for quantitative fitting of the DNP spectra lineshapes using four different datasets acquired at 3.4 T and 7 T. We show that the DNP mechanism changes with increased excitation bandwidth. While with narrowband continuous-wave irradiation, the DNP mechanism is a combination of the solid effect (SE) and iCE, it shifts toward iCE with increasing excitation bandwidth until, at high bandwidth, the iCE completely dominates the DNP– this effect was not accounted for previously. An additional effect that was never accounted for in the iCE model, is T1e anisotropy. We present preliminary results of adding the T1e anisotropy into the iCE model.

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PO145

Purified parahydrogen-hyperpolarized fumarate for preclinical in-vivo metabolic magnetic resonance imaging

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Introduction:
We present a method for the preparation of purified, hyperpolarized [1-13C]fumarate as a contrast agent for preclinical in-vivo metabolic MRI, via parahydrogen-induced polarization (PHIP). We compare our process to dissolution Dynamic Nuclear Polarization (d-DNP) and find that both methods achieve similar concentrations and polarization, while PHIP can provide a preclinical injection dose every 10 minutes. Metabolic conversion of fumarate to malate in tumor mice models was imaged.

Methods:
Using a dedicated polarizer, [1-13C]acetylenedicarboxylate was reacted with 10 bar parahydrogen at 90°C using a trans-selective catalyst to yield [1-13C]fumarate. The hyperpolarization is transferred from the hydrogen to the carbon nuclei via a magnetic field sweep. The hyperpolarized fumaric acid is precipitated and washed to remove residual starting material, catalyst and reaction side products. The purified solution was injected into mice tumor models inside a 11.7 T Bruker preclinical MRI, where the metabolic conversion was observed via a chemical shift imaging (CSI) sequence.

Results:
We achieve 45% 13C hyperpolarization in fumarate with a concentration of 180 mmol/l. 20% 13C polarization and a fumarate concentration of 100 mmol/l remains following purification. We investigated the efficiency of the purification process by monitoring the contamination concentrations at different stages of the process. Cell-toxicological studies were executed with the purified and unpurified solutions, s howing that the purification process is crucial for running ethical preclinical studies with PHIP hyperpolarized fumarate. We demonstrated the use of the hyperpolarized [1-13C]fumarate for in-vivo perfusion and metabolic imaging of necrotic tumors.

Conclusion:
We demonstrated that PHIP hyperpolarized fumarate is a viable cost-effective method for preclinical metabolic MRI. In terms of polarization and concentration, our system is comparable to a state-of-the-art d-DNP system. We found that the purification process decreases the concentrations of contaminants to non-toxic levels.

Cross-Polarization for Bullet-Dynamic Nuclear Polarization

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In Bullet-DNP [1, 2] a hyperpolarized sample is rapidly transferred with pressurized gas to the secondary magnet and dissolved only upon arrival. This approach may avoid excessive dilution and the associated signal loss, a problem often encountered in small volume samples.

Cross-polarization has been successfully used with Dissolution-DNP [3] and its popularity is increasing.

We report cross-polarisation results using a cross-coil DNP probe design in which two single loop coils are used to create two independent radio-frequency fields. The coils have diameters of approximately 7 and 10 mm only, and consequentially achieve a relatively high B1 field for a given power level.

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PO148

13C solid-state photo-CIDNP on a flavoprotein embedded in glassy sugar matrix

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Phototropin is a blue light receptor containing flavin mononucleotide (FMN) in its LOV domain. After photoexcitation with blue light, the FMN forms a photoexcited triplet state which in natural occurring LOV domains reacts with a conserved nearby cysteine to form a covalent adduct. By mutation of the cysteine to a serine, the adduct formation is prohibited and the lifetime of the photoexcited 3FMN is elongated. This induces a one-electron transfer from a tryptophan of the protein matrix (1 nm edge-to-edge distance) which in turn forms a radical pair as an intermediate leading to photochemically induced dynamic nuclear polarization (photo-CIDNP) signal enhancement in NMR which is observable in both solid and liquid states [1]. Here, isotope enrichment of 13C and 15N allows the selective detection of the hyperpolarized signals from both partners involved in the electron transfer.

The measurement time under illumination, however, is limited because of the generation of singlet oxygen by the photoexcited triplet FMN [2] which enhances the photodegradation of the protein. Besides, expensive and not straightforward isotope labelling can prohibit the search for photo-CIDNP in newly discovered proteins and impede further investigations on known ones. Therefore, an increase in stability of the protein under illumination can enable the use of two-dimensional solid-state photo-CIDNP NMR techniques for further investigations.

Here, we present an approach where the protein is embedded into a sugar glass matrix and measured with 13C solid-state photo-CIDNP NMR. This has the advantage that the solid-state experiment can be performed at room temperature while simultaneously prolonging the measurement time to investigate unlabeled protein samples here presented for LOV1 of phototropin C57S.

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PO150

Magnetic Resonance Imaging based on spontaneous emission

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The spatial resolution of MRI is fundamentally limited by the width of Lorentzian point spread functions associated with the exponential decay rate of transverse magnetization ($1/T_2$*). Here we show a different contrast mechanism in MRI by establishing RASER (Radio-frequency Amplification by Stimulated Emission of Radiation) in imaged media. RASER imaging bursts emerge out of noise and without applying (Radio Frequency) RF pulses when placing spins with sufficient population inversion in a weak magnetic field gradient. The population inversion is generated by SABRE on dissolved pyrazine, a molecule with four chemically and magnetically equivalent spins giving a single peak, ideal for imaging. A small difference in initial population inversion density creates a stronger image contrast than conventional MRI. This contrast is based on the cooperative nonlinear interaction between all slices. On the other hand, the cooperative nonlinear interaction gives rise to imaging artefacts, such as amplitude distortions and side lobes outside of the imaging domain. Both the contrast and the artefacts are demonstrated experimentally and predicted by simulations based on a proposed theory. This theory of RASER MRI is strongly connected to many other distinct fields related to synergetics and non-linear dynamics. The initial results motivate background-free RASER MRI as the RASER signals solely stems from negatively polarized molecules at low concentration. In this way, high spatial resolution is obtained applying weak gradients and in the absence of any RF-excitation.

Hybrid BDPA-Nitroxide Polarizing Agents for High-Field, and Variable Temperature MAS DNP

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MAS DNP is increasingly establishing itself as a powerful technique to boost sensitivity of NMR. At 9.4 T (400 MHz) and 100 K, DNP enhancements of 200–300 are now possible with several polarizing agents (PA) (AMUPol[1], TEKPol[2], and more recently[3] SPIROPOL,[4] PyPolPEG2OH,[5] bcTol,[6] AsymPolPOK,[7] O-HydrOPol,[8] …). Despite the excellent performance at 9.4 T, these dinitroxide biradicals are much less efficient at 18.8 T or higher field, and the need for more efficient PA stimulated the research of biradical with a narrow EPR line,[9] such as for trityl in TEMTriPol[10] and more recently NATriPol.[11]

Here we show how hybrid biradicals, based on the Cross-Effect mechanism and designed by coupling BDPA with a nitroxide unit, provide very high DNP enhancements at 100 K: up to 185 at 18.8 T and 40 kHz MAS,[12] and up to 200 at 21.2 T and 65 kHz MAS.[13] We also show that this exceptional enhancement obtained with HyTEK2 is also persistent with temperature increase, when it is dissolved in a suitable rigid glassy phase like ortho-terphenyl (OTP)[14]. Enhancements up to 60 are observed at the glass transition temperature (243 K; –30 °C) and at 18.8T.[15]

In particular, we will discuss how the DNP performance of HyTEK2 depends on a combination of several factors, from the magnetic properties of the polarizing agent to the role played by spin-diffusion, including simulations to interpret the role of the electron relaxation times in the variable temperature performances. This radical formulation has the potential to make accessible the characterization of materials at high field over a large range of temperatures.

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Posters

PO152

A pulsed field-independent PHIP-SAH method to hyperpolarize [1-13C]pyruvate in clean water solutions for biomedical applications

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Diseases are often correlated to metabolic dysfunctions. Signal enhanced methods in NMR and MRI opened windows for investigating metabolic processes in vitro and in vivo, with the aim to assess disease progression as well as therapy responses.[1,2]

Para-hydrogen based methods[3] (PHIP) augmented by side-arm hydrogenation[4] (SAH) provides a convenient way to enhance the NMR signal in biological relevant substrates.

Here, we introduce a field independent pulsed PHIP-SAH method, to rapidly generate high polarization levels in 13C labelled substrates in neat water solutions at physiological conditions. The method relies on a purposely designed sidearm in combination with an effective pulse sequence to transfer spin order from para-hydrogen into the target heteronuclear spin. We demonstrate the method on a portable custom-built system at 22.5 mT (~1 MHz) in order to polarize [1-13C]pyruvate. After hydrogenation and polarization transfer in situ, we measured 22% polarization level (~12 million signal enhancement) on the [1-13C]pyruvate in the precursor. Following a rapid cleavage and purification and transport procedure, accomplished in less than 20 s, we report ~10% polarization level on [1-13C]pyruvate in clean water solution at 7 T. We show the capability of the method by presenting time-resolved profiles of the pyruvate to lactate conversion in cancer cell lines. Chemical shift resolved metabolic imaging is demonstrated as first step towards in vivo applications. Finally, we discuss improvements that can ultimately lead to a widespread use of the method to obtain 13C-labelled hyperpolarized substrates in magnetic resonance for biomedical applications.

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PO153

Room-temperature Dynamic Nuclear Polarization Enhanced 13C NMR Spectroscopy of Small Biological Molecules in Water

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Nuclear magnetic resonance (NMR) spectroscopy is a powerful and popular technique for probing the molecular structures, dynamics and chemical properties. However, the conventional NMR spectroscopy is bottlenecked by its low sensitivity. Dynamic nuclear polarization (DNP) boosts NMR sensitivity by orders of magnitude and resolves this limitation. In liquid-state this revolutionizing technique has been restricted to a few specific non-biological model molecules in organic solvents. Here we show that the carbon polarization in small biological molecules, including carbohydrates and amino acids, can be enhanced sizably by in-situ Overhauser DNP (ODNP) in water at room temperature and at high magnetic field. An observed connection between ODNP 13C enhancement factor and paramagnetic 13C NMR shift has led to the exploration of biologically relevant heterocyclic compound indole. The QM/MM MD simulation underscores the dynamics of intermolecular hydrogen bonds as the driving force for the scalar ODNP in a long-living radical-substrate complex. Our work reconciles results obtained by DNP spectroscopy, paramagnetic NMR and computational chemistry and provides new mechanistic insights into the high-field scalar ODNP. In addition to our published results, we will further present new classes of biological molecule that can be explored now by high-field room-temperature ODNP in water.

PO154

Spinning Driven Dynamic Nuclear Polarization with Optical Pumping

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We propose a new, more efficient, and potentially cost effective, solid-state nuclear spin hyperpolarization method combining the Cross Effect mechanism and electron spin optical hyperpolarization in rotating solids. We first demonstrate optical hyperpolarization in the solid state at low temperature and low field, and then investigate its field dependence to obtain the optimal condition for high-field electron spin hyperpolarization. The results are then incorporated into advanced Magic Angle Spinning Dynamic Nuclear Polarization (MAS-DNP) numerical simulations that show that optically pumped MAS-DNP could yield breakthrough enhancements at very high magnetic fields. Based on these investigations, enhancements greater than the ratio of electron to nucleus magnetic moments (>658 for 1H) are possible without microwave irradiation. This could solve at once the MAS-DNP performance decrease with increasing field and the high cost of MAS-DNP instruments at very high fields.

PO155

Third dissolved-phase xenon-129 resonance in blood caused by elevated glucose level

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Hyperpolarized(HP) xenon-129(129Xe) dissolved-phase imaging of highly perfused organs (such as lungs(1,2), brain(3-6), and kidneys(7)) is currently under extensive development. HP 129Xe dissolved phase imaging requires a sufficiently long T1 time in blood and well-distinguished 129Xe resonances. Therefore, understanding the dependence of the HP 129Xe signal on the chemical composition of blood is important for further HP 129Xe MRI development. 129Xe has two resonances in blood, that correspond to xenon dissolved in plasma(P) and in the red blood cells (RBC)(8-11). Although there are multiple studies that evaluated the effect of blood oxygenation on the HP 129Xe signal(8-11), the role of the blood glucose level is presently unknown.

In this work, citrated sheep blood was mixed with a glucose solution to obtain a range of glucose concentrations in the blood. Naturally abundant 129Xe was hyperpolarized up to 56% using a XeBox-10E polarizer (Xemed LLC). Blood samples were saturated with HP 129Xe by using a mixing module (SuperphobicMicroModule 0.5X1 G680) inside a Philips Achieva 3.0T clinical MRI scanner. HP 129Xe MR spectra,T1, and T2* relaxation times were measured.

We observed line broadening for the 129Xe-RBC resonance with the increase of blood glucose concentration. In addition, the T1 of the 129Xe-RBC resonance increased substantially for glucose concentrations higher than 25 mM, indicating slow 129Xe exchange between plasma and RBC. These observations could be explained by the presence of two different RBC pools: glucose-unaffected(RBC1) and glucose-affected(RBC2) RBCs. Therefore, this model was implemented for data analysis. The RBC1 peak was mainly unaffected by hyperglycemia while the RBC2 peak shifted downfield with a rate of (0.015 ± 0.003)ppm/mM. The 129Xe-RBC1 T2* increased non-linearly with the increase of glucose concentration, whereas T2* for plasma and RBC2 peaks were unaffected.

PO156

The Beneficial Instability of Frémy's Salt for Dissolution DNP

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Dynamic nuclear polarization (DNP) relies on an efficient magnetization transfer from an unpaired electron to nuclei in its vicinity; hence the importance of optimization and tuning of polarizing agents (PA) for specific tasks. In Dissolution DNP (DDNP) experiments, PAs are used to generate nuclear hyperpolarization in a frozen solid before rapid dissolution and transfer to an NMR spectrometer for detection. However, the presence of the radical that plays a key role in solid-state DNP, becomes a source of paramagnetic relaxation effects in solution. To avoid rapid relaxation, efforts have been made to remove the radical after dissolution, e.g., through reduction [Mieville, 2010], filtration [Vuichoud, 2016] or annealing of photo-induced radicals [Capozzi, 2017].

Here we present an alternative route to PA neutralization while providing excellent ^{13}C polarization in the solid before dissolution. We use Frémy's salt ($K_2[NO(SO_3)_2]$) as PA, which we found provides a $P(^{13}C) > 20\%$ at 298 K after dissolution. Also, the proton polarization is particularly good. Importantly, this radical can be quantitatively neutralized with 0.5 equivalents of ascorbate in less than 500 ms. To do so, we developed a hybrid pneumatic/hydraulic sample transfer system that rapidly mixes the hyperpolarized solution containing the PA with an ascorbate solution while on its way to the NMR spectrometer for detection. As a result, the obtained signal enhancements after dissolution for sodium pyruvate-1-^{13}C as well as $^{13}C_3$ and sodium acetate-1-^{13}C could be doubled and the relaxation times prolonged.

The combined efficiency, inexpensiveness, and instability render Fremy's salt a simple alternative to established PAs while offering a handle on paramagnetic relaxation of hyperpolarized samples. The extended hyperpolarization lifetimes are not only of great interest for already established methodologies but have the potential to improve the application of DDNP.

PO158

Inductive detection and coherent manipulation of electronic-nuclear multi-spin clusters

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At sufficiently high concentrations, randomly occurring paramagnetic centers in a solid interact with each other to form variable-size clusters integrating electronic and nuclear spins in the material host. These many-spin systems feature nearly-degenerate, hybrid states whose dynamics is central to processes involving spin diffusion, but their characterization has proven notoriously difficult mostly due to their relative isolation and low relative numbers. Here, we combine field-cycling experiments with selective radiofrequency (RF) excitation to probe transitions between hybrid multi-spin states near energy-matching conditions between nitrogen-vacancy (NV) centers in diamond and surrounding paramagnetic impurities. Using bulk ^{13}C spins as a long-term reservoir, we implement a time resolved scheme featuring periodic intervals of optical illumination, radio-frequency excitation, and free evolution. We find that slight changes in the duration or amplitude of the radio-frequency pulses have a large effect on the observed bulk nuclear signal, hence indicating that the hybrid spin cluster serving as the polarization seed remains coherent on a time scale exceeding 100 µs. Besides shedding light on the microscopic mechanisms of spin thermalization in a solid, our approach portends intriguing pathways to generating nuclear polarization without the use of optical or microwave excitation.

Bullet-Dissolution Dynamic Nuclear Polarization and Ligand Binding

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In bullet DNP, a sample is hyperpolarized at cryogenic temperatures and rapidly transferred to a second magnet where it is dissolved and liquid-state nuclear magnetic resonance (NMR) spectra are recorded [1]. We have recently reported 13C spectra with polarization levels of approximately 30 % with a resolution of 2 Hz, and less than 10-fold dilution [2]. The low dilution is advantageous in particular for ligands with a low solubility in aqueous solutions. Here we discuss bullet-DNP enabled 13C NMR based ligand binding experiments with and without isotopic labelling.

PO160

Direct Observation of Calcium Carbonate Prenucleation Clusters via Dissolution DNP

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Calcium carbonate is one of the most important inorganic solids in the world. It has numerous polymorphs and is usually formed from the reaction between Ca^{2+} and CO_3^{2-} in aqueous solutions. However, the exact mechanism of this process remains elusive to this date. Precursors that include prenucleation clusters, liquid-like precipitates and amorphous particles have been proposed recently. As the carbon atoms in these proposed precursors have different chemical environments, ^{13}C NMR could be a powerful tool to study the nucleation mechanism of $CaCO_3$. However, standard NMR methods are limited by their low sensitivity and furthermore, it is challenging to observe dilute, short-lived species using NMR. Recent advances in dissolution dynamic nuclear polarization (dDNP) have made it possible to observe chemical species with low concentrations and short lifetimes in solutions.

Here we investigate the early-stage nucleation process of $CaCO_3$ using ^{13}C dissolution dynamic nuclear polarization (dDNP) NMR. When Ca^{2+} ions are combined with hyperpolarized CO_3^{2-} ions, we observe in situ a previously unseen signal with a chemical shift higher than 169 ppm, which is a typical feature of $CaCO_3$. In addition, this unseen signal decays at a slower rate than the free carbonate signal. After the completion of the reaction and decay of hyperpolarization, this signal is no longer observed. We ascribe this signal to the short-lived pre-nucleation clusters of calcium carbonate which is the first direct observation of such species using NMR. The results we report here directly complement recent studies in this area using other methods such as cryotransmission electron microscopy. This work opens up possibilities to perform in situ characterization of chemical processes using dDNP.

PO161

Experiences with TPPM DNP at 1.2 T

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Pulsed dynamic nuclear polarization (DNP) is a promising new approach to enhancing the sensitivity of high-resolution magic-angle spinning (MAS) NMR. The TOP (Time-Optimized Pulsed) DNP pulse sequence in 2019 by Tan, et al.,[1] managed to deal with the matching condition problem at high fields by incorporating a modulation frequency. Based on a similar principle, recently we introduced the XiX DNP[2] pulse sequence which was found superior to TOP DNP at Q band (51 MHz/1.2 T/34 GHz), using a nutation frequency of 18 MHz. The larger scaling factors of XiX DNP enable a faster e--^{1}H polarization transfer and we suspect that they are behind the improved performance. In the process of improving the performance of pulsed DNP sequences at Q band, we explored the TPPM DNP sequence, which is a more generalized version and has a larger parameter space. To find the optimal phase (Φ) and pulse length (τp) we used numerical simulations with the SPINACH[3] libraries. We found a simpler way to deal with the incommensurate frequencies problem in the calculation of the interaction frame trajectory analysis to find the scaling factors and provided a detailed theoretical description along with a possible theoretical maximum of the enhancement factor. The possibility of quantitative prediction of experimental enhancement factors using SPINACH simulations along with more experimental observations will be discussed at the poster presentation.

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PO162

Detecting oligopeptides via parahydrogen hyperpolarization

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Oligopeptides are short peptides which consist of 2…20 amino acids. They perform vital functions in living organisms and might be related to the cause and treatment processes of neurological disorders like Alzheimer and Parkinson's disease. They are also used in tumor-targeted therapy, nutrition and skin conditioning and revitalization products. Because of their biological importance, analytical methods for oligopeptide detection are desired However, observing oligopeptides with NMR in biological mixtures is difficult due to inherently low sensitivity of NMR. To overcome the sensitivity problem parahydrogen (pH2) hyperpolarization (HP) can be exploited for the detection of oligopeptides.

We use non-hydrogenative (nh-PHIP) pH2 HP [1], a variant of parahydrogen-induced polarization (PHIP), to detect oligopeptides. nh-PHIP does not require sample manipulation like labelling or sidearm addition and is therefore a relatively simple technique. It is a chemoselective method which works quantitatively orders of magnitude below the usual NMR LOD. It detects pH2 derived hydrides of short-lived complexes of analytes with iridium based HP catalysts and allows to adopt NMR in drug quality control or drug detection (e.g. doping testing, pharmacokinetics [2]) and disease biomarker analysis [3].

In this work we show that nh-PHIP can be applied for the detection of oligopeptides. It is possible to distinguish different oligopeptides in artificial as well as in biological mixtures without the need of extensive sample preparation. We demonstrate that oligopeptides exhibit similar binding modes to iridium catalysts as amino acids [1], and give rise to distinctive hydride resonance frequencies.

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PO163

Physical mechanisms underlying large 31P enhancements in triphenylphosphine in liquid state DNP

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Dynamic nuclear polarization (DNP) is a technique developed to overcome the sensitivity issue in nuclear magnetic resonance (NMR). There, higher spin polarization of polarizing agents (PA) is transferred to coupled target nuclei via microwave irradiation. In liquids, the Overhauser effect dominates the polarization transfer, which is based on spin cross-relaxation driven by the time modulation of the hyperfine coupling between the unpaired electron of the PA and the target nuclear spin.[1]

Besides [1]H- and ^{13}C-DNP, ^{31}P is an interesting target nucleus for NMR. Few ^{31}P-DNP studies on triphenylphosphine (TPP) show large NMR signal enhancements (~180) at various magnetic fields. [2,3] It is important to understand the underlying mechanisms of the polarization transfer to establish ^{31}P-DNP. To this purpose, we performed ^{31}P-DNP at 1.2 T on trivalent TPP and pentavalent triphenylphosphine oxide (TPPO). TPP showed large enhancements ($\varepsilon = 150 \pm 15$) with BDPA, a carbon-based radical, while the enhancement was limited to $\varepsilon = 37 \pm 4$ when using nitroxide radical TEMPONE. Interestingly, TPPO showed no enhancement under the investigated conditions. Supported by DFT calculations of the radical/target molecule complex, we observed that BDPA and TPP forms a complex, where the phosphorus is pointing with its lone pair to the center of the BDPA, where the spin density is the largest. This interaction, which is specific for BDPA radical, results in large hyperfine couplings (A ~ 11 MHz). This is possibly the reason behind the large ^{31}P enhancements observed in TPP over the whole magnetic field range. This close encounter is hindered for TPPO by the oxygen bound to the phosphorus resulting in much smaller hyperfine couplings (A ~ 0.5 MHz) and lower enhancements.

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PO164

Rapid SABRE Catalyst Scavenging Using Functionalized Silicas

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In recent years the NMR hyperpolarization method signal amplification by reversible exchange (SABRE) has been applied to multiple substrates of potential interest for in vivo investigation.

Unfortunately, SABRE typically requires an iridium-containing catalyst that is cytotoxic and thus unsuitable for biomedical applications. This work utilizes inductively coupled plasma-optical emission spectroscopy (ICP-OES) to investigate the potential use of metal scavengers to remove the iridium catalytic species from the solution. The most sensitive iridium emission line at 224.268 nm was used in this analysis.

We report the effects of varying functionality, chain length, and scavenger support identity on iridium scavenging efficiency. For the solid supports the impact of varying the quantity of scavenger utilized is reported for the three scavengers with the highest iridium removed from initial investigations: 3-aminopropyl, 3-(imidazole-1-yl)propyl, and 2-(2-pyridyl) functionalized silica gels. Exposure of an activated SABRE sample (1.6 mg mL-1 of iridium catalyst) to 100 mg of the most promising scavenger resulted in <1 ppm of iridium being detectable by ICP-OES after 2 min of exposure.

We propose that combining the approach described herein with other recently reported approaches, such as catalyst separated-SABRE (CASH-SABRE), would enable the rapid preparation of a biocompatible SABRE hyperpolarized bolus.

Additionally, several homogenous analogues of the solid supported scavengers were investigated and found to be effective at deactivation of the iridium catalyst. Following catalyst deactivation there was a 99.6% reduction of observed SABRE signal when only two equivalents of the most effective deactivating ligand were utilized. Catalyst deactivation results in substrate longitudinal relaxation times returning to levels commensurate with pure substrate, enabling SABRE hyperpolarized signals to be observed far longer than without catalyst deactivation.

PO165

Radio Frequency Sweeps at µT Fields for Parahydrogen Induced Polarization of Biomolecules

Magnetic resonance imaging (MRI) of the metabolic processes within the human body opens up new methods of studying cancers by assessing tumors. It is accomplished through the monitoring of the 13C NMR signal of a metabolite, used as a tracer (e.g., pyruvate), and its downstream metabolic products. This signal has such a low intensity that measuring it is almost impossible, and hyperpolarization offers an attractive solution to this problem. Parahydrogen-induced polarization (PHIP), where parahydrogen is chemically added to an unsaturated precursor molecule, generates a highly polarized spin state in the product molecule. A transfer step is then undertaken to convert the spin-order of the hyperpolarized protons into observable 13C magnetization. A short ultra-low magnetic field sweep is a quasi-adiabatic way to transfer the polarization that has been shown to be highly effective1.

It can be beneficial to introduce deuterium labels into molecules, as this reduces relaxation rates of nearby spins. Additionally, we show that it can increase the efficiency of polarization transfer through spin networks, by breaking the symmetry of mediating CH2 groups. However, the introduction of deuterium also has downsides. 2H frequently behaves as a "polarization sink" at ultra-low fields, where it is strongly coupled to 1H, owing to its quadrupolar relaxation. Simple magnetic field sweeps then perform poorly as they transfer polarization to the deuterium, where it is lost.

In this work, we present a novel method using radio-frequency (RF) ramped sweeps at low static magnetic fields in the presence of deuterium. These RF sweeps are a robust method of polarization transfer that enable the use of deuterated molecules. For selectively deuterated cinnamyl pyruvate, an important example, we achieve a 40% polarization improvement. We demonstrate this method on a variety of molecules and find that its performance either matches or exceeds the performance of magnetic field sweeps.

PO166

Dendritic macromolecules as possible Cu(II) sensors using nuclear singlet state NMR

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The singlet states of coupled nuclear spins offer several interesting properties. For one, these states can be populated using specialized sequences, and their lifetime (Ts) is relatively long, often exceeding the longitudinal relaxation time (T_1). These features allow for populating, storing, and retrieving spin order at a later time. Once retrieved, the singlet-derived spin order be used to generate observable signal, while the bulk part of non-singlet-derived signals is suppressed. This allows for specific imaging of singlet-labeled molecules - an interesting approach to bring molecular specificity to MRI. One interesting system are dendrimers, highly symmetric macromolecules with applications as e.g. filters, in catalysis or as drug delivery systems. In this study the influence of different paramagnetic ions on the Ts of dendrimers has been investigated, showing a selective effect of Cu(II) on Ts which could not be observed in the presence of other ions. This effect was observed not only in D_2O but also in H_2O and buffered H_2O solutions. High concentrations of Cu(II) are associated with protein aggregates found in patients with neurodegenerative diseases, making this effect in combination with the capability to remove background signals a promising candidate for a Cu(II) sensor under physiological conditions that can be observed directly. This possibility was further investigated by attaching a fluorescent marker to the dendrimers and incubating different cells with it, showing an accumulation of the dendrimer in the cell nuclei as well as on the cell membranes. An effective filtering of any background signal could be shown using nuclear singlet state NMR even in the presence of cells making the direct observation of the dendrimer possible. Overall the investigated dendritic structure make for an interesting candidate for a directly observable Cu(II) sensor in cell experiments and possibly even medical applications in magnetic resonance imaging applications.

Long-lived, transportable reservoir of nuclear polarization used to strongly enhance solution-state NMR signals

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A fundamental problem in Dynamic Nuclear Polarization (DNP) is that the same polarizing agent (PA) required for DNP is also responsible for shortening the lifetime of hyperpolarization. As a result, long time storage and transport of hyperpolarized samples is severely restricted and the apparatus for DNP is necessarily located near or integrated with the apparatus using the hyperpolarized spins.

We produce a polarization reservoir, charged hundreds of kilometers away, and use it in the recently developed intermolecular-NOE-mediated hyperpolarization transfer system (HYPNOESYS, doi. org/10.1021/jacs.1c09119) to hyperpolarize proton sites of target molecules up to three orders of magnitude.

In our method, the reservoir of hyperpolarized proton spins with an extremely long relaxation time is transported to the receiving user instead of the frozen polarized sample itself. This reservoir, a crystal of polarized naphthalene, is then utilized by dissolving it in a solution of target analytes, resulting in polarization transfer via the intermolecular Nuclear Overhauser Effect (NOE), which provides large NMR signal enhancements on a range of substrates. Essential for the proposed strategy is the use of the short-lived photo-excited triplet state of pentacene to polarize the naphthalene protons to about 80%. Without optical excitation, the pentacene rapidly decays to its diamagnetic ground state and the PA decay channel is eliminated, which results in polarization decay time constants of 60h at 80K and 0.5T, and more than 800h at 5K and 20mT. This allows for long time storage or transport of the hyperpolarized sample. For transport, a module based on a small Halbach magnet yielding 0.75T and a conventional cryogenic dry shipper operating at liquid nitrogen temperature is used.

Here we present results on samples transported from Paul Scherrer Institut in Villigen to NVision Imaging Technologies GmbH in Ulm.

PO168

Nuclear Magnetic Ordering in Naphthalene

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Analogous to electronic magnetism, a nuclear spin system cooled below a critical temperature in the order of 10^-6-10^-7 K, can undergo a transition from a paramagnetic to a ferromagnetic or antiferromagnetic state subject to mutual dipole-dipole interaction. This exotic phenomenon, known as nuclear magnetic ordering (NMO), has only been observed in a few cases that form simple atomic single crystals.[1-4]. We recently extended the theory to describe the phenomenon in molecular crystals, which exhibit more possibilities of ordering, since each molecule contains more than a single spin[5]. In our case of a naphthalene single crystal, Weiss field calculations predict a longitudinal ferromagnetic structure. The ferromagnetic domains are thin disks with their axis along the B-field and alternating polarization direction.

The critical temperature Tc, with kTc comparable to the dipolar interaction, can be reached by hyperpolarization of the crystal using triplet-DNP[5] and subsequent adiabatic demagnetization in the rotating frame (ADRF) that reduces the effective field on the spins to zero.

Since the external magnetic field is still present after the ADRF, NMR can be used to investigate the configuration of the spins. Asymmetric NMR signals are observed that show a broadening compared to the polarized signal, which can be interpreted as a signature of a long-range ordered state. In addition, the naturally abundant 13C spins repolarised after ADRF were used as local probes of the order[6]. Their NMR signal is split into a doublet or a quadruplet depending on whether an order has been achieved. Furthermore, measurements for the transverse susceptibility as a function of the initial polarization show the expected plateau for negative spin temperature[1].

To confirm the nuclear magnetic ordering and to verify our theory, we have performed polarized small-angle neutron scattering experiments.

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Posters

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Rapid 1H→13C hyperpolarization transfer via adiabatic field inversion

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Dissolution dynamic nuclear polarization (dDNP)[1,2] is a method of choice for the preparation of hyperpolarized 13C metabolites such as 1-13C-pyruvate used for in vivo applications including the real-time monitoring of cancer cell metabolism in human patients[3]. The approach consists of transferring the high polarization of electron to nuclear spins via microwave irradiation at low temperature (1.0-1.8 K) and moderate magnetic field (3.3-7 T). The solid sample is then dissolved and transferred to an NMR spectrometer or MRI scanner for detection in the liquid-state.

Common dDNP protocols use direct hyperpolarization of 13C spins reaching polarizations of >50% in ~1-2 hours. Alternatively, 1H spins are polarized before transferring their polarization to 13C spins using cross polarization (CP)[4], reaching similar polarization levels as direct DNP in only ~20 min. However, it relies on more complex instrumentation.

Here, we explore an alternative route using 1H dDNP followed by an inline adiabatic magnetic field inversion in the liquid-state during the transfer. 1H polarization of >70% in the solid-state are obtained in ~5-10 min. As the hyperpolarized sample travels from the dDNP polarizer to the NMR spectrometer, it goes through a field inversion chamber, which causes 1H→13C polarization transfer. This transfer is made possible by the J-interaction between the heteronuclei, which mixes the Zeeman states at zero-field and causes an anti-level crossing[5].

We report liquid-state 13C polarization of ~10% for 3-13C-pyruvate and 13C-formate. The instrumentation needed to perform this experiment in addition to a conventional dDNP polarizer is simple and readily assembled.

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PO170

DNP juice as skin lotion

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The outermost skin layer, the stratum corneum, painfully reminds us of its existence during cold winters since it experiences osmotic stress in dry conditions. It is obvious and well established that one should apply creams which contain small polar molecules that has shown to replace the water while retaining lipid and protein fluidity.[1] But exactly where in the stratum corneum these polar molecules are located has previously not been directly observed.

A powerful 5-dimensional DNP-facilitated $^1H \rightarrow {}^{13}C$ HETCOR NMR spectroscopy acquisition framework can investigate $^1H \leftrightarrow {}^1H$ spin diffusion with correlations at the Ångström to hundreds of nanometer scale[2] at ~100 K, DNP build-up with distance to radical up to the µm scale[3,4], and cross polarisation with sensitivity to ^1H-^{13}C distances at the Ångström scale. Such features are applicable to corneocytes (~1 µm) and multilayer lipid bilayers (~4 nm/bilayer) brick and mortar like assemblies at ~20 µm thicknesses of the stratum corneum in humans. We demonstrate that the principles of DNP facilitated HETCOR NMR spectroscopy can be applied to investigate the localization of the moisturizing agent glycerol as observed from in-situ pig's skin stratum corneum samples, exploiting that glycerol is also a commonly used glassy matrix for DNP experiments. We find that the glycine (keratin) within the corneocytes "bricks" are neighboring glycerol at approximately <1 nm with a homogeneous dispersion, while the multilayer lipid bilayer "mortar" experiences a more heterogeneous dispersion of glycerol.

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PO171

SABRE-enhanced real-time pure shift NMR spectroscopy

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The combination of poor signal dispersion, highly prevalent homonuclear scalar (J) coupling, and numerous peaks present in spectra, hinders ^{1}H NMR spectral analysis of all but the simplest molecules. Effective suppression of homonuclear J-evolution to produce spectra that comprise a single signal for each chemical environment, using pure shift ^{1}H NMR methodologies, enables acquisition of ^{1}H NMR spectra at digital resolutions approaching the inherent limits of the hardware used and sample studied, and facilitates spectral analysis of complex molecules and mixtures based on chemical shift alone.[1] However, a significant limitation of broadband pure shift ^{1}H NMR methods[2–4] is their reduced sensitivity. At low sample concentrations, the poor sensitivity of broadband pure shift methods necessitates extensive signal averaging and collection of useful spectra becomes impractical due to long experiment durations.

Hyperpolarization techniques, which create non-Boltzmann distributions of nuclear spins amongst energy levels, have previously been used to overcome NMR sensitivity limitations, including in pure shift ^{1}H NMR experiments.[5] Here, we demonstrate compatibility of signal amplification by reversible exchange (SABRE) hyperpolarization with real-time pure shift experiments.[6] Pure shift ^{1}H NMR resonances of pyridine are enhanced by up to a factor of 60 in a single scan experiment. We extend this methodology to mixture analysis, where the favourable slow relaxation properties of components unamenable to SABRE hyperpolarization means that their signals are not observed in these pure shift ^{1}H NMR experiments.

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Detection and discrimination of enantiomers via non-hydrogenative parahydrogen Induced Polarization

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Reversible association of small molecules and parahydrogen to an iridium catalyst forms the basis of SABRE techniques for nuclear spin hyperpolarization in solution. Under suitable conditions, hyperpolarization of the transient complex resulting from this reversible association can also be exploited to detect specific analytes at concentrations much lower than routinely observed in thermal NMR measurements. In this respect the iridium catalyst employed in SABRE can act as an NMR chemosensor, allowing the selective detection of dilute analytes in complex mixtures, while removing the large signal background originating from other species in solution. Here, the most recent NMR applications concerning the detection and discrimination of enantiomers by non-hydrogenative PHIP will be presented. Our results demonstrate that it is possible to quantitatively discriminate enantiomers in biofluids or natural extracts without any prior functionalization or separation.

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Nonlinear Chaotic Dynamics in DNP –Hyperpolarized Spins at 1.2K: Simulation and Experimental Control

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Nonlinear dynamics of hyperpolarized spins due to radiation damping in Dynamic Nuclear Polarization (DNP) experiments at cryogenic temperature are presented. Due to the large magnetizations achieved in DNP experiments, the strong coupling between spins and the resonant circuit gives rise to efficient Radiation damping [1-2] despite line widths of several tens of kHz. In these conditions, the combination of two competing processes, namely the hyperpolarization to negative temperatures through DNP, and the precession of the magnetization towards the equilibrium direction driven by radiation damping, leads to sustained M/RASER [4].

In recent DNP experiment performed at 1.2K, we observed long series of MASER bursts occurring at random time intervals, and for durations over 100 seconds, suggesting the presence of chaotic spin dynamics related to the above phenomena.

Besides, multiple MASER bursts usually exhibited asymmetric profiles, which was ascribed to the presence distant dipolar field effects that become efficient for such large magnetizations. A classical description of the magnetization dynamics based on the Bloch equations with dipolar interactions between magnetic moments, the Maxwell equations for radiation damping, and the Provotorov equations to account for thermal mixing DNP (including the flow of spin temperatures between the various nuclear Zeeman and electron non-Zeeman reservoirs) was used to provide a qualitative analysis of the phenomenon.

In parallel, to achieve controlled MASER pulses, we show preliminary experiments of an electronic feedback device aiming to control radiation damping in DNP experiments. The possible applications of such an approach for maser control will be discussed.

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Protein Folding Studies by DNP Enhanced-NMR Spectroscopy in Frozen Solution

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Some proteins do not have only one well-defined structure in equilibrium, instead they may partially or fully consist of more than one conformation. We recently implemented DNP-enhanced solid-state NMR-spectroscopy of frozen solutions to investigate structural ensembles of (disordered) proteins [1]: At temperatures below 120 K the dynamics is frozen out, and all conformations of the ensemble coexist, which leads to inhomogeneous line-broadening of NMR resonances. Analysis of the line-shapes of these inhomogeneously broadened peaks can reveal the conformations in the ensemble. To reduce spectral overlap selective isotope labeling is mandatory to be able to analyze selected peak shapes: we used different sparse isotope labeling patterns which allow for analysis of backbone conformations and contacts in selected sites [2] or for analysis of the conformational space sampled by Ile side-chains [3].

Here, we present a study on protein (un)folding of the model protein PI3K-SH3, a globular protein domain which can unfold and form amyloid fibrils at low pH. Comparative studies by low temperature solid-state NMR specrtoscopy and solution NMR spectroscopy reveal a substantial degree of conformational freedom even in the folded protein, and gradual unfolding and aggregation of the protein leads to changes in the relative populations and tertiary contacts.

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Natural abundance 15N nuclei explain anomalous field dependence in 1H SABRE experiments

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SABRE (Signal Amplification by Reversible Exchange) is a parahydrogen based hyperpolarization technique capable of quickly generating large polarization levels in a wide range of substrates without requiring chemical modification of the target substrate. Though the underlying spin dynamics that govern polarization transfer are generally well characterized, a recent phenomenon was uncovered which caused an unexpected in split the magnetic field dependence of a SABRE-relay experiment, producing at least two distinct regions of maximal polarization transfer efficiency to the primary amine substrate, benzylamine. Further probing showed coupling to natural abundance spin-½ 15N nuclei as the cause of the observed phenomenon. Additionally, increased efficiency of the SABRE process is observed when using non-labeled benzylamine as opposed to fully labeled material. Investigation of active exchange kinetics and Ir complex species are still underway.

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Signal Amplification Waveform (SAW) for Enhanced Benchtop 15N NMR Investigations of Ir Organometallic Chemistry

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SABRE (Signal Amplification by Reversible Exchange) is a physicochemical technique that allows transferring spin order from parahydrogen (pH2) to a molecular substrate of interest, providing renewable liquid-state hyperpolarization by merely flushing pH2 gas through the solvent. The process is typically performed at low or ultralow magnetic fields via coherent spin mixing or at high magnetic fields through cross-relaxation. Several high-field (6-10 T) radiofrequency (RF) based approaches have been developed enabling signal enhancement directly at the field of NMR measurement. However, these approaches are not readily translatable to the benchtop (1 T) NMR work. In particular, it is more challenging to address spins (for example, bound SABRE-ligands) selectively given low Larmor frequency separation. To overcome this limitation, we developed an approach called SAW (Signal Amplification Waveform) based on the recently demonstrated approach, APSOC-SABRE, which converts the spin order of parahydrogen into 15N magnetization in situ inside a 40 MHz benchtop NMR spectrometer. SAW was experimentally tested with 15N labeled fam-pyridine as the substrate and methanol as the solvent and its efficiency was confirmed. The re-polarization technique is also tested where the pulse sequence was applied multiple times and a significant improvement of the signal enhancement was achieved. The hyperpolarized 15N signals were then used to study the organometallic chemistry of the SABRE complex Ir(IMes)H2(FamPy)3Cl. A long-term (30-40 s) buildup of the 15N signal from the axial ligand was observed. This indicates either a slow exchange between the axial and the free ligand in the solvent or an internal ligand rotation in the SABRE catalyst which swaps the axial and the equatorial ligands.

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Parahydrogen-Induced Polarization Mediated by Metal-Free Biradicaloids and Hydroborane Catalysts

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Low sensitivity of NMR often leads to insufficient signal strenghts to perform reliable analysis. Spin hyperpolarization techniques can increase the sensitivity. Parahydrogen-induced polarization (PHIP) has been succesfully utilized for mechanistic studies and NMR sensitivity boosting [1]. Most of the PHIP studies were performed using metal containing catalysts. Recently, it has been shown that metal-free catalysts can also demonstrate PHIP effects. In the present work we study activation of parahydrogen by a number of 4- and 5-membered pnictogen biradicaloids [2]. The mechanism of activation allowed observing strong nuclear spin hypepolarization effects in 1H and 31P NMR experiments. The measured signal enhancement exceed three orders of magnitude at 9.4 T, and this is a record high signal enhancement for metal-free catalysts. Also, for the first time we demonstrate metal-free hydrogenation with hyperpolarized product using ansa-aminoborane HCAT [3]. The use of PHIP enables detecting signals of key catalytic cycle intermediates of the hydrogenation of alkynes in 1H, 11B and 15N NMR spectra which was not accessible with thermal polarization. We also observe effects of regio- and stereo- selectivity of alkyne addition to HCAT and difference in reaction rate with different isomers.

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Advancing Parahydrogen-Induced Polarization Based on the Use of Metal-Free Catalysts: Findings and Perspectives

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Hyperpolarization using parahydrogen finds a growing range of applications including reaction monitoring and imaging, mechanistic elucidations, biomedical imaging and mixture analysis, etc. Chemical activation of parahydrogen molecules plays a key role in deriving enhanced NMR signals in this method. Commonly, metal catalysts are employed to mediate such activations to produce hyperpolarized substances. At the same time, more biogenic main group catalytic systems are known as metal-free activators for parahydrogen [1]. In this communication, we present an overview of findings in PHIP and SABRE based on the use of metal-free catalytic systems.

We show that unimolecular pairs of sterically separated ('frustrated') Lewis acids and bases (FLPs) are promising metal-free parahydrogen activators that provide hyperpolarization of protons and heteronuclei (15N, 11B, 31P) in FLP-H2 adducts [1-3] and free FLP molecules. Recently, we demonstrated that metal-free FLPs catalyze hydrogenation of alkynes, providing at least two orders of magnitude signal enhancements for alkene products at 9.4 T [4]. Besides FLPs, pnictogen biradicaloids can lead to strong hyperpolarization effects in parahydrogen activation allowing detection of strongly polarized biradicaloid adducts with parahydrogen [5]. We discuss role of kinetic parameters, dihydrogen bonding and structural features in the context of observed metal-free hyperpolarization effects and provide a perspective for the use of these systems in NMR applications.

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NMR methods and devices for the characterization of flows and transfers in milli-channels

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Milli-fluidics is the technology of flows in channels with characteristic dimensions of a few hundred microns. At this scale, capillary and viscosity effects are more important than volume forces and diffusion is often dominant over advection. The flows through milli-fluidic systems are implemented in several domains such as synthesis chemistry, biology or process engineering. The study of phenomena in milli-fluidic devices faces two major problems. One is that microfabrication techniques require costly investments and a good technological knowledge. Second, the geometric complexity of the systems induces difficulties due to optical access.

NMR/MRI methods are adapted to the study of such complex systems, provided that a specific instrumentation is developed in order to improve the sensibility of the measurement. In this context, our study aims to implement, with a low-cost methodology, specific devices to study flows and transfer phenomena in milli-fluidic systems and to optimize NMR/MRI methods for their characterization.

Two applications have been developed. The first one consists in a study of the growth of a biofilm in a capillary of submillimeter dimensions and the characterization of the hydrodynamics of the flow in presence of this biofilm. The second one is the study of the flow regime and hydrodynamic instabilities occurring in micromixers. For each application, a specific device was set up, including the milli-fluidic system and the radio frequency (RF) coil adapted to the geometry and dimensions of the system. The milli-coils were fabricated by etching on flexible copper/Kapton® substrates. With the considered geometrical parameters, the RF simulations showed that the milli-coils produce an intense and homogeneous field and the MRI measurements demonstrate an improvement in signal to noise ratio of a factor about 7 to 10 compared to the commercial MicWB40 probe which allows the detailed analysis of the above-mentioned phenomena in the context of milli-fluidic applications.

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A cryogen-free 400 MHz MAS system for high resolution Solid State NMR

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We present a narrow (54 mm ID) bore, 9.4 T (400 MHz) Magic Angle Spinning (MAS) system based on a cryogen-free magnet. The system consists of a Tecmag Redstone NMR console, a Phoenix NMR 4 mm diameter MAS probe, a cryogen free 9.4T magnet and a rack with electrical devices. In the rack there are a main magnet and a cryoshim power supply, a temperature monitor and a computer. A LabView based temperature monitoring software suite allows the user to record the temperature of key parts inside the cryostat.

The magnet is compact with a height of the top flange being only 1 m and can be accommodated in a room with a ceiling height as low as 1.5 m. A benefit of the cryogen free magnet is that, in the unlikely event of a field quench, there are no quench hazards and no loss of helium. After the quench the magnet temperature is recovered automatically within a few hours. The main magnet and cryoshim current leads are permanently mounted inside the cryostat, so the magnet is always ready to be ramped. We showed that within an hour after the magnet ramp, the magnetic field can be made stable enough for high resolution NMR measurements. The temporal magnetic field distortion associated with the cold head operation is around one part per billion (ppb) with respect

to the static magnet field. The mechanisms responsible for the conversion of the cold head operation into the temporal field distortion are discussed. The method used to measure and reduce the temporal field distortions is shown. The unique property of the cryogen-free magnets makes them an ideal tool to perform MAS NMR experiments at different static fields. The field value can be changed every day without compromising the measurements resolution.

Towards Automated Bullet-Dynamic Nuclear Polarization

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Bullet-DNP [1] is a form of dissolution-dynamic nuclear polarization which offers substantial potential for scaling to small volumes and increased throughput. In bullet-DNP the hyperpolarized target material and a suitable radical are dissolved in a glass-forming solvent, and the solvent is placed into a bullet (a little bucket made from PTFE). The sample is then hyperpolarized under standard D-DNP conditions. Afterwards the bullet carrying the hyperpolarized sample is ejected from the polarizer using pressurized gas, and shot into an injection device where the material is dissolved in solvent (0.5 mL), and the solution is injected into an NMR tube for detection. We recently presented a semi-automatic bullet system, in which such experiments can be carried out repeatedly without the need to remove the injection device from the solution NMR magnet, with polarization levels as high as 30%, approximately 10-fold dilution, and 2 Hz resolution [2]. The instrument presented in Ref [2] still needed manual intervention between experiments, namely for preparing and loading the bullet into the polarizer, re-connecting the bullet-path of the DNP system to the injection device and, after the experiment, for ejection of the empty bullet into a waste bin.

Here we present a revised system which comprises a device for preparing the bullets, and routines and custom-designed switches for loading the bullet into the polarizer without manual intervention. The system is still under development, but will facilitate unsupervised DNP experiments in the near future.

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Design and Construction of 14 Tesla DNP / EPR spectrometer

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Dynamic nuclear polarization (DNP) has been a rapidly developing field in the past two decades due to its ability to enhance signals in nuclear magnetic resonance (NMR) experiments by orders of magnitude. The signal enhancement is a result of polarization transfer from electron spins to nuclear spins. However, to get the maximal benefit from the DNP experiments, they have to be performed at high magnetic fields typical for modern NMR experiments.

Rational design of improved DNP experiments requires an understanding of the quantum mechanical mechanisms underlying the polarization transfer between electron and nuclear spins. It was shown that EPR experiments such as electron-electron double resonance (ELDOR), performed under DNP conditions (high magnetic field and low temperatures), provide a wealth of information about the DNP mechanisms [1]-[3].

In this poster, we present the design of the 14 T static DNP / EPR instrument that was built by our group at Tel-Aviv University. The instrument is designed for investigation of DNP mechanisms and DNP of challenging nuclei. It allows for CW, pulsed EPR, and DNP (NMR) experiments at 7 Tesla (200 GHz) and 14 Tesla (400 GHz) and low (< 20 K) temperatures.

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Streamlined LN2-based triplet DNP polarizer for fast turnaround HYPNOESYS experiments

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Sensitivity in NMR spectroscopy depends crucially on the level of polarization. Increasing this level by nuclear spin hyperpolarization provides a promising route to enable new advances ranging from applications in life sciences to medicine. The methodology of dynamic nuclear polarization via photoexcited triplet states in molecular crystals has been well established. These systems can offer unique properties such as close-to-unity ^{1}H polarization at moderate magnetic fields and temperatures and contain several tens of molar spin densities. Only in recent years have applications transitioned from traditional fields in particle physics to hyperpolarization in NMR.

We present an optical polarizer which sources pentacene-doped naphthalene crystals for hyperpolarization in solution-state NMR when combined with our recently developed HYPNOESYS technology [Eichhorn et al. J. Am. Chem. Soc. 2022, 144, 6, 2511–2519]. Seamless polarizer operation is facilitated by cold nitrogen vapor cooling, pulsed laser excitation, AWG-based microwave pulses for polarization transfer, polarization buildup monitoring via NMR and automated sample shuttling for loading and unloading allowing fast sample turnaround.

With the aid of optically detected magnetic resonance (ODMR), we access important material performance parameters, including sample alignment, as well as yield and kinetics of the photoexcited triplet state of the pentacene electron spins. Furthermore, we improve DNP performance using quantum optimal control of the microwave pulse [Marshall et al.arXiv:2112.15021 (2021)] leading to > 20% polarization within one hour in 40 mg samples.

PO185

3D Printed Magic-Angle-Spinning Hardware

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Magic angle spinning (MAS) in solid-state NMR is the most important technique to achieve high-resolution where time averaging of rank 2 interactions is sufficient. Commercial double-bearing setups typically consist of a ceramic rotor with a plastic or ceramic cap and a stator which consists of multiple materials including precision bearings. The high required precision of a few micrometers is reflected in the impressive prices of MAS modules. We have shown [1] that 3D printing can be used to print stators in a single-piece reducing the materials cost of a stator to a few cent using standard printer for fused filament fabrication. The process presented achieves a suitable printing quality both with different target materials including ABS, PC and ZrO2, which is suprising given one considers the nozzle-diameters of such printers.

Such techniques allow for rapid prototyping of stator designs. We show applications to low-field MAS NMR and printing of different MAS accessories.

Posters

Design of Cryogenic, 14 Tesla DNP / EPR Probe with Fast Sample Exchange

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Dynamic Nuclear Polarization (DNP) is a promising method to overcome the main limitation of NMR - its low sensitivity. DNP is based on polarization transfer from unpaired electrons to the neighboring nuclei. The polarization transfer requires on-resonance irradiation at the Larmor frequency of the electron spins (~400 GHz for experiments at 14 Tesla). In addition, DNP experiments are best performed at ultra-low temperatures (< 20 K) due to the combined benefit of high initial electron spin polarization and prolonged electron spin relaxation times. The challenge in DNP probe design is to provide efficient 400 GHz irradiation without compromising the NMR performance. The requirement for operation at ultra-low temperatures adds additional challenges.

In this poster, we present a new, home-built DNP / EPR probe designed for static experiments at 14 Tesla and ultra-low temperatures. The probe combines 600 MHz NMR and 400 GHz DNP / EPR capabilities. In addition, the probe allows for fast sample exchange at cryogenic temperatures. The probe is designed for use with the new 14 Tesla DNP / EPR spectrometer constructed over the past three years in our group at Tel-Aviv University. The spectrometer and probe design have unique features such as 400 GHz irradiation from underneath the sample, with the quasi-optical bridge located below the magnet. Furthermore, removal of long waveguide used in the designs with the quasi-optical bridge above the magnet allows for ample space in the probe for easy sample exchange and RF circuitry. An additional benefit of the waveguide removal is the reduction of the heat load on the cryostat.

PO187

Miniaturized tri-axis biplanar coils for atomic and nuclear spin sensors

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Magnetic field sensing technologies using alkali-metal vapors are among the output of the ``second quantum revolution'' (DOI: 10.1098/rsta.2003.1227) setting new boundaries in precision metrology. These spark a wide interest from gyroscopes for navigation and autonomous control, biomagnetism measurements (e.g. magnetoencephalography and myography), and also more recently, nuclear magnetic resonance signal detection in the kHz band as an efficient alternative to inductive-pickup coils, SQUIDs, and other sensors.

The miniaturization and mass-manufacture of atomic devices is in general a highly active area of research to find an optimal balance of size, power, cost and sensitivity. Sensitivity and dynamic range, especially, rely upon careful control of magnetic fields in three dimensions.

Here we discuss the design of miniaturized coils for three-dimensional, localized and uniform field control by direct placement around the sensor, as a flexible and compact alternative to global approaches used previously. Coils are designed on biplanar surfaces using a stream-function approach and then fabricated using standard printed-circuit techniques (arXiv preprint: 2204.01370). Application to a ^{87}Rb magnetometer of sensitivity ~ 20 fT/$\sqrt{\text{Hz}}$ is shown. We also demonstrate the performance of a coil set measuring $7 \times 17 \times 17$ mm³ that is optimized specifically for magnetoencephalography, where multiple sensors are operated in proximity to one another. Internal and external (stray) field profiles are measured using magneto-optical effects in a MEMS ^{87}Rb vapor cell, inside and outside the coil set. Ways in which low-field NMR can profit from these advances will be discussed.

PO188

Planning and Installing a Helium Liquefication Plant

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The world helium supply was finally supposed to have a major reprieve this year (2022) form the multi-year shortages and resulting restrictions in helium delivery. But we all found out the hard way that this might not be the case. Several fires at the Russian state-owned production site of Gazprom in Amur, a very likely impact by the Ukraine war, as well as unexpected maintenance issues at the BLM plant in Texas since July 2021 have severely impacted the supply chain in the last months again. This lead to renewed helium shortage with allocations at the 60% levels.

This cycle of shortages and allocations has been a re-occurring story in the last 15-20 years, which prompted us at Vanderbilt University to invest into our own helium re-liquefying plant. While the concept is straight forward, specific information on many details was hard to come by when planning this plant, which prompted us to present this poster.

The poster describes our solution and provides many of the necessary detail when planning for, installing, and operation such a plant. We will discuss pressure issues, magnet hookup and safety considerations, choosing proper dimensions and their impacts, accessories that you might need, but are not necessarily provided or even discussed in the planning stage.

NMR STUDIES OF MULTIFERROIC XMn7O12 (X = Sr, Bi) AND BiMn3Cr4O12

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Promising multiferroic materials for further applications are those which display the highest ferroelectric polarization, high magnetoelectric coupling, and critical temperatures near room temperature. Interesting candidates with these properties are so-called quadruple perovskites with chemical formula RMn_7O_{12} (R= Pb, Cd, Ca, Sr, Bi, …), where R-atoms mix with three Mn atoms in perovskite A positions, while the octahedral B sites are solely occupied by Mn atoms. Only one of these compounds, $CaMn_7O_{12}$, has been studied in detail until now [1]. There is a general agreement that in these materials the ferroelectricity is driven by spin order of Mn atoms. However, local spin structure of such materials was only partially confirmed by neutron diffraction [2].

Origin of the ferroelectric phase transition of $(BiMn_3)Cr_4O_{12}$ is different. In this material the ferroelectricity is driven classically by the soft phonon describing Bi cation displacement [3]. None of these compounds have been studied using NMR so far, although NMR is powerful technique for local magnetic structure investigations and can help us answer the question about the connection of local spin structure and ferroelectricity.

We are reporting 55Mn NMR studies of $SrMn_7O_{12}$ ceramics within temperature range $4.2 - 25K$ in zero external magnetic field, 55Mn NMR measurements of $SrMn_7O_{12}$ in magnetic-field $0 - 2.5$ T at 4.2 K, 55Mn NMR spectrum of $BiMn_7O_{12}$ and 55Mn NMR spectra of $BiMn_3(Mn_{0.4}Cr_{3.6})O_{12}$ measured in zero external magnetic field at 4.2 K. Comparison of the measured spectra with DFT calculations and possible interpretation will be presented.

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PO190

Probing the atomic-level structure of LiPON amorphous electrolytes of microbatteries using solid-state NMR

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Amorphous lithium phosphorus oxynitride (LiPON) thin-films benefit from good electrochemical stability against lithium and are currently the commercial standard electrolytes for all-solid microbatteries. Furthermore, these amorphous electrolytes can be prepared by radiofrequency sputtering in a wide compositional range and lack grain boundaries, which can result in structural i nhomogeneities that facilitate lithium dendrite propagation. Nevertheless, the structure of these amorphous materials of limited volume is challenging to characterize. Recently a structural model of LiPON prepared by radiofrequency sputtering has been proposed by combining solid-state NMR experiments and ab initio calculations.1 Nevertheless, the relationships between composition, atomic-level structure and transport properties of LiPON thin-films remain elusive.

To investigate these relationships, we characterized using 31P and 7Li solid-state NMR spectroscopy the changes in the atomic-level structure between LiPON thin-films prepared by radiofrequency sputtering containing various amounts of nitrogen.2 The 31P NMR spectra of all LiPON samples exhibits four distinct P sites corresponding to orthophosphate, PO_4^{3-}, pyrophosphate, $[P2O7]4-$, PO3N4-, and P2O6N5- anions. 2D 31P homonuclear correlation experiment indicates that these anions are randomly distributed in the LiPON thin-films in agreement with structural models derived by ab initio molecular dynamics. Furthermore, higher nitrogen content increases N/P ratio as well as the fraction of bridging nitrogen sites with respect to that of the non-bridging N atoms. These bridging N sites attract the Li+ ions less than the non-bridging N atoms, which results in lower coordination and higher mobility of Li+ ions as seen from the deshielding and narrowing of 7Li NMR signal, respectively. These modifications in the atomic-level structure and dynamics of LiPON revealed by NMR explain the increased ionic conductivity of LiPON samples with higher nitrogen content.

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PO191

NMR studies of intracrystalline dynamics in polyesters

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Synthetic polymers can only crystallize partially, as they consist of long polydisperse chains. The resulting semicrystalline morphology is governed by the presence of intracrystalline chain motion [1, 2]. In order to complement and further test these findings, a combination of Nuclear Magnetic Resonance (NMR) techniques has here been implemented to investigate the intracrystalline dynamics (ICD) of Polybutylene succinate (PBS). 1H low-field NMR measurements of Free Induction Decay (FID) and Magic Sandwich Echo show that the PBS sample exhibits constant crystallinity around 41% at different isothermal crystallisation temperature, suggesting that PBS may be a crystal-fixed polymer [1]. The independence of the second moment from the crystalline part of the FID signal upon heating elucidates no possible intracrystalline dynamics on a timescale faster than a microsecond. A combination of cross-polarization (CP) and direct polarization 13C MAS spectra with short recycle delay were used to differentiate the 13C resonances originating from the amorphous and crystalline domains. The Torchia 13C CP MAS experiment was implemented as it is currently the most convenient pulse sequence to detect any crystal chain diffusion by probing the change of T1-relaxation via chain diffusion [3]. Our analysis detected slow T1-relaxation longer than 90s for the crystal resonances from 30°C to 90°C, indicating the PBS crystal chain was highly rigid; no slow chain dynamics up to 1s was detected. Lastly, the DIPSHIFT measurement shows that the 13C-H dipolar coupling of PBS crystal chains almost reaches the rigid limit of 23kHz, supporting the claim that the PBS polymer does not exhibit intracrystalline dynamics.

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PO192

The functionality of Stacking Faults on the Ionic Conductivity of Sulfide Solid Electrolytes

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All-solid-state lithium batteries, with a high theoretical energy density and high safety, have emerged as potential next-generation energy storage devices. The key to developing ASSLB lies in the development of high-performance solid electrolytes (SEs). Among all SEs, sulfide solid electrolytes (SSEs) stand out as the most promising candidates as they simultaneously meet all the significant demands with regard to practical utilization, such as ultra-high ionic conductivity (around 10-2 S·cm-1 at room temperature), satisfactory mechanical strength whilst performing excellent wettability,

flexibility, and processability. Further improvement of the ionic conductivity of SSEs still is a hot topic of research, as they haven't reached the theoretical limit yet. The incorporation of guest cations into base SSEs to reduce activation energies for ion-hopping and/or increase the concentration of vacancies, both facilitating the rapid diffusion of conductive ions, is the most feasible strategy that has reached the limit, however. Alternative strategies, therefore, need to be developed. Recently observed stacking faults in halide-based solid electrolytes have been shown to influence their ionic conductivity. Hence tuning the stacking faults of SSE to form more defects/vacancies is a promising way to further boost their ionic conductivity. In this work, we look into more detailed structural signatures relevant to stacking faults with 31P refocused inadequate 2D solid-state nuclear magnetic resonance (NMR) and X-ray diffraction (XRD) techniques. Then we correlated them with Li+-ion mobility (determined by electrochemical impedance spectroscopy (EIS) and variable temperature 7Li static NMR determining the spin−lattice (T1) relaxation constant) and built the structure-property relations by density functional theory (DFT). The results presented in this work will reveal insights into a novel strategy for boosting the ionic conductivity of SSEs by regulating the lattice stacking faults.

Orientation and Dynamics of Water Molecule in Beryl

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Orientation and dynamics of water molecules in cavities of beryl single crystal were systematically studied by means of ^{1}H and [2]H Nuclear Magnetic Resonance (NMR) spectroscopy. NMR spectra, measured in a temperature range 5–360 K, support the generally accepted notion that the water molecule is not oriented randomly, but preferably points with its H–H line along the hexagonal axis of the beryl crystal. However, the number of NMR spectral lines and their characteristic splitting clearly indicate that hydrogen atoms in water molecule are not equivalent and the equilibrium orientation of H–H axis thus must be appreciably deviated from the direction of hexagonal axis of beryl. Based on detailed analysis of ^{1}H dipolar interaction and ^{2}H quadrupolar interaction – and in contrast to the generally accepted concept of water behavior in beryl – we suggest that the water molecule is about 18° tilted from the hexagonal axis of beryl and is oriented by its O–H bond towards one of oxygen atoms in the cavity walls, which allows forming a hydrogen bond. The molecule performs two types of movement: rapid oscillations around the hydrogen bond axis and occasional jumps between the twelve possible binding sites available in the cavity. The frequencies of the oscillatory motions are evaluated from a simple thermodynamic model and agree well with wavenumbers of libration motions observed by optical experiments in available literature.

PO194

Design and Synthesis of Fluorine-Based Nanocrystals for ^{19}F-MRI Applications

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Until very recently, ^{19}F-MRI was applicable only when using organo-fluorine formulations, with no reports of the use of inorganic fluoride-nanocrystal (NCs, i.e., nanofluorides). Proposing small (d ~10nm) nanofluorides as inorganic colloids that tumble fast in solution to average out homonuclear dipolar interactions, of the fluoride within their solid core, result with a new type of nanotracers for ^{19}F-MRI applications.

Specifically, the characteristic ^{19}F-NMR spectra of alkaline-earth based nanofluorides (MF_2) span over wide range of chemical shifts (δ CaF_2 = -109ppm, δ SrF_2 = -88ppm, δ BaF_2 = -10ppm) thus making them ideal for "multicolor" ^{19}F-MRI.

In addition, their chemical modifiability allows to decorate their surface for targeting purposes. Nevertheless, their very long T_1 results with a limited signal-to-noise ratio (SNR) at a given time of acquisition and raise challenges when implementing them in vivo.

Here, we show that doping nanofluorides with paramagnetic lanthanides (Ln^{3+}), expressing paramagnetic relaxation enhancement (PRE) properties, results with shortening of their T_1 relaxation times by ~200 (from 15 sec to ca. 70 ms) resulting in x8 improvement in their ^{19}F-MRI SNR.

We also show improvement in their target-ability by coating their surface with multivalent lactose moieties, obtaining paramagnetic glyconanofluorides, enhancing their uptake by active immune cells to provide background-free in vivo mapping of both local inflammation and neuroinflamation, using ^{19}F-MRI.

Finally, we demonstrate the principles to obtain dispersed BaF_2 colloids showing for the first time their use as ^{19}F-MRI tracers. Using CaF_2, SrF_2 and BaF_2 colloids allowed their presentation in a multicolor ^{19}F-MRI fashion showing their potentiality to be further developed for multiplexed imaging purposes in the future.

Investigating the Effects of Post-Synthetically Treated MAPbI3 Using solid-state NMR and Synchrotron X-ray Diffraction

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Hybrid perovskites, consisting of an organic cation inside a metal halide scaffold, arose in 2009 [1] as low-cost photovoltaic devices. Methylammonium lead iodide ($MAPbI_3$) is one of the most extensively studied hybrid perovskite materials since it exhibits high photovoltaic conversion efficiencies (> 20%), while substantially reducing production costs over existing technologies.[2-5] However, thermal and moisture stability issues, along with structural variability over the general operating temperatures of a perovskite solar cell (15 – 70 °C), affects their overall photovoltaic performance.[6]

Our research focuses on developing post-synthetic annealing treatments that allow us to manipulate the hybrid perovskite materials in the bulk. Most notably, we have stabilised a new cubic phase of $MAPbI_3$ at room temperature. Using ^1H, ^{13}C, and ^{14}N solid-state NMR, we are able to probe the dynamics of the methylammonium cation in isolation to the surrounding inorganic octahedra.

We show that post-treatment of $MAPbI_3$ under various conditions alters the motions of the cation which correlates to the change in tilting of the PbI_6 octahedra observed in X-ray diffraction. This demonstrates the importance of using a combination of techniques to characterise the structural modifications in the treated phases, while demonstrating the synergy between the structure and dynamics in these systems. This opens up the opportunity for a range of new related hybrid perovskite materials through topotactic transformations that can have properties tuned according to the requirements of their application.

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PO196

Solid-State NMR Study of Hydrogen Bonding in Mesogenic Ionic Liquids

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The occurrence and stability of the liquid-crystalline phase in mesogenic ionic liquids are strongly related to a balance between hydrogen bonding (HB), electrostatic interactions, and dispersion forces. We have recently demonstrated a profound role of HB in mesophase stabilization in ionic liquid crystals [1-6]. Indeed, the temperature range of the smectic phase is strongly correlated with the HB ability of counterions [2,3,5]. In the present study, the HB interactions in imidazolium-based mesogenic ionic liquids are examined and compared in solid, isotropic liquid, and liquid crystalline states as well in ionic liquids nanoconfined in porous solids. An important finding in our study is that HB strength decreases only slightly on the transition from isotropic to mesophase despite the high molecular order and layered assembly in the smectic phase. The HB is not disrupted owing to the formation of an ionic sublayer where anions can approach polar groups of cations. This dynamic ionic sub-layer is formed irrespective of the structural position of the charged group in the cation. When ILs are nanoconfined in porous silica, the cation-anion HB is locally broken by anion H-bonding to the solid interface. The proximity of cations and anions to the solid surfaces was probed using a solid-state heteronuclear correlation NMR spectroscopy.

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PO197

Assessing the quantification of acetylation in konjac glucomannan via ATR-FTIR and solid-state NMR spectroscopy

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Dietary fiber like konjac glucomannan (KGM) is important in maintaining good human health. There is no established method for quantifying the average degree of acetylation DA of this polysaccharide. Polysaccharides are notoriously difficult to dissolve. In this study, KGM could not be fully dissolved in common solvents and was characterized in the solid state. ATR-FTIR spectroscopy enabled a fast qualitative assessment of acetylation, selective to the outer layer of KGM particles, and identifying excipients like magnesium stearate. Average DA was quantified for the first time with solid-state 13C NMR in KGM: semi-quantitative measurements on the same arbitrary scale by cross polarization (1 to 2 days) were calibrated with a few longer single-pulse excitation measurements (approximately 1 week). DA values ranged from 4 to 8 % of the hexoses in the backbone, in agreement with previously reported values. This method could be used for quality control and standardization of KGM products.

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PO198

Magic Angle Spinning Pulsed Filed Gradient NMR of Ionic Liquids Confined to Carbon Black

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The investigation of ionic mobility plays a crucial role for the understanding and quantification of electrochemical systems such as batteries, fuel cells, or electrolyzers [1]. Important parameters include transference numbers, diffusion, and aggregation in liquid- and ionic liquid-based electrolytes or ion mobility in ion-conducting solid electrolytes [2]. The investigation of species inside porous matrices, which are of importance in metal-air batteries, or in solid ceramic or hybrid ceramic-polymer electrolytes for solid-state batteries is particularly challenging [3]. In both cases, ions move in different environments or across interfaces; hence, multiple environments must be distinguished to obtain a comprehensive description of dynamic processes.

This contribution focuses on ionic liqids (IL) adsorbed in carbon black (CB) as a promising candidate for porous electrodes. Insight into the dynamics of such systems is imperative for tailoring electrochemical performance. 1-Methyl-1-propylpyrrolidinium bis(trifluoromethylsulfonyl)imide and 1-Hexyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide are studied in CB host using spectrally resolved Carr-Purcell-Meiboom-Gill (CPMG) and 13-interval Pulsed Field Gradient Stimulated Echo (PFGSTE) Magic Angle Spinning Nuclear Magnetic Resonance (MAS- NMR). Data are processed using a sensitivity weighted Laplace inversion algorithm without non-negativity constraint. Previously found relations between the alkyl length and the aggregation behavior of pyrrolidinium-based cations are confirmed and characterized in more detail. For the IL in CB, a different aggregation behavior is found as compared to the neat IL, adding the surface of a porous electrode as an additional parameter for the optimization of IL-based electrolytes. Finally, the suitability of MAS is assessed and critically discussed for investigations of this class of samples.

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Structural insights into germanium halide perovskites via 133Cs and 73Ge solid-state NMR

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Metal-halide perovskites remain top candidates for better-performing photovoltaic devices but concerns with Pb-based materials remain. Much work in lead replacement has been focused on Sn perovskites, with photoconversion efficiencies having already surpassed 10%. Ge perovskites remain understudied for use in solar cells compared to their Sn-based counterparts, with only modest efficiencies being reported to date (0.11-0.57%). Detailed structural information is essential for overcoming stability and efficiency issues for these materials. Extensive structural studies exist for both Pb and Sn perovskites, with solid-state nuclear magnetic resonance (NMR) spectroscopy being a key technique in the exploration of perovskites today. Herein, we discuss a combined ^{133}Cs and ^{73}Ge NMR and DFT approach to investigate bulk $CsGeX_3$ (X = Cl, Br, I) perovskites. We show how small structural variations within germanium halide perovskites have major consequences on their ^{73}Ge and ^{133}Cs NMR parameters – with a cubic-like CsGeCl3 phase at room temperature being present, whilst having severe local Ge octahedral distortion. Quantum chemical computations are shown to be effective at predicting the structural impact on NMR parameters for ^{73}Ge and ^{133}Cs. Together, NMR and DFT provide an effective route to understanding polyhedral distortions in perovskites on an atomic level.

Cation Dynamics and DNP in Hybrid Perovskites

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Hybrid lead halide perovskites are promising materials for photovoltaic applications due to their high power-conversion efficiencies, tunability, and inexpensive solution processing. Among various favourable optoelectronic properties, the long charge-carrier lifetimes of hybrid perovskites have been attributed in part to the fast rotational dynamics of the organic cations, which reside in the cuboctahedral cavities of the inorganic lattice; however, these dynamics remain poorly understood, with conflicting results in the literature. Here, we perform quadrupolar ^2H and ^{14}N relaxometry to determine the cation dynamics of the most technology relevant cation, formamidinium (FA+, $CH(NH2)2+$), in FAPbI3. By simultaneously fitting all the ^2H and ^{14}N T_1 relaxation constants as a function of temperature with an anisotropic rotational diffusion model, we determine the activation energies and correlation times for rotation about each of the three distinct principal axes. This contrasts with the majority of previous studies for which only an average rotational rate was considered. We then extend this methodology to investigate how the dynamics change in mixed cation perovskites, as used in photovoltaic devices. Finally, by understanding the relaxation properties of the organic cation, we are able to design dynamic nuclear polarisation (DNP) experiments which achieve far higher signal enhancements than were previously possible, enabling the observation of the extremely mass-limited surface coating on a single thin-film perovskite sample.

PO201

^{17}O High-Field Solid State-NMR for characterization of hydrogen bonding in pharmaceutical compounds

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It was recently demonstrated that mechanochemistry can bring promising routes for fast and low-cost ^{17}O labelling of organic and inorganic compounds [1]. This is of high practical interest in the pharmaceutical industry: carboxyl and hydroxyl functional groups are ubiquitous in active pharmaceutical ingredients (API) and, due to their propensity to form hydrogen bonds, are very often used to engineer new solid forms of APIs, with improved solubility. In such cases, ^{17}O ss-NMR can provide direct probes for characterization of the established hydrogen bonding networks. We have successfully enriched ibuprofen in ^{17}O and subsequently use the ^{17}O enriched Ibuprofen, to form Ibuprofen – Bipyridine cocrystal and Ibuprofen – Tris salt. The three samples are fully characterized using high field and fast MAS solid-state NMR experiments on ^{1}H, ^{13}C and ^{17}O. The experimental results are compared with the NMR parameters calculated on the X-ray reported structures using the gauge-including projector-augmented wave (GIPAW). NMR DFS-ECHO-MAS experiments on ^{17}O performed at different temperatures shows that for ibu and for Ibu-bipy cocrystal there is exchange between the protonated oxygen and the non-protonated oxygen of the carboxylic group. At -10 ⁰C in a 20 T magnetic field the two ^{17}O signals are 14 kHz apart, the exchange is much slower than 14000 exchanges / second and the two signals are clearly separated, whereas at + 40 ⁰C the exchange is much faster than 14000 exchanges / second and only one resonance signal is observed. The results demonstrate that ^{17}O ss-NMR may represent a fast structural characterization tool for identifying whether the API and the excipient are forming a salt or a cocrystal.

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PO202

NMR Study of Ion Adsorption in Activated Carbon

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The charge storage in supercapacitors relies on the formation of the electric double layer at the electrode surfaces. In the aqueous system, whilst several electrochemical studies on different electrolyte salts have been carried out, the possible roles of hydroxide ions (OH-) and hydronium ions (H3O+) in the electric double layer formation have yet to be investigated and the mechanism of the charge storage in aqueous electrolyte remains unclear.

In this work, nuclear magnetic resonance (NMR) spectroscopy has been used to study the ion adsorption at the electrode-electrolyte interfaces. NMR experiments performed on the electrolyte anions and cations demonstrate that three environments are observed in the carbon sample soaked with aqueous electrolyte. Further variable temperature experiment reveals that one of the signals originates from the partial exchange between the bulk electrolyte ions and ions adsorbed inside carbon nanopores. Thus, the quantification of total in-pore ion population needs to take account of both the apparent in-pore ion intensity as well as the contribution of in-pore ions to the exchange peaks.

The method developed for in-pore ion quantification was then applied to study the electroneutrality of the total charge given by the electrolyte ions. The apparent imbalance of charges inside the carbon pores was investigated by solid state NMR. Combining with the pH measurements for YP-50F carbon, we obtained a holistic description of the net charge neutrality in the nanoconfined region. These results show that the participation of charged species (OH- and H3O+) from water dissociation in the electric double layer plays an important role in charge balancing, and explain the pH dependence of the capacitance from a molecular level.

PO203

Li+ Ion Diffusion in Solid State Electrolyte Li3InCl6 measured by 7Li Liquid State NMR

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Conventional lithium-ion batteries have changed modern life. However, concerns remain about their energy density limit and safety risks introduced by flammable liquid electrolytes. One alternative is all-solid-state Li batteries (ASSBs) which are not only safer but also can achieve higher energy densities. One of the most vital components of ASSBs is the solid-state electrolyte (SSEs). The optimization and design of SSEs is still underway, of which ion conductivity, Li+ diffusion mechanisms, and their stability are key. For the study of ion conduction mechanisms and Li+ diffusion, pulsed gradient spin echo (PGSE) NMR methods are ideal. The PGSE method provides diffusion time dependent 7Li apparent diffusion coefficients (D) and distances on the scale of 0.2-10 micrometers. In the short diffusion time limit, the electrical conductivity is directly proportional to D via the Nernst-Einstein equation. In the long diffusion time limit, D scales with tortuosity and restricted motion, such as the effect of grain boundaries.

In this study, the Li+ diffusion mechanisms in Li3InCl6 are studied as a function of treatment condition. Li3InCl6 is a halide-based SSEs with a stable monoclinic crystal structure and good ionic conductivity. It is known that the ionic conductivity increases after annealing; however, the mechanism by which conductivity increases is unknown. The results indicate that it is stable when exposed to air and that the Li+ D is enhanced by annealing even though the presence of intraparticle restrictions increase. The Li3InCl6 samples are also characterized by D-T2, T2, T2-T2, and T1 measurements as well as FESEM, TEM/STEM and XRD.

PO204

Harnessing water to enhance quadrupolar NMR spectroscopy and imaging

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^{17}O and ^{14}N are attractive targets for in vivo molecular NMR spectroscopy and imaging, but their low gyromagnetic ratios (γ) and fast relaxation complicate these observations. This work explores indirect ways for detecting some of these sites, with the help of proton-detected double resonance techniques. As standard coherence transfer methods are of limited usefulness for such indirect detections, alternative routes for probing the quadrupolar spectra on ^{1}H were tested. These centered on modulating the broadening effects imparted onto protons adjacent to the low-g species via J-couplings through either continuous wave or through spin-echo double resonance decoupling/recoupling sequences. As in all cases the changes imparted by these double-resonance strategies were small due to the fast relaxation undergone by the quadrupoles, these effects were amplified by porting their effects onto the abundant water ^{1}H signal. These amplifications were mediated by the spontaneous exchanges that the labile ^{1}Hs bound to ^{17}O or ^{14}N undergo with the water protons. For the experiments designed on the basis of double-resonance spin echoes, these enhancements were imparted by looping the transverse encodings together with multiple longitudinal storage periods, leading to Decoupling-Recoupling with EXchange (D-REX). For the experiments designed on the basis of continuous on/off quadrupolar decoupling, these solvent exchanges were incorporated into chemical-exchange saturation transfer schemes, leading to the Decoupling-Recoupling with Saturation Transfer (D-REST) sequence. Both of these variants successfully harnessed sizable proportions of the easily detectable water signals, in order to characterize the NMR spectra and to image with atomic-site specificity the ^{17}O and ^{14}N species.

PO205

Molecular Dynamics in Polymer-Ionic Liquid Systems Studied by Magnetic Resonance Methods

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In this contribution we present a study of molecular dynamics of Ionic liquids (ILs) incorporated in polymer matrices (IL/pol). The ILs consist of organic cations based on imidazolium rings and different types of anions. Depending on the anion type, the polymers form either a rigid matrix with porous structure where the confined ILs express high mobility, or a gel-type matrix where the mobility of the ILs is reduced with respect to the bulk. By studying molecular translational diffusion and NMR relaxation times, we recover information about the molecular interactions taking place in the system. In addition, we are interested in the study of the mobility and interactions of stable organic radicals (nitroxide, porphyrins), with the polymers and ILs, with the aim of studying mechanisms of electron polarization transfer to the NMR active nuclei in the system, i.e. 1H and 19F. For this purpose, X-band EPR and DNP experiments were performed. Moreover, NMR Relaxation Dispersion (NMRD) is used to distinguish between different dynamical models expressed in terms of their spectral densities [1].

All this information is used to obtain a deeper understanding of the basic principles underlying charge transport processes, molecular dynamics, and physico-chemical stability in these systems, essential for any practical application in the field of material science focused on sustainable energy supplies and energy storage materials. For instance, ionic conductivity is related with translational mobility and Nuclear Magnetic Relaxation (NMR) is used to assess information on microscopic rotation and translational dynamics in these systems.

The current state of our research related to the ILs emim-TFSI, emim-DCA combined with Poly(vinyl alcohol) (PVA) and nitroxide radicals will be given, specifically about molecular rotation and translation correlation times retrieved by analyzing advanced models on the electron-nuclear spin interaction.

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PO206

Real time monitoring of the through thickness moisture profile of thin sheets using NMR

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The through-thickness distribution of liquids and liquid migration over time in thin, porous media is highly relevant for the production and product properties of materials such as textiles, membranes, paper, barrier materials, and thin films. We present a 1D NMR profiling method for the analysis of through-thickness liquid distribution during liquid absorption, migration, and drying of thin sheets [1].

The good temporal resolution (1.3 s) and high spatial resolution (10 μm) make it possible to study changes of liquid distribution in sheets of about 100 μm thickness. A key aspect of our method is the simultaneous scanning of a liquid saturated reference sheet. Using a reference, local liquid saturation and absolute amount of liquid present over time can be evaluated. We studied the drying of water-based inkjet ink on paper substrates with differing water absorption behaviors. Differences in z-directional liquid distribution, penetration depth, and drying kinetics over time could be observed.

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PO207

NMR Insights into the impact of Al incorporation on the structure and dynamics of β-Li3PS4

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The ever-growing demand of high-energy-density and high safety energy storage devices has driven intensive research on all-solid-state lithium batteries which largely hinge on developing highly ion-conductive and reliable solid electrolytes. Aliovalent element doping is a generic way of improving the ionic conductivity of solid-state electrolyte via introducing vacancies or interstitials. Solid-state NMR has been the technique of choice to reveal the doping mechanisms and conduction pathway. Aluminum doping has been widely used in oxide electrolytes to stabilize the structure and enhance the Li ionic conductivity. Herein, we elaborated a set of Al-doped sulfide electrolytes based on the β-Li3PS4 platform. Electrochemical measurements indicated that an appropriate doping amount would result in the highest ionic conductivity. Further increase of Al caused sudden collapse of ionic conductivities due to the destruction of the β-Li3PS4 crystal structure. The impact of Al incorporation on local structure and Li ion dynamics was investigated comprehensively by solid-state NMR. The analysis of the temperature dependence of 7Li linewidth showed that the Li ion jump attempt frequency increased substantially because of the generated vacancies in the Li channels due to Al doping. Variable-temperature static 7Li NMR relaxation revealed that the activation barrier for long range Li ion diffusion decreased a lot. 27Al, 31P as well as 6Li MAS NMR also showed interesting differences which allows a deeper analysis of the Li ion transportation mechanism.

PO208

Moisture-induced CO2 species in amine-based solid adsorbents: molecular-level study from solid-state NMR and molecular modeling

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Solid sorbents are viable candidates for post-combustion CO_2 capture due to their good sorption capacity, stability, ease of handling and reusability. The nature of CO_2 species interacting with porous surfaces determines many of the properties of the adsorbents. However, an atomic-level understanding of the CO_2 sorption process remains elusive, hindering our ability to design improved sorbents to help mitigate CO_2 emissions. The lack of advanced spectroscopic studies, tailored to elucidate the structure of adsorbed gas species, has also been a bottleneck for further progresses in understanding gas-solid interfaces.

In recent years, our group has focused on understanding CO_2 chemisorption processes in amine-modified mesoporous silicas (SBA-15) combining solid-state NMR and computer modeling.[1] We've shown, for instance, that control over amine surface density enabled the detection of proton-transfer, among distinct CO_2 species, using NMR CSA.[2]

Here we present a comprehensive study of the influence of moisture and CO_2 partial pressures on CO_2 speciation upon CO_2 chemisorption on SBA-15.[3] Solid-state NMR and molecular modeling are used in tandem to shed light on the nature of such surface gas species, under dry and wet conditions, providing molecular-level details on the formation mechanism of moisture-induced CO_2 species. This work extends the current understanding on the nature of chemisorbed CO_2 structures under wet conditions.

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PO209

Multidimensional Lead Halide Perovskites: Insights into 35/37Cl Chemical Environments Using Solid–state NMR Spectroscopy

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Lead halide perovskites (LHPs) are forerunners in the field of next–generation energy materials and have found use in photovoltaics, displays, lasers, detectors, batteries, and catalytic applications due to their phenomenal optoelectronic properties. The availability of n–dimensional (n = 0, 2, 3) LHPs opens a wide range of applications, which makes this particular family of semiconducting materials appealing. Solid–state nuclear magnetic resonance (ssNMR) spectroscopy has been shown to be a robust analytical characterization method for studying LHPs using readily accessible nuclei such as ^{133}Cs and ^{207}Pb in the A and B sites, respectively [1]. Comparatively, more challenging quadrupolar halogen nuclei are largely overlooked due to the challenges associated with experimentation time and sensitivity. Halogens are the intrinsic linker between A and B sites, which can reveal crucial details about doping, vacancies, chemical environments, and dynamics in LHPs [2,3]. Here, we discuss the efficacy of ultra–wideline NMR approaches for rapid acquisition of ^{35}Cl and ^{37}Cl ssNMR spectra of 3D organic–inorganic hybrid (A = FA and MA) and Cs–based 3D, 2D, and 0D LHPs from moderate (7.05 T) to ultrahigh (21.1 T) magnetic fields. We further investigate the quadrupolar NMR parameters (CQ and Đ), that exhibit distinct signatures towards unique Cl chemical environments affected by both A–site variation and dimensionality; additional support was provided by quantum chemical computations using GIPAW–DFT approach in CASTEP. These findings may pave the way for routine and robust spectroscopic analysis of Cl chemical environments in halogen–based semiconducting materials.

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PO210

Packing of polyanions in polelyectrolyte complexes – a combined PFG and solid-state NMR study

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Polyelectrolytes and complexes of oppositely-charged polyelectrolytes find wide application in surface modification, water treatment, paper production or controlled drug release. Such complexes exhibit better performance and stability against changes in the surrounding media in particular for applications for the environment or biological applications. Therefore knowledge of the inner structure of such complexes is desirable.

The conformation of polyelectrolytes in solution depends on the repelling force from the charges along the polymer chain. For a weak polyanion poly(maleic anhydrite-co-ethylene) it depends on pH and ionic strength. The effective charge is determined from electrophoresis NMR while diffusion NMR yields the hydrodynamic size as a measure for the conformation. With increasing pH the weak polyacid dissociates generating more charges and thus a more stretched conformation.

Separating 1H spectra in two-dimensional single-quantum-double-quantum correlation spectra distinguishes between acid protons hydrogen bonded to other acid protons. Thus polyanion-rich regions in solid polymers or complexes are identified and can be quantified by two-dimensional integration of the on-diagonal and off-diagonal signals of the acid protons. At low pH (weak charge) this are reduced by a factor of three in the complexes compared to the pure polyanion. At higher pH (high nominal charge) with a more stretched conformation almost none acid-acid contacts are found in the complexes. pH of the solutions is adjusted by NaOH. In 22Na spectra signals from NaCl and sodium maleate are distinguished and quantified. Even at the highest pH when all of the polyanion is dissociated about one quarter of the sodium is detected in maleate in the complexes proving so-called extrinsic charge compensation. In the partially dissociated poly(maleic anhydrite-co-ethylene) very strong hydrogen bonds are observed.

PO211

Rheo NMR - stress response and flow visualization

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Rheological NMR, NMR under the application of external shear, is applied to study the response of materials to external stress. Proton T2 has been used to probe polymer chain mobility. Often at least two components are observed, a short one for the fraction close to entanglements and a longer one for the more free chains. It has been found that for entangled polymers both T2 components become longer under continuous shear and the fraction of the short T2 decreases. This is interpreted as a loss of entanglements resulting from the shear. This finding is contrary to the common expectation that the dominating effect of shear would be shear-induced orientation. Further studies involve oscillatory shear with a crank-swing gearbox allowing to vary both the frequency and the amplitude of the deformation. This oscillatory shear reveals that this effect sets on at a minimal deformation of about 100 at all frequencies. While there is less mobility in proteins shear can trigger orientation and subsequently aggregation. Orientation effects have as well been found in molecular dynamics simulations. In silk fibroin the shear-induced orientation results ß sheets and rapid precipitation.

Flow NMR imaging in addition enables to study the flow filed. For the double-cylinder (Searle) cell used here counter-rotating flow has been observed depending on the gap size and viscosity under oscillatory motion close to the point reversing the velocity which has been confirmed in CFD simulations. This results in strong velocity gradients and thus shear rates which can be three times larger than at the point of maximal rotational velocity. This needs to be considered in the interpretation of rheological data of low-viscosity materials. Another type of counterflow is observed if the inner cylinder is placed off the center of the outer, which then results in complex flow pattern and shear rates.

PO212

Application of ssNMR to study structure and dynamics in natural biopolymers

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Velvet worms, or Onychorphora, are soft-bodied carnivorous organisms that range from a few cm to 60 cm in size. They are nocturnal predators who squirt a sticky slime in their defence or immobilise prey. The slime hardens quickly and turns into a mesh of strong threads. The slime of velvet worms consists mainly of proteins, which make up 55% of its dry mass. In a local species found in Singapore, we identified multiple different proteins, with molecular weights ranging from 12 kDa – 231 kDa. High molecular weight proteins in slime are the main components for the slime fibres. However, further molecular information about the physicochemical properties of slime remains elusive. Our work is the first detailed study on the molecular composition of slime from tropical species found in Singapore. We used solid-state NMR combined with biochemical studies to understand the interactions between different proteins in slime. We fed 13C Glucose to the velvet worms to isotopically label the slime proteins for NMR studies. Molecular dynamics information obtained from 13C ssNMR spectra of the collected slime provided exciting insights into the protein sequences which undergo structural transitions mainly responsible for converting the crude liquid slime into dry fibres. We believe our study is pivotal to expanding NMR applications in material science to study the structure and dynamics of natural biopolymers.

Posters

PO213

Spin isomer conversion in endohedral molecules in C60

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Formaldehyde, like molecular hydrogen and water [1, 2], exists in two spin isomers corresponding to total nuclear spin 1 (ortho) and 0 (para) of the two hydrogen nuclei [3]. The ortho and para states can be identified as the antisymmetric and symmetric rotational states corresponding to rotations about the molecule's C=O bond. Rotational spectroscopy shows the ground state to be a para state, and silent to 1H NMR. Thus, the conversion between ortho and para states of formaldehyde can be observed by following the change in NMR signal intensity as a function of temperature. A similar treatment for methane shows the existence of three spin isomers, with the ground state having a total nuclear spin of 2 due to the four protons [4].

We will present 1H NMR measurements at cryogenic temperatures on formaldehyde and on methane encapsulated in the fullerene C60, synthesised by the 'molecular surgery' technique previously used for other endofullerenes [5]. Such encapsulated molecules are known to undergo rotations even at very low temperatures. Temperature jump experiments to measure the 1H NMR signal contrast between low temperatures, where almost all the population resides in the ground state, and moderate temperatures, where a large fraction of the population is expected to be in the excited states, will be presented, along with spin isomer conversion time estimates. Initial progress towards Quantum Rotor Induced Polarisation [6] in the formaldehyde system will also be presented.

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PO214

Solid-state NMR spectroscopic investigation of supported novel imidazolium-based task-specific ionic liquids for catalytic applications

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Supported Ionic Liquid Phase (SILP) catalysts are a new class of materials, obtained by depositing on a solid support a thin liquid film of an ionic liquid (IL), which contains a dissolved molecular catalyst. Control of the catalytic properties of the active sites and wetting behavior of the IL is obtained by combination of ionic liquid with suitable supports. Understanding the behaviour of the IL film and its interactions with the support is therefore essential to fully exploit the potential of SILP catalysts [1]. This allows for the design of new, tailor-made ILs with a specific arrangement on the surface and favourable interactions with both catalyst and substrate. These targeted interactions will preferentially direct the location of the active complex, either in the gas/IL or in the IL/solid interface, to obtain an interface-enhanced SILP catalyst.

We investigate novel imidazolium-based task-specific ionic liquids, synthesized to orient perpendicularly to the support, and their interaction with a silica surface. Four task-specific alkyl fluorinated ILs were deposited on 29Si-enriched SBA-15. 1H, 19F, 29Si MAS NMR spectra as well as Cross Polarization proved fluorine interaction with the surface. Furthermore, two-dimensional correlation methods, such as DQMAS and HETCOR, allowed to study the IL molecule conformation on the silica surface. Finally, REDOR experiments gave insight of the internuclear distance of the interacting spin pair, 29Si and 19F.

PO216

Understand the Effect of H-bonding in Photocured Polymer Films using NMR

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Polyacrylates are important polymers widely used in the pharmaceutical industry such as drug coatings due to their low cost, processability, and ease of functionalisation. Chemical functionalities (e.g. H-bonding) can be easily included to modulate the transport of low molecular weight drug-like entities through the network. Understanding how such microscopic structural modifications determine macroscopic diffusion is critical for designing next-generation responsive polymers. In this study, we used a series of NMR techniques (DQ NMR, diffusometry, relaxometry) to understand the effect of hydrogen bonding on the topological and morphological properties of these photocured polymer membranes, as well as its relationship with the diffusion of small molecules inside these polymer matrices. Additionally, detailed IR analyses on cured samples show that networks made from structurally distinguishable mono-acrylate have a significantly different extent of H-bonded monoacrylate present. By correlating DQ NMR data, we found that this is induced by differences in the relative reaction rate between homo-polymerization and copolymerization of the relevant monoacrylates and crosslinker (PEGDA). Although 'pre-organization' of H-bond capable monoacrylates was observed for those tested formulations prior to crosslinking, which should significantly increase the homo-polymerization rate, it was found that the co-polymerization rate was also significantly increased. Furthermore, DSC thermoporometry showed a significant increase in the average network mesh size potentially due to the pre-organization of H-bonding containing monomer during network curing. By introducing the H-bonding disrupter, $LiClO_4$, it was found that the diffusivity of solute became positively correlated to the average mesh size across the series of networks. Hence, a modified diffusion model based on hydrodynamic theory is proposed to separate the direct (solute-network) H-bonding contribution to solute diffusion from the indirect contribution arising from monomer pre-ordering induced mesh size reduction.

Posters

A mechanistic understanding of nanoplastic toxicity in the intact zebrafish embryo using HR-MAS NMR

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Plastic pollution, especially by nanoplastics (NPs), has become an emerging topic due to the widespread existence and accumulation in aquatic environment. The uptake and bioaccumulation of nanoplastics via food chain as well as the release of contaminants adsorbed on NPs might pose significant threats to organisms. The large-scale studies mainly employed chemically synthesized polystyrene model particles. However, the uptake, accumulation and toxicity effect of NPs from polyethylene terephthalate (PET), which is widely used for packaging material, have been poorly investigated. In the present study, we utilized state-of-the-art high-resolution magic angle spinning nuclear magnetic resonance (HRMAS NMR), applied to intact zebrafish (Danio rerio) embryos, as a model of vertebrate development, to elucidate toxicity effects and changes in metabolic profiles associated with PET NPs exposure.

HRMAS NMR analysis of intact zebrafish embryos exposed to sub-lethal concentrations of PET NPs afforded well-resolved spectra, and in turn, identification of several metabolites to be significantly altered, relative to controls. Complemented by several alternative analytical approaches (i.e., in vivo visualization and in vitro assay), HRMAS NMR identified robust and dose-dependent effect of PET NPs on several relevant metabolic pathways suggesting a multifaceted toxicity of PET NPs. A comprehensive model of nanoplastic toxicity is constructed and will be presented

PO218

Rapid Metabolomic Profiling by NMR Imaging

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In vitro fermentation models have been a long-standing method for probing substrates' propensity for fermentation, production of gas, and physiologically relevant metabolites, such as short-chain fatty acids (SCFAs). The primary SCFAs produced in the large intestine are acetate, butyrate and propionate, with all three having important physiological roles in hepatic gluconeogenesis, influencing metabolic homeostasis markers, such as hormones PYY and GLP-12, improving B-cell function, as well as insulin secretion. Dysregulation of the production of these SCFAs has been linked to cardiovascular disease (CVD), metabolic syndrome, and diabetes mellitus.

However, most current in vitro fermentation models involve complex set-ups, featuring multiple vessels, frequent sampling, and often introducing oxygen to the anaerobic fermentation set-up, which may perturb the life cycle and metabolism of anaerobes and obligate anaerobes, such as Bacteroides, Faecalibacterium, and Ruminococcus.

In this work, we demonstrate a new, rapid semi-automated method for the spatially resolved quantification of small molecular metabolites directly in an NMR tube, with minimal sample preparation or sampling. Our method is based on the chemical shift imaging (CSI) methodology, developed by Wallace et al., and further allows for the dynamic and accurate determination of pH, as a function of fermentation time, which has been shown to be useful in the rapid diagnostics of colorectal pathologies and bacterial dysbiosis.

Microcoil NMR and automated segmented-flow sample transfer for target identification and quantification of nanomole quantities

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NMR spectroscopy is well-known for its advanced and accurate NMR elucidation and quantification analysis of complex mixtures. However, the analysis of minor compounds in complex mixtures is still a major challenge. Separation techniques can provide microliter-size aliquots of sufficient purity to be elucidated and quantified by NMR [1]. Microcoil NMR would be well suited to assess such mass and volume limited compounds[2] but requires accurate sample handling and consecutively also a microfluidic transport.

In this work we address this challenge by working with Helmholtz NMR microcoils and automated segmented-flow sample transfer [3]. The robotic autosampler in combination with the microfluidic setup was adapted to prepare NMR samples of (sub)microliter volumes. They were placed as slug between fluorinated FC43 oil which allowed for a smooth sample transfer to both FEP tubing storage and the Helmholtz NMR microcoils. The microcoil insert was optimized for sensitive volumes of 0.1 µl with a resolution below 3 Hz obtained for the water peak. This enabled recording high-resolution NMR spectra with SNR of 10 for 32 scans for 1 nanomole quantities of peptides.

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PO220

Metabolic characterization of medaka inbred strains - a possible link between genotype und phenotype

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Understanding the relationship between genetic variation, environmental factors and phenotypic traits remains a fundamental challenge in modern biology. Analysis of metabolites that report on physiological pathways can help to establish direct causal and functional associations. The teleost medaka (Oryzias latipes) is a long-established model organism with several near-isogenic inbred strains providing a paradigm to study the influence of the genetic background on the metabolism in vertebrates [1]. However, no comparative studies addressing the influence of the genetic background on the metabolome have been reported yet.

NMR-based metabolomics provides the perfect tool for this purpose as it allows untargeted screening of a large sample cohort. First, we examined whether this method could reveal strain-specific differences in the metabolome using liver extracts of two classical medaka inbred strains iCab and HO5. Fifteen metabolites could be detected in uni- and multivariate statistical analyses that showed strain-specific levels. Moreover, the observed differences could be assigned to specific metabolic pathways [2]. Based on these findings, we have started to analyse embryonic stem cells from several medaka inbred strains that showed different behaviour in derivation of retinal organoids. Preliminary results also indicate differences in their metabolome. Our aim is to uncover a metabolic profile of stem cells that is characteristic for pluripotency and linked with efficient derivation of retinal organoids.

Our results show that NMR spectroscopy is a suitable method to reveal variance of the metabolome caused by subtle genetic differences. Thus, it has the potential to fill a gap in genotype-phenotype association studies in medaka. The correlation between the metabolome and genetic background will be addressed in future studies combining our findings with data from RNA sequencing and GWAS (genome-wide association studies).

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PO221

Flow encoding established by optimal control RF pulse

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Flow encoding MRI is traditionally achieved by applying bipolar gradients which cancel the even terms of Taylor expansion of the magnetization phase with respect to time, and the odd terms lead to a predominantly velocity-dependence of the phase. In this study, we show that velocity can be encoded into phase during the excitation time. First, a mathematical model is designed. Then, optimal control (OC) theory is used to design the required flow encoding RF pulse. Also, GRAPE algorithm is employed to minimize the cost function in the OC problem, and a constraint is added to make the RF pulse slice selective.

Since in this method encoding is achieved based on a mathematical model, it opens new doors to many beneficial abilities and flexibilities which are not accessible by traditional methods. First, during this process, encoding is established without the reliance on switching the gradients. This avoids the adverse effects of gradient induced eddy currents. Second, it enables one to achieve shorter echo times hence enhance signal-to-noise ratio. Furthermore, bipolar gradients lead to a linear relationship between phase and velocity; but in this method, a non-linear relation can be made which improves phase-SNR of regions with lower flow rates, for example close to vessel walls. Regarding further studies, many other parameters can be taken into account in the mathematical model. For example, OC problem can be asked to improve contrast in multiphase flows or address adverse effects of acceleration in turbulent flows.

PO222

Quantitative MR imaging and 2D velocimetry of ethane

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Thermal reactions using heated gases are the driving force behind industrial production processes. However, little is known about the distribution of the gas flow inside the reactor, hampering the optimization of chemical processes. Here we aim to investigate the gas flow inside the closed packed cell as a reactor model using magnetic resonance imaging (MRI) and 2D-MR velocimetry (MRV).

Static and flowing low-pressure ethane (C_2H_6) was imaged with a pre-clinical 7T MRI scanner (BioSpec 70/30, Bruker). For static MRI, 4 bar ethane was filled into a 38 ml borosilicate glass flask together with the 3D printed urethane dimethacrylate cylinders. A fast low-angle shot (FLASH) sequence was used for imaging: TE = 2.532 ms, 48x48 mm² field of view (FOV), 0.375x0.375 mm² pixel size, 49 averages.

Flow MRI: MRV maps of the 2.5 bar ethane flow were performed using a 2D flow compensated gradient echo sequence (35x35 mm² FOV, 36 averages, 50 cm/s velocity encoding, slice perpendicular to the flow). The ethane flow was controlled using a mechanical gas flow meter set to 9.03 L/h. Two pixel sizes were tested: 0.70x0.70 mm² and 0.27x0.27 mm².

The static and flowing ethane was successfully measured. A static ethane gave bright contrast with an SNR of 4.7±0.8, where 3D-printed cylinders resulted in signal voids. A flow rate of flowing ethane was 11.83 L/h and 10.85 L/h measured by the MRV at low and high spatial resolution, respectively. High resolution study resulted in better gas flow estimation close to the value set by the flow meter. Moreover, the flow imaging was performed just under 3 minutes.

Thus, 2D MRV appears to be well suited to map the flow profile of ethane, an essential step for measuring the multi-dimensional gas flow in complex structures.

PO224

Characterization of commercial iron oxide clusters as potential Magnetic Resonance Imaging contrast agent

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Superparamagnetic iron oxide particles are intensively used for molecular and cellular MR imaging in preclinical studies on small animals [1]. Clusters of iron oxide cores are especially efficient because of their huge effect on T2. The Polymag iron oxide clusters have initially been developed for magnetofection [2] but are here studied as potential contrast agents for MRI thanks to a multidisciplinary characterization including dynamic light scattering, electron microscopy, magnetometry and NMR relaxometry.

The clusters present a hydrodynamic diameter of 180 nm with a large polydispersity index (PDI = 0.15). The diameter of the cores constituting the clusters, determined by magnetometry, is assumed to follow a lognormal distribution with $d = 4.9$ nm and $\sigma = 0.53$. The magnetization of the cores is $Mv = 371000$ A/m. The transverse relaxivity of the clusters is remarkably high: $r2 = 470$ s-1mM-1 at 1.41 T and 37°C, which is close to the maximum achievable relaxivity of $\simeq 750$ s-1mM-1. The transverse relaxation is independent of temperature and of the interecho time used in the measurement sequence. From the theoretical point of view, the clusters should be mainly in the Static Dephasing Regime [3]. These clusters are thus good candidates for cellular MRI since they can be internalized into cells using the transfection protocol. However, one disadvantage of such large clusters is their reversible clustering within the magnetic field, which has been proven thanks to the evolution with time of the measured relaxation times.

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Posters

PO225

Investigating turbulence and mixing within the ambr® 15 microbioreactor using operando MRI

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Biopharmaceuticals such as monoclonal antibodies (mAbs) accounted for 50% of the ten best selling drugs in 2020,[1] and more than 800 are currently in clinical trials.[2] Miniature bioreactors are used widely in the biopharmaceutical industry. As a part of upstream bioprocessing, these systems allow for the reduction of process development costs and timescales. The ambr® 15 (Sartorius) system is capable of running up to 24 vessels in parallel, with automated sampling and monitoring of key system characteristics. The performance of cell cultures run in the system show similarities to that of bench-top bioreactors.[3] However, due to the asymmetric configuration of the ambr® 15 compared to traditional bioreactors, assumptions about the performance cannot be made without further understanding of the hydrodynamic behaviour within the microbioreactor.

Building on previous work,[4] operando magnetic resonance imaging (MRI) experiments have been utilised to investigate the effects of turbulence and mixing within the ambr® 15. 3D velocity flow maps have thus been generated. The use of compressing sensing has reduced experiment times by up to 80%, enabling rapid data collection with minimal loss of image quality. Review of the 3D velocity field shows good agreement with previously published 2D data. Additionally, areas of turbulent flow have been visualised in 3D, allowing for greater understanding of the hydrodynamic processes occurring within the microbioreactor during operation. This represents the first use of MRI to investigate and quantify turbulent kinetic energy within a microbioreactor.

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PO226

Magnetic resonance microimaging methods to access muscle wasting in zebrafish model of Leptin deficiency

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In this study we used state-of-the-art magnetic resonance microimaging (µMRI) methods to probe muscle wasting in leptin deficient (lep-/-) zebrafish model. Leptin is a hormone that plays a key role in controlling food intake and energy homeostasis. Skeletal muscle is an important target for leptin and recent studies show that leptin deficiency may lead to muscular atrophy. However, leptin deficiency-induced structural and metabolic changes in muscles are poorly understood. The zebrafish has emerged as an excellent model organism for studies of vertebrate diseases and hormone response mechanisms. In this study we used wild-type (control) and lep-/- zebrafish. Measurements were performed on a vertical wide-bore 7T Bruker spectrometers using birdcage radiofrequency coil (10 mm). Anatomical images were acquired using rapid acquisition with relaxation enhancement (RARE) sequence. The fat mapping was performed using chemical shift selective imaging. Multi-spin multi-echo (MSME) pulse sequence was used for T2 mapping. For T2 multicomponent analysis, nonnegative least-squares algorithm was implemented using the MATLAB codes, along with phasor plot analysis approach. RARE imaging shows significant structural changes in the muscles of mutant lep-/- zebrafish. The T2 relaxation times were changed at various regions in the muscles of lep-/- as compared to control zebrafish. The T2 multicomponent analysis in zebrafish muscle shows well-distinguished components which have been altered in lep-/- zebrafish related to control. The results show that muscles of lep-/- zebrafish undergoes several microstructural changes in comparison to control zebrafish, such as the increase of T2 values, the increase of fat infiltration within the muscle fiber, the lower muscle cross sectional area and the difference of T2 multicomponent characteristics. The outcome of this study also demonstrates that µMRI provides excellent means to non-invasively study the microstructural changes in the muscles of zebrafish model.

PO227

Use of Flow-Assisted Magnetic Resonance Imaging for Rheological Characterization of Whey Protein/Xanthan Gum Pickering Emulsions

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Magnetic Resonance Imaging (MRI) is non-destructive analysis technique that can be used to develop various quality control methods. With the development and widespread use of low-frequency, low-resolution, and low-cost imaging systems, they have been widely used to characterize various physicochemical changes that occur in food systems. In addition, the Continuous Flow Assisted Magnetic Resonance Imaging (CFA-MRI) emerges as a promising technique that allows monitoring rheological changes in food systems. In recent years, pickering emulsions and their applications have drawn intense interest due to their special properties which include enhanced stability, lower toxicity, and easy preparation. In contrast to classical emulsions, pickering particles stabilize emulsions through irreversible absorption to the droplet surface and can change the droplet interface by eliminating the need for amphiphilic compound. In this study, it is aimed to use flow assisted Magnetic Resonance Imaging technique to understand the rheological behavior of pickering emulsions designed by xanthan gum an whey protein isolate. Stock solutions of protein (10%) and polysaccharide (2%) were mixed at a biopolymer ratio ranging from 0.01 to 0.1. Emulsions was prepared by keeping the protein concentration 5% w/w and oil 20% (v/v), respectively. With the proposed MR technique, velocity profiles of pickering emulsions were obtained and this experimental method of flow visualization was used to characterize rheological behavior of emulsions. Rheological experiments and mean droplet size measurements were also conducted to complement the MR results.

PO228

In vitro 1H MT and CEST MRI of protein breakdown in the stomach

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Protein digestion starts in the stomach and is affected by food processing, such as heat treatment. Understanding the link between processing and digestion can help design food products with optimized digestibility. Magnetic Resonance Imaging (MRI) techniques, such as Magnetization Transfer (MT) and Chemical Exchange Saturation Transfer (CEST), can indirectly detect changes in the state of proteins via their ^{1}H exchange with water, and have the potential to non-invasively monitor in vivo protein digestion.

In this work, we performed MT and CEST ^{1}H MRI measurements on a 7T vertical MRI spectrometer to study the digestion of unheated and heated skim milk (SM) in a static in vitro digestion model. Within one minute of a single scan, we obtained both the semi-quantitative MT ratio (MTR) and the MTRasym parameters as markers of protein digestion. The MTR enables monitoring of the amount of semi-solid proteins, and it is expected to decrease during in vitro digestion due to the breakdown of the initially formed protein coagulum. For unheated and heated SM after 1h of simulated digestion, such decrease was 87% and 75%, respectively. The MTRasym measures the amount of dissolved proteins and peptides: for unheated and heated SM, it was found to increase by 67% and 57%, respectively. The in vitro MT and CEST ^{1}H MRI measurements were also performed on 3T and 7T clinical MRI scanners for validation under in vivo conditions. We show that B_0 and B_1 inhomogeneities make CEST measurements on a clinical scanner more challenging. Therefore, fast B_0- and B_1-field mapping is required for dynamic in vivo studies of protein digestion. Our pioneering in vitro findings open a new way to future quantification of protein digestion in vivo with MRI.

PO229

Microcapillary flow-MRI setup for imaging and quantifying sub-mm confined flow of colloidal dispersions

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Flow of complex dispersions, with particle sizes in the order of $10\text{-}10^2$ μm through sub-mm geometries occurs in many industrial and everyday-life situations, such as 3D printing, mastication or extrusion. It is now well established that, under such strongly confined conditions, flow of particulate dispersions can become cooperative. Flow cooperativity causes fluidization of the flowing material, and leads to enhanced velocities and viscosity heterogeneities as compared to theoretical flow predictions in the absence of cooperativity. However, current food processing and product design rules fail in accounting for and, thus, in quantifying, cooperativity effects, due to the lack of comprehensive high-resolution velocimetry studies of complex optically-opaque dispersions under varying flow confinement and wall-slip conditions.

To overcome all these experimental limitations, in this work we study cooperativity by flow-MRI velocimetry on a high-resolution 14T wide-bore spectrometer, using flow capillaries with diameters in the range 0.1 – 1 mm, with either smooth or rough walls, connected to a pump able to apply pressures up to 8 bar. We acquire 2D flow maps of both Newtonian fluids and colloidal dispersions using the pulsed-field gradient spin echo (PFG-SE) or stimulated echo (PFG-STE) sequences. We show that the setup enables correct quantification of local flow properties in Newtonian fluids, and provides access to wall-slip, yield-stress behavior and confinement-induced cooperativity effects in the colloidal dispersion. We foresee that our setup with tunable flow geometry will propel further applications of flow cooperativity studies, especially on industrially-relevant optically-opaque dispersions.

PO230

In-situ NMR & MRI characterization of proton exchange membranes for fuel cells

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NMR and MRI represent effective means to measure dynamically, quantitatively and in-situ the distribution of water inside the central elements of proton exchange membrane fuel cell (PEMFC), in particular the electrolyte membrane, which takes the form of a thin polymer film [1]. However, the use of NMR techniques is limited due to the complexity of the experimental setup, which must allow the application of controlled humid gases on both sides of the membrane inside the NMR apparatus.

In the present paper we present an experimental protocol based on the use of a humid gas controller and NMR/MRI hardware and sequences to investigate water in the polymeric membrane in terms of NMR parameters (($_^1$)H chemical shift, T_1, T_2) and membrane properties (water profiles, water diffusion coefficient, water flux, membrane thickness). We describe how the method can be used effectively to record the temporal evolution of these parameters when the membrane is exposed to cyclic humidity conditions at room temperature. We also present results on membranes on which platinum electrodes have been sprayed and discuss the influence of the electrode layer on water transfer at the interface.

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PO231

Optimal control design of preparation pulses for higher contrast imaging

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Magnetic Resonance Imaging (MRI) is an imaging technique that has gained a lot of attention in the medical community for its ability to visualize the internal body in a non-invasive manner. This visualization is achieved based on the contrast originating from intrinsic tissue properties, such as relaxation times. These differences can be emphasized by the manipulation of acquisition parameters, for example, RF pulses, with the standard acquisition strategies being T1 and T2 weighting. These contrast acquisitions generally start with a standard pulse, called preparation pulse. However, this standardized preparation pulse does not always represent the optimal contrast. This work is focused on a more tailored approach, where we present how to find an optimal pre-acquisition sequence based on optimal control theory for higher contrast between specific tissues[1]. In addition, it allows for robust control pulses even when introducing B0- and B1-inhomogeneities. More precisely, we employ the Pontryagin Maximum Principle to numerically find optimal solutions to the underlying Bloch equations by implementing the GRadient Ascent Pulse Engineering (GRAPE) algorithm. We focus particularly on the theoretical and experimental limits of the optimizations, such as short acquisition times and tissues with similar relaxation values.

Keywords: MRI, Optimal Control, Contrast, Bloch Equations

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PO232

Magnetic Resonance Imaging of zebrafish (Danio rerio) at ultra-high magnetic field (1.2 GHz)

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Magnetic Resonance Imaging (MRI) at high magnetic fields is an important tool for studying pathological animal-models non-invasively. However, getting access to cellular resolution in small animals is still challenging and demands high-spatial resolution and Signal-to-Noise Ratio (SNR). Very recently, the state-of-the-art magnetic field strength of 28.2 T (f(1H) 1.2 GHz) has been installed in the uNMR-NL facility in Utrecht (NL) and became available for imaging, which is currently the highest magnetic field strength commercially available for MRI. In the present study, we will present the first data of Magnetic Resonance Imaging of zebrafish performed at 28.2 T. As a proof-of-concept, several anatomical and chemical-shift selected MR imaging sequences were optimized and applied to young adult zebrafish and the results are compared to those obtained at 17.6 T (f(1H) 750 MHz) and 22.3 T (f(1H) 950 MHz) with identical subjects. Improvement in SNR leads to visualization of various anatomical structures at cellular resolution in significantly short scanning time. Furthermore, we will show the effect of ultra-high field strength on relaxation times in various tissues in zebrafish and how this should be taken into account during method optimization. Finally, we will discuss the technical challenges and outlook towards future in vivo MR microscopy at 1.2 GHz. Experiments at the 28.2 T (1.2 GHz) and 22.3 T (950 MHz) NMR instrument were supported by uNMR-NL. We also thank Ruud Aspers for technical support at Nijmegen University, Karthick B. Sai Sankar Gupta and Alfons Lefeber for technical support at Leiden University, Andrei Gurinov for technical support at Utrecht University, and Volker Lehmann and Thomas Oerther (Bruker BioSpin GmbH) for the technical help provided.

Posters

Novel Multifrequency STD NMR Tools to gain 3D Structural Information on Weak Protein-Ligand Complexes

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STD NMR is a powerful ligand-based NMR technique for weak ligand screening of protein targets and to gain quantitative structural information from biologically relevant protein-ligand complexes of interest in drug discovery.[1] The approach is appropriate for small/medium-sized molecule binders of medium-weak affinity (dissociation constants from high nM to low mM), there is no upper limit for protein size, and labelling is not required. In this presentation, the investigation by STD NMR of molecular recognition processes of ligands by biologically relevant protein receptors will be presented with a focus on the application to specific cases of recognition of glycans by proteins.[2-4] Protein-glycan interactions are very relevant protein-ligand interactions in Nature and are processes typically falling within the range of fast chemical exchange (weak affinity) but still showing high specificity. Along this communication the novel methodological developments in STD NMR produced in our lab will be presented, as the development of novel multifrequency STD NMR approaches,[5,6] with a focus on the "DiffErential EPitope mapping STD NMR (DEEP-STD NMR)" method[5] that allows for the first time to identify the nature of the protein-ligand contacts in the bound state from STD NMR approaches, facilitating ligand orientation in the binding pocket, of great value for the rational design of improved binders in drug discovery efforts.

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PO236

Evaluation of the Benefit and Informing Capability of 2D NMR Experiments for Computer-Assisted Structure Elucidation

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The most significant improvement in the 50-year history of Computer-Assisted Structure Elucidation (CASE) [1,2] came after the introduction of routine 2D NMR experiments in the 1990s. The increased information content of these experiments allowed substantially more complex problems to be addressed than ever before. With the simultaneous advances in computing, CASE has now become an established tool for resolving the unprecedented structures of natural products [3]. Today, further advancements in CASE involve the evaluation of advanced NMR experiments and the determination of the minimum set of experiments required to solve a structure. Although these answers vary with problem complexity, we begin to address both questions through a few examples in this poster.

In the first example, we use the very simple molecule 2-Ethylindanone to examine the benefits of basic NMR experiments and their effect on calculation time. Then, we examine the benefits and potential consequences of using modern experiments like LR-HSQMBC, in addition to the traditional HSQC and HMBC using the complex natural product Spirodactylone [4]. The influence of manually adjusting atom properties (e.g. hybridization and connection to heteroatoms) is also examined using this molecule. Finally, a xanthone-class natural product [5-6] is used to consider the inter-relation between HMBC and INADEQUATE in CASE.

Details of spectra, calculations, and a comparison of the achieved improvements in performance will be shown.

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PO237

NMR of C60 endofullerenes and endofullerides

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Since its discovery[1], the C_{60} fullerene molecule has raised great interest of scientists and mathematicians due to its fascinating truncated icosahedron shape, with a very high degree of symmetry. Endofullerenes are supramolecular complexes where one small (endohedral) atom/molecule is completely confined within a bigger, fullerene, molecule which acts as an enclosing cage.[2] Endofullerenes offer an ideal "particle in a box" nano-laboratory to observe quantum mechanical phenomena. A selection of topics related to the NMR of endofullerenes will be presented, outlined below.

A J-coupling between ^3He and ^{13}C in the C_{60} cage was observed due to confinement, in the $^3He@C_{60}$ endofullerene. This is attributed to a ^0JHeC-coupling, where the "zero" represents the number of chemical bonds between Helium and Carbon.[3] Signs of similar ^0J-couplings between ^1H and ^{13}C are observed in endofullerenes containing endohedral protons. Furthermore, ^3He NMR measurements of $^3He@C_{60}$ in solution and solid state were performed from room temperature down to cryogenic temperatures.

When the C_{60} molecule is filled with an endohedral species, the C_{60} ^{13}C resonance is shifted downfield depending on the species. The shift appears to reflect the "pressure" a single atom/molecule exerts on its container, in this case the C_{60} molecule. Furthermore, rather strong paramagnetic shifts have been observed for the ^{13}C resonance of the paramagnetic endofullerene $NO@C_{60}$.

We hope to report on low temperature NMR measurements of superconducting endofullerides $Rb_3(A@C_{60})$, where A = H_2, HD, H_2O and ^3He. The NMR behavior of the endohedral species reports on the electronic structure of the negatively charged C_{60} fulleride anions. As the endofulleride passes through the superconducting transition (Tc~30K), changes in the electronic behaviour are expected to be observed.

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PO238

Real-time flow NMR monitoring of organic reactions with ultrafast 2D COSY

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Monitoring by flow NMR is a powerful approach for the real-time analysis of batch and flow reactions.[1] An important benefit of flow NMR is that makes it possible to monitor reactions carried out in the conditions of interest, which may not be replicated in an NMR magnet.[2] 2D NMR experiments provide diagnostic structural information and allow to overcome the signal overlap problem that affects the analysis of reacting mixtures through 1D 1H NMR. One of the challenges in the use of 2D experiments for investigation of reaction kinetics and mechanisms is for their duration to be compatible with the reaction timescale. Fast 2D NMR experiments are particularly relevant in that context, and their use for online monitoring in flow conditions raises a series of challenges.[3]

Here we describe the development and optimization of tailored ultrafast (UF) 2D NMR experiments for real-time monitoring of chemical reactions by flow NMR. UF NMR uses spatial parallelization to collect 2D data sets in a single scan. We developed flow-compatible UF COSY pulse sequences including solvent suppression modules and gradients along 3 orthogonal axes. Experiments were optimized for sensitivity, resolution and spectral width by incorporating interleaved acquisitions while minimizing interferences between flow and spatial encoding. The optimization of the acquisition parameters, in accordance with the hardware capabilities, and the developing of suitable processing will be presented, together with applications to the monitoring of organic reactions.

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Posters

PO239

NMR-based structure elucidation of novel regioisomeric 3(5)-(1H-pyrazol-4-yl)-5(3)-phenyl-1,2-oxazoles obtained from pyrazolo-chalcones

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Heterocyclic chalcones are an important class of compounds in medicinal chemistry, as most biologically active chemical entities contain a heterocyclic scaffold. For instance, incorporation of a pyrazole or 1,2-oxazole (isoxazole) nucleus is a common practice to develop new drug-like molecules with anti-cancer, anti-diabetic, anti-viral, anti-inflammatory, anti-bacterial, anti-fungal, antineurodegenerative, anti-tubercular, anthelmintic, and antimalarial properties [1,2].

Herein, we report the synthesis and structural elucidation of novel, diverse pyrazole-chalcone derivatives via the base-catalysed, Claisen-Schmidt condensation reaction of 4-formyl or acetyl-1-phenyl-1H-pyrazol-3-ols and appropriate acetophenones or carbaldehydes. The aforementioned pyrazole-based chalcones were easily used as an intermediate compounds in the preparation of the regioisomeric 3(5)-(1H-pyrazol-4-yl)-5(3)-phenyl-1,2-oxazole derivatives.

In order to avoid any ambiguity in the structure assignment of regioisomeric 1,2-oxazoles, the 15N-labeled pyrazole-isoxazoles were synthesized. Treatment of chalcone with 15N-hydroxylamine hydrochloride gave an inseparable mixture of regioisomers in a ratio of about 8:1. The formation of novel pyrazole-chalcones and isoxazoles was deduced by an in-depth analysis of NMR spectral data, which were obtained through a combination of standard and advanced NMR spectroscopy techniques.

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PO240

Efficient early drug discovery of RNA drug targets using NMR and machine learning

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Non-coding RNAs are functional RNA molecules that are transcribed from DNA but not translated into proteins. In general, these RNAs regulate the expression of proteins and provide a potential avenue for therapeutic intervention, especially for difficult-to-target proteins or complex signal transduction pathways. Indeed, with over 200,000 RNA transcripts in the human transcriptome, it is a vast, untapped reservoir of potential pharmaceutical targets.

We have integrated fragment-based screening (FBS) by NMR, in-silico machine learning, and cellular assays for drug discovery. FBS is a very efficient way to sample the chemical space with only a few thousand compounds. NMR data on fragments that bind or do not bind to the drug target are routinely used to generate AI models to identify available larger compounds that combine two fragments that bind to near pockets of the drug target. These larger compounds are obtained and tested in cellular and biochemical assays.

The combination of fragment screening by NMR and compound selection by machine learning is very efficient and allows us to select leads for follow-up chemistry within weeks of starting a new project. Because of this efficiency, NMR plays a crucial role in the selection of RNA drug targets, since validation of potential binding pockets for selective ligands becomes available early in the project.

Examples of lead finding efforts for multiple structural motifs are shown: pseudoknot (SARS CoV-2), bulge (ribosomal RNA, miRNA), and three-way junction (spliceosome).

PO241

Micromolar concentration interaction studies on a benchtop NMR spectrometer with secondarily 13C-labeled hyperpolarized ligands

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Hyperpolarization by dissolution-DNP [1] provides a route for enhancing 13C NMR sensitivity by more than four orders of magnitude on a wide range of small molecules for numerous applications in chemistry, metabolomics, drug discovery, etc.

One important drawback in the dDNP approach is its lack of generality since it generally relies on slow relaxing spins like low natural abundance 13C (1.1%) nucleus. In 2009, Wilson et al. proposed an approach where amine groups in amino acids were easily labeled with [1,1-13C] acetic anhydride [2], and subsequently hyperpolarized. This secondary labeling approach was revisited by our team in the context of NMR drug screening [3]. There, we showed how ligands could be secondarily labeled, and finally used to probe interactions with target proteins through 13C NMR relaxation.

We have more recently combined this concept with d-DNP to demonstrate hyperpolarization-enhanced interaction studies. This provides dramatic sensitivity boost (>10,000x), thus decreasing required ligands concentrations and allowing observation of stronger affinity interactions. We show here that hyperpolarized 13C-based relaxation studies considerably improve the detection and identification of several ligands in a mixture on an 80MHz benchtop NMR spectrometer.

Studies are being carried out to demonstrate the potential of the method by i) extending the chemical library and ii) by conducting competition experiments between molecules with and without labelling. We believe this approach may improve and accelerate the discovery of new drug candidates.

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PO242

Advanced NMR methods for targeting K-Ras using the NMR molecular Replacement and photo – CIDNP

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Recent progress in Nuclear Magnetic Resonance (NMR) techniques, including photo – Chemical Induced Dynamic Nuclear Polarization (CIDNP) [1] and NMR Molecular Replacement (NMR²) [2] could potentially speed up the process of structure-based drug discovery.

The goal of this work was to use these advanced NMR techniques to find molecules binding to the oncogenic protein K-Ras. Their affinities were measured using conventional NMR and photo – CIDNP NMR. Finally, their complex structures were elucidated using NMR².

A screening with 900 small fragments of K-Ras G12V, bound to a GTP analogue, was performed using STD NMR. The dissociation constants in the low millimolar range were determined for the best fragment hits using [^{15}N,^{1}H] - HSQC titration. Additionally, the binding constants were measured using a method based on photo – CIDNP NMR, in which no protein labelling is required. Filtered NOESY spectra with different mixing times were acquired to elucidate the NMR² structures of the seven best fragments in complex with K-Ras. Five of the seven compounds showed intermolecular NOE cross-peaks. Three compounds were measured with a conventional ^{13}C,^{15}N – filter in the NOESY pulse sequence. One of those compounds and the two other compounds were measured using a T_1 and T_2 relaxation-based filter in the NOESY pulse sequence. The filter takes advantage of the slow relaxing ligand compared to the fast-relaxing protein. No ^{13}C labelling is required to get the structural information. The binding pocket next to the switch-II of K-Ras was identified using CSP. Finally, progress toward NMR² structures of the complexes was made.

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Posters

PO244

Scrutiny of the supramolecular structure of bio-based Low Transition Temperature Mixtures by NOESY and PFG-NMR

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Deep Eutectic Solvents (DES) or Low Transition Temperature Mixtures (LTTM) constitute an appealing class of neoteric solvents that mostly fulfill the criteria of green chemistry. For their industrial implementation, water addition is often required to achieve the fluidity suitable for processes. Therefore, the effect of water content on the internal self-organisation and the physical and chemical properties of these solvents is an emerging issue in scientific literature [1-4]. In this frame, the present work deals with the fine characterization of bio-based ternary mixtures composed of glycerol (G), fructose (F) and water (W)5. In addition to the examination of their macroscopic properties such as thermal transitions, changes in viscosity and activation energies, NMR experiments were performed for understanding interactions at the molecular level. More specifically, NOESY and PFG-NMR experiments were conducted to point out the effect of molecular composition and temperature variation on the supramolecular organization.

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PO245

STD-NMR for ligand design and refinement

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Glycosyltransferases (GTs) are ubiquitous enzymes involved in important biological processes and therefore are of high interest both for understanding their molecular mechanism of action and as potential pharmaceutical targets.

However, most of GT inhibitors described so far contain the pyrophosphate unit to increase affinity to the enzyme, but this renders the inhibitor unable of permeating through lipidic membranes and consequently incompatible with any in vivo activity.

Our aim is developing inhibitors against human GTs presenting lower polarity than natural Leloir donors. For this purpose, we have carried out a complete process of design based on the available crystallographic structures of GTs and the synthesis of a library of compounds, all of them containing the nucleoside moieties necessary for protein recognition.

In order to evaluate the activity of our compounds and their further improvement, we have used STD-NMR as the best tool to map the changes in the ligand epitopes along with the structural changes of our ligands. This offers extremely valuable feedback to further design and improve the affinity and activity of the ligands.

New applications of STD-NMR methodology are explored, such as interligand STD, competition STD experiments and DEEP-STD.

Posters

Investigation of the extraordinary self-assembly of a simple organic salt by multinuclear NMR in liquid-state

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Recently, we have isolated four new crystalline phases of fampridine hydrochloride (4-APH+Cl−), a simple organic compound used for the symptomatic treatment of multiple sclerosis.[1] The four crystalline phases comprise two analogous sub-hydrate forms 4-APH+Cl−·1/12H2O (phase 1) and 4-APH+Cl−·1/90H2O (phase 2), a monohydrate, 4-APH+Cl−H2O (phase 3) and an anhydrous form, 4-APH+Cl− (phase 4).

Of particular interest was the observation of phases 1 and 2, featuring polyhedral molecular self-assemblies similar to those observed in clathrate hydrates, and a large number of molecules in the asymmetric unit (Z′=30 and 15, respectively). This serendipitous discovery represented the first observation in small organic molecules of Frank-Kasper (FK) phases,[2] a particular family of topologically close-packed structures identified more than 60 years ago in metal alloys and, over the recent years, also observed in a variety of systems.

To broaden the understanding of how such a simple molecule may crystallise as an FK phase, we monitored the crystallization of complex phase 1 and simple phase 3 by liquid-state NMR, to identify any differences in the composition, intermolecular interactions and aggregation between the precursors of phase 1 and 3.[1] In particular, the samples were investigated by ^{1}H, ^{13}C, ^{14}N, and ^{35}Cl NMR as a function of the concentration of 4-APH+Cl− until the moment when precipitation of the crystalline phases occurred. Variations of chemical shifts, T_1 relaxation times of ^{13}C signals, and full-width at half-maximum of the signals of quadrupolar ^{14}N and ^{35}Cl nuclei were measured. The results indicate the formation of different self-assembled clusters prior to the nucleation of complex phase 1 and simple phase 3.

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PO248

Aggregation of aqueous surfactant mixtures

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Surfactants are substances that lower surface tensions of liquids. At higher concentrations, they create self-assembled molecular clusters called micelles. Application of surfactants include their use in households as well as in industry as detergents, wetting agents, emulsifiers, and food ingredients. Surfactants are also a common component of aerosols and can change the surface tension of cloud droplets.

To get more insight into the molecular structure of mixed micelles it is important to have a deeper understanding of surfactants behavior and properties in aqueous solutions. Aggregation of pure surfactants has been widely studied by NMR1,2 but aggregation of mixtures has not been investigated so much. Here, we study the mechanism of mixed micelle formation in binary surfactant aqueous solution systems. We exploit relaxation and diffusion measurements to obtain insights into the microscopic structure of mixed micelles in surfactant solutions. Diffusion NMR measurements reveal the self-diffusion coefficient of molecules, while relaxation times reflect both rotational and translational motion of molecules. The critical micelle concentrations of each individual component in the mixed surfactant solutions were obtained by analyzing changes in relaxation times and by measuring the diffusion constants of the individual peaks.

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Complete Resonance Assignment of a Pharmaceutical Drug by combining DNP-Enhanced Solid-State NMR and DFT calculations

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Solid-state dynamic nuclear polarization enhanced-Magic angle spinning (DNP-MAS) NMR measurements coupled with density functional theory (DFT) calculations display how resonance assignment can be carried out for a complex pharmaceutical molecule. Employing DNP-MAS can solve two main drawbacks of solid-state NMR for the structural investigation of pharmaceutical drugs (i) the low sensitivity of NMR active nuclei such as 13C, 15N at natural abundance, and (ii) long 1H T1 relaxation times of many pharmaceuticals. The use of DNP will enhance the NMR signal and reduce the time required to get a sufficiently good two-dimensional correlation NMR spectra at natural abundance. This is particularly important for the structural characterization of organic molecular crystals like pharmaceutical compounds. We demonstrate that DNP-enhanced MAS NMR experiments combined with DFT calculations can significantly improve the structure elucidation process for complex organic molecules.

Herein, we combined DNP-enhanced multinuclear solid-state NMR experiments with DFT calculations to explore the structure of a complex organic molecular crystal; for this purpose, we choose sitagliptin phosphate monohydrate, a phosphate salt used as an oral antidiabetic drug for the treatment of type 2 diabetes. Sitagliptin is a long and flexible molecule with plenty of C-C single bonds and nitrogen atoms that disrupt the carbon backbone, making it impossible to solve the structure by using 13C-13C correlation spectrum alone. Hence, we used multinuclear DNP MAS NMR experiments combined with DFT calculations for the complete structural assignment of sitagliptin phosphate.

Posters

PO251

A Pipeline for Accelerating Drug Discovery: Screening and Affinity-Ranking of Fluorinated Ligands with CSAR

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Modern drug discovery is based on screening libraries of potential ligands and subsequently selecting the ligands with highest affinity for further development into a drug. NMR is a highly sensitive method for screening of ligands: 3000 fluorinated ligands can be screened in 24 hours and typically just a milligram of unlabelled protein is required. However, for selecting the most potent ligands among the initial hits, NMR is not efficient. To determine affinities of the identified ligands, lengthy titrations need to be performed, which require large amounts of expensive isotope labelled biomolecules. This second step typically takes weeks, and is often not feasible with challenging proteins.

We have developed the method CSAR (Chemical Shift anisotropy-based Affinity Ranking), which allows comparing affinities of ligands using simple ligand-observed NMR experiments, without the need of isotope labelled protein, titrations or known reporter ligands. CSAR is based on thorough understanding of CSA-relaxation and control of exchange effects – which both don't correlate with affinity, and therefore hampered analysis up to now. In CSAR, CSA tensors are calculated using quantum chemical calculations, and exchange broadening is suppressed experimentally by strong, on-resonance spin lock pulses. The clean data obtained in this way is proportional to affinity and can be used to rank ligands.

Based on CSAR, we have designed a novel, efficient early drug discovery pipeline. It consists of a public "CSAR-library" of fluorinated fragments, for which CSA tensors have been calculated. Using this library, we have screened diverse targets and established a ranking for the identified ligands, including estimation of KD values. The characterized ligands cover a range of affinity of three orders of magnitude.

Our newly established CSAR pipeline removes an important bottleneck in the drug discovery workflow and enables research on challenging contemporary drug targets.

PO252

STD-NMR reveals that an arginine-glycosylating SseK1 mutant recovers FADD activity without impacting donor recognition

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Salmonella enterica expresses three innate immune system suppressing effectors, SseK1, SseK2 and SseK3[1]. These effectors are unique as they possess the remarkable ability to act as arginine N-glycosyltransferases. This is important as historically glycosyl transfer has been limited mainly to serine and threonine for O-glycosyltransferases, and asparagine for N-glycosyltransferases. Moreover, despite possessing high sequence identity, the SseK effectors have diverse acceptor recognition and activity profiles[2]. To explore the selectivity of these enzymes, we performed site-directed mutagenesis of SseK1, successfully recovering activity towards the Fas Associated Death Domain Protein (FADD)[3]. This work, however, focused only on acceptor recognition (protein-protein interactions) and did not consider the impact on ligand binding (donor recognition) from mutation.

To assess whether the site-directed mutation of SseK1 also impacted donor ligand recognition, Saturation Transfer Difference (STD) NMR was used. STD NMR is a robust technique that can recover detailed information on the recognition of a ligand by a protein. In this study the binding of the natural donor substrate, UDP-GlcNAc, UDP, and other derivatives to the FADD-inactive SseK1 and FADD-active SseK2 and SseK1 mutant was measured. We observed that all the tested ligands bound the receptors and were able to map the contacts the ligands make to the effectors. By comparing their recognition features, we determined whether the mutation of SseK1 led to altered ligand recognition between the effectors. Overall, we conclude that, for the sugar nucleotides tested, there were significant differences in recognition between SseK1 and SseK2, whereas there were subtle changes in ligand recognition by SseK1 and its FADD-active mutant. This implies that the binding of the sugar-nucleotide donor is not coupled to acceptor recognition and activity profiles.

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PO253

Increased Protein Dynamics Defines Druggability

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Targeting Protein-Protein Interactions (PPIs) is challenging, and there are no reliable methods for accurate prediction of the ligandability and druggability for new targets. The majority of currently employed ligandability prediction techniques rely on static topological and structural features, a severe limitation that does not account for the dynamic state of proteins. Drug discovery campaigns targeting PPIs have limited success,and improved ways of assessing the ability to identify small-molecule ligands are required. BTB domains are promising targets for developing novel anti-cancer drugs, and to date, small molecule inhibitors have been developed only for a single member of this family, namely BCL6. Here, we evaluated the ligandability of conserved BTB domains from the cancer-relevant proteins LRF, KAISO and MIZ1. Using fragment screening, we discovered that MIZ1 binds multiple ligands. However, no ligands were uncovered forthe structurally related KAISO or LRF. To understand the principles governing ligand-binding by BTB domains,we performed comprehensive NMR-based dynamics studies and found that only the MIZ1 BTB domain exhibits backbone µs-ms time scale motions. Interestingly, residues with elevated dynamics correspond to the binding site of fragment hits and recently defined HUWE1 interaction site.In summary,we discovered that protein dynamics strongly correlate with the ligand-binding capabilities of the MIZ1 BTB domain and the presence of the µs-ms time scale motions reveal the presence of the cryptic binding sites. In this work,we describe,first to our knowledge,small molecule ligands binding to MIZ1 BTB domain. These compounds can be further developed into valuable probe compounds or anti-cancer drugs. Protein dynamics is for the first time documented to be a decisive feature that distinguishes druggable and non-ligandable members of structurally highly similar family of protein targets.Our data argue that examining protein dynamics using NMR can identify cryptic binding sites, and predict the ligandability of novel challenging targets.

PO254

Targeting Intrinsically Disordered Regions (IDRs) in Viral Proteins via Molecular Recognition Features (MoRFs) Analysis

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In order to replicate, viral proteins hijack the host machinery. They interact with numerous viral proteins using molecular recognition features (MoRFs- induced folding upon binding) located inside intrinsically disordered regions (IDRs) during the infection cycle. IDRs are widely distributed in viral genomes and are often found to be associated with the pathogenicity and oncogenicity caused by the viruses. This study looked into the IDRs/MoRFs found in the emerging viral diseases Human parainfluenza 4A virus (HPIV-4A) and Chandipura virus (CHPV). A rare infection, HPIV-4A, produces a milder respiratory disease. CHPV, on the other hand, is linked to encephalitic illness. In previous studies, Measles morbillivirus virus (MeV) and Vesicular stomatitis virus (VSV) were chosen as representative family viruses of HPIV-4A and CHPV, respectively, because they are densely ornamented with IDRs. Since phosphoprotein is highly disordered and needed for replication and transcription, it could be a promising drug target. The IDRs/MoRFs found in phosphoprotein were targeted using conserved domain analysis and molecular docking. The compounds used in this docking study were then divided into three categories: phytochemicals, repurposed drugs, and antiviral drugs. To summarise, when compared to anti-viral drugs, repurposed drugs (derived from plants) and phytochemicals are the most effective inhibitors of MeV phosphoprotein. Antiviral approaches have so far been used to target viral structural proteins such as polymerase and protease. Unfortunately, pharmacological inhibition of these structural proteins is sometimes surprisingly ineffective due to drug resistance. Inhibiting conserved MoRFs is a novel alternative to this strategy.

PO255

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Posters

Weakly bonded hydrogens in different roles

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Hydrogen atoms have use in MRI when exchanging in a form of protons. Amidic protons exchange has been widely used in protein structure studies, but also in amide proton transfer chemical-exchange saturation transfer (APT-CEST).

The hydroxyl groups of carbohydrates have been researched in promising techniques such as GAG-CEST for cartilage studies or Gluco-CEST for study of glucose concentration or as a pH-sensitive biomarker. There has been serious uncertainty about the exchange rates of glucose, chemical shifts of hydroxyl protons and their pH dependence. Their exchange rate in medium-fast regime complicates their determination. To evaluate the measurements or assess their feasibility, it is invaluable to have an accurately parameterized detailed model. Since such model is missing in literature, we aimed to fill this gap. We have recorded proton NMR spectra of glucose solutions in a phosphate buffer in series of pH and temperatues. By advanced fitting of the lineshapes, we obtained an unprecedently detailed and accurate model. Our parameterization does not reach the physiological pH and temperatures, it is nevertheless a solid base for further studies using indirect techniques such as CEST or spin-lock experiments.

Instead of exchanging in a form of protons, exchange of weakly-bonded molecular hydrogen is a topic in parahydrogen-based sensors. We have investigated the bonding situation of ansa-aminoboranes. Reversible addition of hydrogen molecule can enable spin polarization from parahydrogen, boosting molecule detectability by orders of magninude. The hyperpolarization depends on the adducted H-H J-coupling. We clarified details about this J-coupling. We further calculated energies and forces between the adducted hydrogen atoms, completing the picture about this dihydrogen bond, important for further development of these compounds.

PO257

Imaging Saturation Transfer Difference (STD) NMR for measuring Dissociation constants in a single NMR tube

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Investigating the molecular recognition processes between biomolecules allows to understand how their specific interactions regulate the essential processes of life. This is of paramount importance both in drug design and fundamental biological investigations. NMR is among the election techniques to carry such investigation, and among the other ligand-based NMR technique, Saturation Transfer Difference NMR (STD NMR) stands out as a solid and easy to apply methodology which can provide invaluable insight in the mode of binding (binding epitope mapping) and strength of the interaction (dissociation constant, Kd) of receptor-drug complexes on the weak affinity range (µM to mM). We have combined STD NMR with Chemical Shift Imaging (CSI) titrations, an advanced Imaging NMR technique developed in our group, which allows us to "condense" in a single tube those experiments which generally require time-consuming titrations. For the first time, we developed "Imaging STD NMR", for the study of receptor-ligand interactions in "a single shot", aiming at determining dissociation constants (Kd).

Traditional STD NMR, although efficient in measuring Kds, is particularly dispendious as it requires the preparation and analysis of a number of samples equal to the desired number of receptor:ligand ratios data point (thus also limiting the quantity of data points, if time and resources are limited or experimental conditions do not allow). Our newly developed Imaging STD NMR pulse sequence allows us to measure dissociation constants in a single NMR sample constituted of a gradient of ligand developing along the vertical axis, against a homogeneous receptor concentration. The analysis is neat, reliable and allows us to increase the number of data points in a fraction (10% to 20%) of the experimental time relative to the traditional approaches. Hence, this novel method does carry the potential to make a difference, especially in pharmaceutical industry settings.

PO258

STRUCTURE AND INTERACTIONS OF AZITHROMYCIN-THIOSEMICARBAZONE CONJUGATES AS SEEN BY NMR

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Macrolides belong to well-known class of antimicrobial agents widely prescribed to treat upper and lower respiratory tract infections. Azithromycin is a semi-synthetic 15-membered macrolide antibiotic derived from erythromycin possessing broad spectrum of antibacterial potency and favourable pharmacokinetics. However, bacterial resistance to marketed antibiotics is growing rapidly and represents one of the major hazards to human health worldwide. Today, there is a high need for discovery of new antibiotics to combat resistance. Novel conjugates of azithromycin and thiosemicarbazones, the macrozones, represent one such class that exhibits promising activities against resistant pathogens.

Solution and solid-state structures of several macrozones have been studied and characterised by one- and two-dimensional NMR spectroscopy. Free and ribosome-bound conformations were further evaluated for selected macrozones with highest bioactivity. Saturation transfer difference (STD), WaterLogsy and transferred nuclear Overhauser effect (trNOE) NMR experiments coupled with molecular modelling studies provided valuable data about binding epitopes and bound conformations. The presented results serve as a good platform for discovery and design of new anti-infectives with improved overall biological effect.

PO260

A new suite of simple NMR experiments to assess antimicrobial membrane interactions and permeability

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Antimicrobial resistance represents a significant challenge to future healthcare provision. The discovery of new antibiotics effective against Gram-negative bacteria is a major challenge due to the permeability barrier of the Gram-negative bacterial membrane and the limited chemical diversity of compound libraries to probe this barrier. Gram-negative pathogens have two membranes with orthogonal characteristics, making it difficult to design drugs that can penetrate both barriers and access the bacterial cytoplasm.

Quantifying small molecule-phospholipid bilayer interactions and permeation is vital to the development of new drug candidates and/or medicinal therapies. However, obtaining these data remains problematic. We have developed new phospholipid nanodisc assays which enable the elucidation of small molecule interaction with the membrane1 as well as their lipid selectivity2 using conventional solution state NMR spectroscopy techniques.

In addition, we have now developed a new NMR-based assay able to quantify the permeability of small molecules through vesicles formed with natural lipids. Together, these assays allow a complete understanding of small molecule interactions and permeability through the membrane bilayer, enabling addressing some of the greatest challenges in antimicrobial research.

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2. Medina-Carmona and Ortega Roldan, Chem Comms, 2021

Posters

PO261

NMR assays for the quantification of weak affinity receptor-ligand interactions

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Biophysical methods are widely employed in academia and the pharmaceutical industry to detect and quantify weak molecular interactions. Such methods find broad application in fragment-based drug discovery (FBDD). In an FBDD campaign, a suitable affinity determination method is key to advancing a project beyond the initial screening phase. Protein-observed (PO) NMR finds widespread use due to its ability to sensitively detect very weak interactions at residue-level resolution. PO assays are extremely useful at the early stages of the project, when no or little information is available about the binding sites, protein conformations and allostery. Once PO-NMR is established, it can be used to screen for a weakly binding reporter molecule that may have utility for a ligand-observed (LO) NMR reporter assay. Such an assay can measure affinities in a similar range to PO-NMR while offering some distinct advantages, especially with regard to protein consumption and compound throughput. Here I discuss an optimal approach to setting up both PO and LO assays for routine use with the aim of getting high-quality, accurate data and good throughput. I will also compare the performance of these assays with other affinity determination methods that are commonly used in early drug discovery.

PO262

Low-temperature NOE/ROE Investigation of Intermediates in the Stereoselective Organocatalytic α-Chlorination of Aldehydes

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Understanding the factors determining stereoselectivity is a fundamental challenge in organic synthesis. Rational catalyst design and selection of a specific reaction pathway may only be used deterministically in organic synthesis if the (relative) configuration, conformation and dynamics of the intermediates are known.[1]

Herein we investigate the enantioselective α-chlorination of isovaleraldehyde.[2] The reaction is catalyzed by an organocatalyst derived from proline which in a first step forms an enamine. This reactive enamine then attacks the chlorinating agent to form a chlorinated aminal as directed by the three-dimensional structure of the chiral organocatalyst. In this reaction, two distinct species are observed, the nature of which has long been debated in literature. The two observed species could conceivably be the different diastereoisomers as they are formed due to the selectivity of the organocatalyst.[3] Alternatively, the two observed species may be slowly interconverting conformers.

Using quantitative 1D and 2D NOE and ROE measurements at different temperatures, we aim to distinguish these two cases. From multiple mixing time series we estimate internuclear distances and interconversion rates and compare them with DFT-optimized geometries and calculated chem-ical shifts to identify the respective species. Knowledge of these species in turn provides detailed insight into the reaction's mechanism and selectivity.

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PO263

UNDERSTANDING ANTIMICROBIAL ACTIVITY IN LIVE CELLS

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Antimicrobial resistance is a current severe threat for global public health. The discovery of new antibiotics effective against Gram-negative bacteria is a major challenge due to the permeability barrier of the Gram-negative bacterial membrane, the currently limited chemical diversity of compound libraries that can negotiate this barrier and the existence of robust efflux systems to maintain low intra-bacterial levels of compounds.

We have developed new NMR methods employing both conventional and in-cell NMR to assess drug permeability and their effect on cell metabolism. We have optimised a new, simple methodology to assess membrane permeability of drugs making use of the paramagnetic relaxation enhancement (PRE) effect in lipid vesicles that is applicable to any type of cell/bacterial membrane. Using this assay, we have been able to find important differences in the permeability of new families of antimicrobial compounds, providing unique information for the further development of this family of compounds. In addition, we have now expanded this assay to live cells making use of a NMR-coupled bioreactor. Using this set up, we are to monitor both the drug permeation into live cells, as well as changes in the bacterial metabolism as a result of the antimicrobial agent added.

In summary, we present here a new method reporting on drug permeability in lipid vesicles or live cells that will allow the design of more efficacious drugs and antimicrobial agents.

PO264

Cross-correlation effects in near equivalent spin-1/2 pairs

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The spin dynamics of strongly-coupled spin-1/2 pairs is of interest in the context of singlet-NMR.[1] However, there has been little treatment of cross-correlated relaxation effects in these systems. Cross-correlation effects between nuclear spins have been utilised in protein NMR via the TROSY effect.[2] Here, the novelty is that this same effect is witnessed between near-equivalent and homonuclear spin-pairs; a doublet is observed with differential line broadening and narrowing. This is due to the cross-correlation between the direct dipole-dipole (DD) and chemical shift anisotropy (CSA) relaxation mechanisms. The molecule hosting the spin system contains alternating single- and triple-bonds along a chain of six carbon atoms, with the two innermost carbons isotopically labelled ^{13}C nuclei. Theoretically, the molecule is treated as a rigid symmetric top[3] and, in this framework, expressions for relaxation rates account for the asymmetric lineshape. A detailed analysis of the signal offers coherence assignment of each peak. Magnetic field value which cancels the DD and CSA relaxation mechanisms is also deduced. Further, we find that this system cannot be treated in the extreme narrowing limit, since the rotational correlation time is on the order of nanoseconds.

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PO265

Structure-property relations for polymeric micelles loaded with different curcumin derivatives using solid-state NMR spectroscopy

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Although advantages of incorporating drugs into polymeric micelles are well known, there is no overall reliable, rational concept for finding a suitable polymer for a specific drug. Previous research shows the time consuming experimental and theoretical approach of investigating combinations of different drugs and polymeric carriers.[1] Also the highest loading is not always desireable.[2]

The triblock copolymer (poly(2-methyl-2-oxazoline)-block-poly(2-n-propyl-2-oxazine)-block-poly(2-methyl-2-oxazoline) can form ultra-high loaded polymeric micelles up to ≈50 wt% with the natural product curcumin as model compound, which was investigated earlier on a molecular level.[2c,3]

In this work, we synthesized different curcuminoid derivates and prepared the corresponding polymer formulations. These were investigated by ^{1}H, ^{13}C and ^{1}H-^{13}C FSLG HETCOR solid-state NMR experiments, to identify key structural features to rationalize the observed variation in maximum loadings. Complementary characterization was based on FT-IR spectroscopy and XRPD.

Interestingly, only small changes in the chemical structure of the model drug led to a significantly decreased loading of the polymeric micelles for four of the curcuminoids. The impact of the keto-enol moiety as well as the hydroxy groups of the curcumin molecule could be verified. Building on these results, the analysis of further derivatives enables a refined understanding about this delivery system on a molecular level and offers the potential to better predict drug-polymer compatibilities for (ultra-) high loaded micelles or generate design ideas for improved carrier systems.

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PO266

1H-detected Characterization of Highly Flexible Species in Insoluble Samples using Magic Angle Spinning NMR

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In the last three decades, the scope of MAS NMR has widened to include complex biomolecules, from large protein assemblies to intact cells. This diversity in macromolecules frequently features highly flexible components that sufficiently average dipolar couplings; however, their insoluble environment precludes the use of solution NMR to study their structure and interactions. In this work we explore two fully protonated, isotopically ^{13}C/^{15}N-labeled systems: the fungal cell wall of Schizophyllum commune[1], and a complex of microtubule-associated protein (MAP) Tau bound to human microtubules (MTs)[2]. We used proton detection, which is an especially powerful technique when sample availability is a limiting factor, either due to the prohibitive expense of sample production or poor protein expression. We obtained spectral fingerprints featuring heteronuclear contacts by employing through-bond polarization transfer and isotropic mixing techniques, which aid to characterize the dynamic regions of these samples.

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Posters

Solid-State NMR of Adsorption in Layered Metal-Organic Frameworks

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Supercapacitors are central to transitioning to clean energy; with higher power densities than batteries, they are ideal for rapid energy storage from intermittent renewable energy sources, and rapid energy release for large electric vehicles. However, their energy densities are currently hindered by the disordered nature of traditional carbon-based supercapacitor electrodes, which makes developing structure-property relationships challenging. Crystalline and porous, several layered conductive metal-organic frameworks (MOFs) have demonstrated potential as supercapacitor electrodes, and their well-defined pore structures make them ideal for understanding how electrode structure impacts supercapacitor performance.[1] [2] Despite this, a lack of experimental studies on the charging mechanisms of MOF-based supercapacitors leaves them poorly understood. In-situ and ex-situ solid-state NMR has been used extensively to characterise the charging mechanism of carbon-based supercapacitors, which motivates using solid-state NMR to develop an understanding of MOF-based supercapacitor charging.[3]

This work aims to reveal the charging mechanisms of MOF-based supercapacitors. In the first stages, solid-state NMR is carried out on electrolyte-soaked samples and ex-situ supercapacitor electrodes of $Cu_3(HHTP)_2$ (HHTP = 2,3,6,7,10,11-hexahydroxytriphenylene) and $Zn_3(HHTP)_2$, with two different electrolytes. From these studies, we were able to assign distinct ex-pore and in-pore electrolyte environments in layered MOFs for the first time. These findings demonstrate the potential for well-established NMR techniques to be used widely in characterising the charging mechanisms of MOF-based supercapacitors and forms a preliminary basis for detailed in-situ NMR studies.

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PO268

Characterisation of backbone conformational heterogeneity in solid-state protein samples by high-dimensional, proton-detected NMR spectroscopy

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Disorder is a vital property of many protein chains, from flexible loops and allosteric sites to intrinsically disordered regions and proteins (IDRs and IDPs). Knowledge of conformational distributions, adopted by specific amino acid residues, can provide valuable insight into mechanisms of protein-protein interactions, as well as trajectory of specific molecule's folding, mis- and refolding. Providing site-specific information is one of the major strengths of NMR.

Conformational heterogeneity has been studied by both liquid- and solid-state NMR. In the solid state, conformational disorder results in large and uneven peak shapes which were observed for freeze-trapped folding intermediates, IDPs, frozen crystalline samples, membrane proteins at room temperature [1].

We present an approach to assess site-specific, relative conformational heterogeneity based on the chemical-shift distribution obtained in a 4D hCBCANH solid-state experiment. High dimensionality of the spectrum diminishes probability of peak overlap in case of a complex spectrum without site-specific labelling. The approach was developed on a model, u-(^{13}C, ^{15}N) GGAGG pentapeptide, heterogenized by freeze-drying from aqueous solution. The four-dimensional alanine cross-peak was analysed by two different routines, one of which is adapted from dihedral angles predictions by TALOS-N [2] and the other – from the search through the curated PACSY database [3, 4].

In addition to the obtained summary φ / Đ distribution, which represents all conformations potentially adopted by the heterogeneous residue, we propose several quantitative metrics of residue-specific disorder. Those metrics can be utilized in future research to numerically characterize differences in heterogeneity between different amino acid residues or a change of a given site between two sample preparations.

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PO269

Making the invisible visible: fast-MAS NMR reveals the evasive hepatitis B virus capsid C-terminal domain

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It regularly happens in structural biology, that parts or entire domains of a protein remain invisible in the acquired data. This is often due to dynamics that lead to absence of well-defined electron density in X-ray and cryo-EM signals. In NMR spectroscopy, while protein domains undergoing fast dynamics can be detected, some intermediate dynamic regimes interfere with the observation of the corresponding resonances. This is the case in the solid-state if the correlation time of the motion is comparable with the magic-angle-spinning (MAS) rotation period (about one microsecond), leading to incoherent, i.e. relaxation induced, line broadening. However, for fast spinning e.g. 100 kHz, the linewidth reduction should allow their detection. An alternative cause for disappearing resonances could lie in inefficient heteronuclear polarization transfers either by cross-polarization or INEPT due to reduced relaxation times.

One example of a vanishing domain is the C terminal domain (CTD) of the hepatitis B virus capsid protein, a central part of this assembly, crucial in regulating nucleic-acid interactions, cellular trafficking, nuclear import, particle assembly, and maturation. However, its structure remained elusive to all current techniques, including NMR. Here we show that the recently developed proton-detected fast-MAS solid-state NMR at >100 kHz MAS is a game-changer allowing to detect this domain. Using 2D exchange spectroscopy (EXSY), we were able for the first time to observe proton homonuclear polarization transfer from the CTD. Its NMR relaxation properties revealed that poor heteronuclear polarization transfer rather than line broadening beyond detection causes the loss of signal. It allowed us to characterize its structural and dynamic behavior and compare it for different capsid states. The developed approaches extend solid-state NMR observations to residues characterized by large-amplitude motion on the microsecond timescale and shall allow shedding light on other flexible protein domains still lacking their structural and dynamic characterization.

PO270

ACCURATE STRUCTURE OF CALCIUM CARBONATE HEMIHYDRATE BY DFT-D CALCULATIONS AND SOLID-STATE NMR SPECTROSCOPY

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Calcium carbonate ($CaCO_3$) is one of the most abundant material on Earth and therefore plays an important role in the geological and biological fields.1 The study of the $CaCO_3$'s biomineralization processes is one of the keys to improve our understanding of phenomena like ocean acidification, rocks formations. $CaCO_3$ is also involved in the global carbon cycle.…

$CaCO_3$'s crystallization pathway does not follow the classical nucleation theory since it is based on the formation of different amorphous precursors2,3 and is then still ill-understood. For example, a hydrated polymorph of $CaCO_3$ has recently been discovered, the so-called hemihydrate polymorph (CCHH).4 However, the proposed structure of this newly discovered polymorph remains elusive. In this work, we propose a strategy based on a combination of 1D and 2D solid-state NMR5,6 experiments with DFT-D calculations7 to investigate and propose a more accurate molecular structure for CCHH. Both DFT-calculations and Solid-State NMR experiments carried out in this study allowed to improve our knowledge of this new solid form and its crystallization pathway.

Incorporation of the Ce3+ activator ions in LaAlO3 crystals:
EPR and NMR study

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^{27}Al, [139]La static and MAS NMR and Ce3+ EPR study of Ce3+ incorporation in $Ce_{(x)}La_{(1-x)}AlO_3$ single crystals. Chemical shift of [139]La is strongly influenced by the Fermi contact interaction with Ce ions and linearly increases with Ce concentration up to 165 ppm at x=0.5. Separated peaks corresponding to individual Ce-O-La spin transfer pathways are resolved in the [139]La NMR spectra. ^{27}Al NMR spectra do not evidence such interaction, but additional satellite peak is present with intensity linearly increasing with Ce content. Ce ions visibly modify the local crystal structure which is also reflected in EPR spectra where orthorhombic symmetry of the Ce3+ g-tensor to is observed.

Solid-state NMR studies on heterogeneous catalysis: chemical structure and C1-C2 chemistry

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The structure-activity relationship is the essence of heterogeneous catalysis. Among the various techniques, solid-state NMR finds its unique contributions to molecular-level understandings not only on chemical structures of catalyst, but also on reaction mechanisms. Here, we showcase various applications of ssNMR on two utmost examples: acid-catalyzed C1 chemistry and cooperative acid-base interplay on ethanol conversion.

First, the CO_2 valorization is studied on the cascade catalytic system using aluminosilicate zeolites as process descriptor. The acidic nature and chemical structure of zeolites was demonstrated not only by direct excitations on ^1H, ^{27}Al and ^{29}Si, but also by their correlation studies (CP, HETCOR and HMQC). The catalytic mechanism is traced using ^{13}C-enriched CO_2 as reactant feed, enabling multidimensional experiments with different magnetization transfer schemes (CP, INEPT and PARIS based 1D and 2D) to decode the structure of trapped organics. We also applied (i) ^{13}C-^{27}Al correlation studies (D-HMQC) by splitting a X-channel frequency into two comparably close resonances (νL 150.9 and 156.4 MHz for ^{13}C and ^{27}Al) and (ii) water confinement effect in zeolite using in situ MASCAT probe at 14.1 T.

Moreover, we address a grand-old research question on ethanol-to-butadiene process over SiO_2-MgO catalysts. Magnesium silicates (Mg-O-Si) have been considered as active sites, but their generation mechanism and catalytic roles are not fully established for 70 years. The chemical structure of as-made catalyst was studied using DNP enhanced ^1H-^{29}Si NMR. The natural abundance ^{25}Mg NMR at high field magnet (21.1 T) was used with CPMG based pulse sequences to enhance signal sensitivity, and the features were analyzed with simulations. Combined with other augmented techniques (e.g., XRD, HR-TEM, DRIFTS-MS), the NMR studies allowed for the identification of the chemical nature of magnesium silicates. We expect this study opens fruitful discussions for the role of ssNMR on heterogeneous catalysis.

PO273

Uncovering the Dynamics of Surfactants – A Combined ²H and DNP NMR Approach

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Surfactants show an interesting behavior in aqueous solution as well as in bulk. Due to their amphiphilic nature, aggregate formation into micelles or lamella can be observed.[1] The dynamics in these kinds of aggregates are oftentimes not fully understood and notoriously hard to probe, despite their relevance to a plethora of different fields like catalysis, separation processes or drug delivery.[2]

In this work, we provide an insight into the behavior of the model system octanol using a specialized DNP technique as well as ²H solid state NMR. ²H ssNMR measurements were performed in a temperature range of 120 K to 270 K. The ²H ssNMR spectra were evaluated using numerical modelling; the composition of the sample and quadrupolar coupling constants at any given temperature were determined.[3] The data gathered by DNP measurements with different radicals at 100 K was deconvoluted and the percentages of the direct vs. indirect polarization transfer paths were determined.[4]

The only observable motional degree of freedom throughout the temperature range is the rotation of the CH_3-group around its C_3-axis until the melting point of the octanol. This observation could be confirmed via DNP NMR, clearly illustrating that the rotation of the CH_3-group cannot be suppressed and therefore plays a fundamental role in the dynamic of the system even at low temperatures. The results also that the methods complement one another to a large extend.

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PO274

NMR Characterization of dynamics of the efficient light harvesting Chlorosomes of wild type Chlorobaculum tepidum

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In nature, the largest light harvesting antennae are the chlorosomes that are found in green photosynthetic bacteria, 'chlorobaculum tepidum'. In the chlorosomes, the bacteriochlorophylls selfassemble in a plastic crystalline state to tailor their dynamics for ultrafast energy transfer. They are subjected to geometric strain due to conformational dynamics that gives stability to other parts of the surrounding chiral matrix, with the whole self-assembly subject to weak crystalline order. MAS NMR allows us to study the structure and dynamics of the restrained dynamics at the atomistic level, making it a powerful tool to study how the bacteriochlorophyll in the self-assembled supramolecular complex are tuned for their antenna function. Results show dynamics in the different parts of the ring which may be related to rotational twisting motion of the macrocycle contributing to hydrogen bond breaking or forming leading to different conformers in the system. Herein we employed CP, DP and INEPT experiments and used the DYSE strategy to quantify rigid and dynamic parts of the system. 2D 13C experiments such as PDSD and PARIS were employed in order to measure the distance constraints and probe the dynamics, both PDSD and PARIS experiments make use of hydrogens for spin diffusion. In addition $T1\rho$ was performed to further analyse the dynamics in the system as spin lattice relaxation of rare nuclei carry pure dynamic information including most of the local motion.

PO275

Solid state NMR spectroscopy for investigating the structure and dynamics of Ca2+ cross-linked alginate hydrogels

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Hydrogels are three-dimensional nanofibrous polymeric networks which require cross-linking for their formation. Cross-linking initiates the sol-gel transformation mechanism via engaging specific ionic forces and hydrogen-bond interactions with the water molecules and the polymeric chain functional group especially the carboxylic ones. Understanding these aspects of hydrogels such as water-matrix interactions, (re)hydration step-wise process, biopolymer dynamics and pore formation provide crucial information used in tuning the performances of under-development smart hydrogels. Solid state NMR spectroscopy with its versatile techniques, ability to extract information from different physical states and wide range of detected nuclei is considered a unique analytical technique in this field, especially in probing the crosslinking and (re)hydration processes in hydrogels. The aim of this project is to exploit the capabilities of solid state NMR spectroscopy in investigating the structure and dynamics of Ca2+ cross-linked alginate hydrogels and its repeatability on different types of hydrogels.

C-A-S-H chain length of composite cementitious suspensions with high solid fraction: zeta potential and NMR

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Evidence that C-S-H or C(-A)-S-H can physically bind chloride ions has been reported in many published studies (1), while there have also been research demonstrating that physical binding has a negligible, or possibly a very small, contribution in total bound chloride contents (4).

Physical binding has however, not yet been directly measured unless being estimating through subtracting the chemically bound chloride content from the total amount of the chlorides bound.

As, physical chloride binding is mainly a phenomenon happening at the surface of C-S-H or C(-A)-S-H, due to the surface charges, studying the electrokinetic potential of the surface of these hydration phases and the relation of that with chain structure of C-S-H or C(-A)-S-H through zeta potential analysis and Magnetic Resonance Spectroscopy (NMR) is documented in literature (1) in attempts towards better understanding of physical chloride binding.

The objective of this article is to investigate the possibility for demonstrating the relation between the zeta potential of cementitious suspensions with a composition closer to that of real binder systems, prior and after exposure to chloride solutions, and the relation of this parameter to chain structure of C-S-H or C(-A)-S-H.

The silicon coordination in the hydrated, pastes were measured by 29Si solid-state nuclear magnetic resonance spectroscopy (NMR), to investigate the changes in the C(-A)-S-H structure.

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PO277

Understanding formation of pharmaceutical co-crystal polymorphs in continuous polymer-assisted mechanochemical processes in-situ using CLASSIC NMR

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Pharmaceutical co-crystals, similarly to other organic solids, are prone to exhibit different packing arrangements in their crystal lattice, i.e. polymorphism, which can affect bioavailability of a drug. However, the mechanisms that direct the nucleation towards a particular crystalline phase are yet to be fully understood. In-situ monitoring of changes occurring in a solid (API, coformer, co-crystal) and a liquid (polymer and its interactions with API and coformer) components of the interacting mixture via CLASSIC NMR (Combined Liquid- and Solid-State In-Situ Crystallisation NMR) experiments may provide an insight into the mechanism of mechanochemical co-crystallisation with the addition of polymers.

This study aims to understand mechanism of mechanochemical co-crystallisation of theophylline (TP) and benzamide (BZ) co-crystal (TP:BZ) using polymer-assisted grinding (POLAG) and Hot Melt Extrusion (HME) with combined application of CLASSIC NMR (supported with in-situ synchrotron measurements). The polymorphic outcome of TP:BZ co-crystallisation can be controlled via modification of processing parameters such as polymer content, polymer polarity and average molecular mass (liquid or solid), as well as temperature of the HME process.

Time-resolved ^{1}H-^{13}C CP/MAS solid-state NMR spectra recorded at room temperature (mimicking POLAG experiment) indicate the co-crystallisation of the form I with visible traces of theophylline residue. At the same time, benzamide dissolving in added polymer (PEG 200) was observed in a liquid phase spectra (^{1}H; ^{13}C {1H}). CLASSIC experiments at higher temperature (reproducing HME process at 70°C) show the formation of the form II of TP:BZ co-crystal. Immediately after the addition of PEG 200 co-crystallisation of form I occurred and 14 h were needed to complete transition towards form II of TP:BZ co-crystal. In contrast, when PEG 20000 was used, the physical mixture present at the beginning co-crystallised into form I within 4 h and did not transform into TP:BZ form II co-crystal during the time of experiment (50 h).

PO278

Homonuclear correlations of half-integer spin quadrupolar nuclei: comparison of DQ-SQ and SQ-SQ approaches

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The observation of spatial proximities between identical isotopes using two-dimensional solid-state NMR experiments is an essential step for spectral assignment and structure determination. Under MAS, the homonuclear dipolar couplings are reintroduced by using rotor-synchronized recoupling schemes. This homonuclear dipolar correlation (D-HOMCOR) information is mostly acquired through either single-quantum - single-quantum (SQ-SQ) [1] or double-quantum - single-quantum (DQ-SQ) 2D-spectra [2-4]. For nuclei with spin I = 1/2, various efficient approaches have been developed. However, these D-HOMCOR experiments are more challenging for half-integer spin quadrupolar nuclei, which represent about 66% of NMR active isotopes, owing to their complex spin dynamics under MAS. Recently, several D-HOMCOR methods, including DQ-SQ and SQ-SQ sequences, have been proposed for half-integer quadrupolar nuclei.

=In the present work, we have analyzed and compared the efficiencies of different DQ-SQ and SQ-SQ D-HOMCOR schemes, at moderate (20 kHz) MAS frequency, of half-integer quadrupolar nuclei with spin I = 3/2 (^{11}B), and I = 5/2 (^{27}Al), for $Li_2O.2B_2O_3$ and $AlPO_4$-14 samples, respectively. For both isotopes, we recommend the DQ-SQ sequences, which allows the observation of spatial proximities, even for resonances that have close or identical frequencies and usually benefits from a higher efficiency than 1Q-1Q variants. For ^{11}B isotope, the chemical shift differences are small or moderate (up to ca. 3.5 kHz at 18.8 T), and we recommend 2Q-1Q scheme using [$SR2_2^1$] or [$BR2_2^1$] bracketed recoupling schemes, which benefits from larger scaling factor of the homonuclear dipolar interaction. For ^{27}Al nuclei, the chemical shift differences are larger (up to ca. 10 kHz at 18.8 T) and we recommend the $BR2_2^1$ un-bracketed recoupling scheme, which benefits from higher robustness to offset.

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PO279

MAS and solution NMR resonance assignment of Zinc Protoporphyrin IX(ZnPP) photo-sensitizer

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Zinc(II)-porphyrins are well-known photo-sensitizers. One prominent application has been the photo-sensitized H_2 generation from H+ in aqueous solutions. Zinc(II)-porphyrins are derived from naturally occurring porphyrins, such as Protoporphyrin IX(PP). These tend to aggregate in aqueous solutions, thus greatly lowering their photosensitization efficiency. Embedding Zinc(II)-porphyrins within binding sites of proteins or small peptides prevents this aggregation by forming complexes with peptides/protein. [1] Zinc(II)-porphyrins protein reconstituted products have been shown to be functional photosensitizers for H_2 generation.[2–4] Here we would present the complete chemical shift resonance assignments of ZnPP chromophore determined with high field magic angle spinning and solution NMR. NMR resonances would be recorded and assigned through the standard liquid and solid 2D correlation techniques at 600 MHz and 750 MHz spectrometers. The characterization of ZnPP is of interest from biochemical to pharmaceutical industries and energy research.

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PO280

Extracting diamagnetic chemical shift tensors parameters in paramagnetic systems with combined SQUID and NMR measurements

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Accessing diamagnetic parameters in paramagnetic systems is a common feat that has been demonstrated in recent publications where methods were proposed to experimentally access isotropic shift or quadrupolar parameters in paramagnetic systems. Many other studies use powerful advances in calculations methods for solid-state NMR to predict all components of diamagnetic and paramagnetic shifts in complex materials.

Here we propose a modest contribution to this line of work by combining SQUID and solid-state NMR measurements at various temperatures and semi-empirical calculations to extract from the temperature dependant CSA parameters in spin 1/2 systems the constant contribution that comes from its diamagnetic component. While it has been demonstrated that isotropic shifts are easily accessible this way, we propose a method to experimentally access the full diamagnetic shift tensor of spin 1/2 nuclei in paramagnetic systems.

We illustrate this work with two examples: an organometallic framework with Ni-metal centres in which Fermi-contact contribution to the paramagnetic shift is important, another with lanthanide complexes for which only the pseudo-contact contribution of several paramagnetic centres surrounding the observed nuclei have to be taken into account.

PO282

5D and 4D experiments for near-complete resonance assignment in solid-state NMR

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The advent of (ultra) fast magic angle spinning (MAS) in combination with ^{1}H detection today allows us to study an increasingly large variety of proteins without deuteration schemes. These advances especially promote the use of protein side chains, including side-chain protons, to report on interactions and distances, yielding more refined structures. Despite increasing spinning speeds and magnetic field strengths, proton and carbon side-chain resonances suffer from limited chemical shift dispersion and low redundancy to link side-chain resonances to the protein backbone in traditional 3D assignment approaches. Even for the protein backbone, these approaches fail for proteins of increasing molecular weight. In this work, we introduce a combined 5D/4D HCCNH/HCCH experiment for unambiguous side-chain-to-backbone assignment of the protein side-chain in a fully protonated sample. We demonstrate on a small model protein, the SH3 domain of chicken α-spectrin, that a nearly complete assignment can be obtained through a combination of high chemical shift dispersion, high redundancy with the protein backbone, and optimized use of residual magnetization. Concatenated with a single 5D experiment for backbone assignment, this reduced set of necessary experiments provides nearly complete backbone assignments within 8 days of measurement time on a midrange spectrometer. Through the application of the SSA algorithm for spectral reconstruction, the assignment of the protein backbone and the side chains can be done intuitively and straightforwardly which can be supported by automated assignment through FLYA. Our new approach provides reliable and outright assignments for downstream applications such as chemical shift perturbations and structure calculation.

PO283

Solid-State NMR Study of Novel Hydrogen-Bonded Supramolecular Aggregates

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Supramolecular aggregates are the structures formed by a complex of molecules held together by noncovalent bonds. We present a novel supramolecular construct - a fully noncovalent molecular tweezer. The supramolecular tweezer was assembled from a set of four building blocks, composed of two identical molecular angle bars based on the bicyclo[3.3.1]nonane and two flat aromatic extension wings, using hydrogen bonding only. The distance between the side walls in a cleft/ tweezer structure is designed to be ~ 12 Å, which roughly corresponds to three typical $\pi-\pi$ stacking distances. This allows for one tweezer to interact with two other tweezers by intercalation, an aggregation process reminiscent of the action of a zipper.

The combination of solid state NMR techniques, such as 1H, 13C/ 15N CP MAS, 1H-1H 1Q-2Q correlation, 1H-13C HETCOR and MAS-J-HMQC, proofed to be a powerful method to elucidate the geometrical organization of the supramolecular aggregates. In particular, the 1H-1H 1Q-2Q correlation experiments provided useful insight on the formation of the zipper-like polymer. Interestingly, the chemical shifts of some protons were observed ~ 3 ppm upfield compared to the chloroform solution. Such a significant shielding is seen in a section of the molecular model of the polymer, where the corresponding N-H protons of the flat unit are located exactly above the centers of the top and bottom neighboring flat units aromatic rings within the stack.

Summing up, A fully supramolecular tweezer assembled solely using H-bonding is demonstrated for the first time and solid state NMR proved to be a sensitive method to study the aggregation process.

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PO284

Studying molecular changes at the cell / extracellular interface with Goldman-Shen experiments

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Membrane interfaces, i.e. the region separating different cell environments are of paramount importance to sustain cell activity. Membrane proteins embedded within the cell membrane as well as the extracellular basement membrane surrounding the cells are responsible for cell adhesion, migration, and provide structural support to the tissue. So far, the study of such interfaces has heavily relied on electron, and fluorescence microscopy for which extensive sample preparation is needed, so that truly native tissues cannot be studied. NMR spectroscopy is a valuable tool to perform structural studies on biological tissues, which requires very little sample preparation. However, conventional NMR spectra are dominated by signal intensity from the bulk of the sample, which obscures the much lower intensity of signals from interfacial regions. Thus, there is an urgent need for NMR techniques to study interfacial regions to allow the benefits of NMR spectroscopy to be conferred to these important biological structures.

Here, we propose that spatial selectivity can be achieved with solid-state NMR using Goldman-Shen-like experiments, which have been previously used to measure domain size of semicrystalline polymers, and to study the orientation of membrane proteins in lipid vesicles.

In this work, we use Goldman-Shen-like experiments to study the interface between bovine vascular smooth muscle cells (VSMCs) and their surrounding 13C-enriched extra-cellular matrix (ECM). The Goldman-Shen experiment rely on i) the selection of 1H phospholipid NMR magnetisation close to the region of interest with a $T_2(1H)$ filter, ii) a spin diffusion time that allows the magnetisation to be transferred to the region of interest, i.e. the surrounding 13C-enriched extra-cellular matrix (ECM) iii) 13C NMR detection after a cross polarisation step. We characterise extensively the spin diffusion dynamics, and present relevant control experiments to remove unwanted $T_1(1H)$ relaxation effects.

PO285

Proton-decoupled 15N R1rho in solid proteins: the study of the slow rocking motion

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The ^{15}N relaxation rate R1ρ is governed by the heteronuclear [1]H-^{15}N dipolar and CSA relaxation mechanisms. Under MAS, both these contributions are proportional to the combination of the spectral density functions J(ωSL±ωMAS) and J(ωSL±2ωMAS), where ωSL/2π and ωMAS/2π are the spin-lock and MAS frequencies, respectively. The smaller the difference between ωSL and ωMAS, the slower motions can be analysed. If this difference is however small, the amplitude of the relaxation decay is close to zero and the amplitude of the initial oscillations of the decay is rather high, so that the relaxation rate cannot be determined reliably from the experimental data (1). This interfering effect can be significantly suppressed by applying proton high power CW-decoupling during the ^{15}N spin-lock pulse. The proton decoupling effectively "switches off" the dipolar relaxation mechanism of the slow molecular motions leaving CSA mechanism untouched. In this case, the difference between ωSL and ωMAS can be set few times smaller than in the standard experiment and thus, much slower motions can be observed. The proton decoupling also suppresses the interfering coherent spin-spin relaxation pathway at low spin-lock fields, which overlaps the Bloch-McConnell (chemical exchange) range of R1ρ dispersions.

The time scale of the protein rocking motion in solid samples (1) is on the edge of the dynamic window of the R1ρ experiment, thus extending it towards slower motions is very important for obtaining reliable dynamic information. The standard and proton-decoupled R1ρ experiments were applied to studying rocking motion of the protein GB1 in two forms, microcrystals and lyophilized amorphous powder. R1ρ's were measured at different spin-lock and proton decoupling fields and different temperatures. The data enable detailed characterization of the motional correlation function and revealing interesting features of inter-protein interactions in solid protein samples.

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PO286

Development of Hyperpolarized and Proton Fast MAS NMR Spectroscopy for the study of performance polymers

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Solid-state nuclear magnetic resonance spectroscopy allows the investigation of structure and dynamics of polymers relating to their functional behavior. NMR probes the local environment of nuclei without relying on their crystalline arrangement. In this work, we will discuss two important methodological developments that can remedy shortcomings of NMR for studying complex polymer systems. We will talk over advantages and disadvantages of ultrafast magic angle spinning (MAS) NMR for high resolution NMR of protons in the solid-state to study highly complex performance polymers like polyurethane (a synthetic polymer) and Arabic gum (a natural polymer). Finally, we optimize and extend Dynamic Nuclear Polarization methodology for low gamma nuclei combined with proton ultrafast MAS NMR to probe these polymeric systems.

PO287

A Lipid Peroxidase complex of monolyso-cardiolipin with cytochrome c probed by solid state NMR spectroscopy.

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The formation of a lipid peroxidase complex between cytochrome c (cyt c) and cardiolipin (CL), an essential phospholipid in mitochondria, plays a key role in apoptosis. Dynamical and structural changes underpin cyt c's conversion into a CL-specific peroxidase capable of triggering apoptosis, rendering their characterization of undoubted interest. In prior work we have shown the power of solid state NMR (ssNMR) spectroscopy to probe these important features of the proteolipid complex. The importance of CL remodeling is underscored by the life-threatening genetic disorder Barth syndrome (BTHS), caused by mutations in tafazzin. The latter reacylates monolysocardiolipin (MLCL) generated from the deacylation of CL, such that BTHS is characterized by accumulation of MLCL in mitochondrial membranes. The effect of MLCL accumulation in BTHS remains unclear, thus understanding the nature of (ML)CL interaction with mitochondrial proteins is key. This includes the question of whether or how the MLCL-cyt-c complex mimics the CL-cyt-c one, and may possess pathogenic peroxidase activity. Here, we probe this question through ssNMR studies of 13C,15N-labeled cyt c in complex with either CL or its three-tailed variant, MLCL. In our experiments, remarkable differences between the two protein/lipid complexes, either containing CL or MLCL, could be observed in terms of both membrane structure and protein structure/dynamics. Multidimensional CP- or INEPT-based 13C-13C or 15N-13C 2D spectra allow both global and local protein features to be observed and compared. On the basis of these data we further our understanding of the dynamic properties of cyt-c as a pro-apoptotic lipid peroxidase, both in general and in BTHS in particular.

PO288

Protein backbone and side-chains motions by simultaneous measurement of 1H-15N/13C dipolar couplings with fast-MAS NMR

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The development of magic-angle spinning (MAS) probes capable of fast sample rotation (100 kHz and above) has produced a real revolution in the field. Coupled to very high magnetic fields, fast MAS enables the direct detection of the larger magnetic moments available from ¹H spins in fully-protonated proteins, enabling structure investigations with a drastic increase in sensitivity.

Here we show that cross polarization with variable contact-time (CPVC) leverages 100 kHz MAS to provide the basis for an exhaustive description of the conformational fluctuations of both backbone and side-chains, using less than 500 µg of a single, uniformly ¹³C,¹⁵N-labelled sample. At these MAS rates, an efficient separation of the proton polarization sources is ensured for ¹⁵N (¹HN) and ¹³C (¹Hα) CP, so that the two ¹H-¹⁵N and ¹H-¹³C recoupling experiments can be recorded simultaneously in a time-shared fashion with negligible intensity losses, and an overall two-fold time saving.

We demonstrate the broad applicability of the approach by testing its performance on microcrystalline samples of globular proteins and on membrane proteins reconstituted in lipid bilayers. The combined investigation of ¹H dipolar couplings with ¹⁵N and ¹³Cα increase the number of probes of protein backbone dynamics, and we predict this approach to be particularly relevant in large systems, for which the 2D ¹H,¹⁵N fingerprints are significantly crowded. In addition, through ¹H-¹³C order parameters it is possible to extend the investigation of motional amplitudes from the protein backbone to the side chains. Importantly, the use of CP-VC combined with 1H-detection and MAS rates above 100 kHz gives access to these information in a rapid way using a single, uniformly labeled sample in sub-mg amounts.

PO289

Molecular elucidation of drug-induced abnormal assemblies of Hepatitis B Virus capsid protein by solid-state NMR

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We investigated, using solid-state NMR, the aberrant assemblies formed by the hepatitis B virus (HBV) core protein (Cp) in presence of a new class of anti-HBV drugs, the capsid assembly modulators (CAMs). While these assemblies apparently show poor order as indicated by electron microscopy (EM), they surprisingly yielded high-resolution NMR spectra which allowed us to characterize them at a molecular level.

CAMs disturb proper nucleocapsid assembly by inducing formation of either aberrant assemblies (CAM-A) or of apparently normal but genome-less empty capsids (CAM-E). Classical structural approaches have revealed the CAM binding sites on Cp, but conformational information on the CAM-induced aberrant assemblies is lacking. Thanks to a combination of solid-state NMR spectra (phosphorus, carbon- and proton-detected spectra, relaxation at fast MAS), we could provide such information and decipher the impact of both types of CAMs on full-length Cp183 in its phosphorylated and unphosphorylated state. We found that in the aberrant assemblies, the asymmetric unit comprises a single Cp molecule rather than the four quasi-equivalent conformers typical for the icosahedral T=4 symmetry of the normal HBV capsids. Furthermore, while in contrast to truncated Cp149, full-length Cp183 assemblies appear unaffected by CAM-A by EM, NMR reveals that Cp183 assemblies are equally aberrant at the molecular level. Site-specific relaxation measurements show that CAMs-A induce a global increase in the dynamics of Cp. Finally, we use NMR of Cp expressed in an eukaryotic cell-free system to reveal how CAMs modulate capsid-RNA interactions and capsid phosphorylation. Our results establish a structural view on assembly modulation of the HBV capsid, and they provide a rationale for recently observed differences between in-cell versus in vitro capsid assembly modulation.

Operando NMR for studying the mechanism of electrochemical ammonia synthesis

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The electrochemical nitrogen reduction reaction has been proposed as an alternative ammonia synthesis method to replace the fossil fuel-based Haber-Bosch process. In particular, lithium-mediated synthesis has received extensive attention due to its high Faradic efficiency. However, the reaction process for lithium-mediated ammonia synthesis is complex, requiring a systematic mechanistic study. Therefore, developing and applying new operando characterization methods to reveal the mechanism of lithium-mediated ammonia synthesis is critical. Compared to traditional operando characterization such as Fourier-transform infrared spectroscopy and X-Ray based methods, NMR is advantageous for its atomic specificity and versatility for studying gas, liquid and solid phases. Particularly, based on ^{6}Li and ^{7}Li NMR spectra, the chemical composition and structure of lithium catalyst during the nitrogen reduction can be identified. This project demonstrate a new operando NMR method to follow the solid-liquid reaction between lithium nitride and ethanol, which is a key reaction step in the catalytic cycle. The produced ammonia was revealed and quantified by ^{1}H NMR. We not only provide answers to some key questions regarding the reaction mechanisms, but also demonstrate the possibility of applying operando NMR to study flow electrocatalytic reactions.

PO291

Open state and Aromatic Network of the SARS-CoV-2 Envelope Protein Unveiled by 19F ssNMR

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The envelope (E) protein of SARS-CoV-2 is a membrane-bound viroporin that conducts calcium and other cations across the host cell's endoplasmic reticulum Golgi intermediate compartment (ERGIC) membrane. This process triggers severe inflammation mechanisms in the cell, thus E is a primary molecular player in viral-induced disease. In mice, inhibition of E greatly attenuated the pathogenic effects of SARS-CoV-2, demonstrating that E is a promising antiviral target.

The structure of the closed state of E Transmembrane Domain (ETM) was recently determined in our lab using ssNMR. However, how the channel pore opens to allow cation transport is unclear. Here we employ 15N, 13C and 1H-detected ssNMR experiments to investigate the conformation and dynamics of ETM at acidic pH in the presence of calcium ions, which mimic the ERGIC and lysosomal environment experienced by E in the cell. Acidic pH and calcium ions increased both the conformational disorder of the N- and C-terminal residues and the water accessibility of ETM channels, indicating that the pore lumen has widened. Moreover, using 19F ssNMR, we identified an intricate aromatic network formed by three regularly spaced phenylalanine (Phe) residues at the center of the ETM sequence. 19F NMR spectra of para-fluorinated Phe20 and Phe26 revealed that each residue adopts two sidechain conformations, forming a complex aromatic arrangement in the channel. The two Phe conformations differ in water accessibility, lipid contact and dynamics. Our results show that channel opening induced by acidic pH and Ca2+ shifts the equilibrium of Phe20 and 26 towards the dynamic lipid-facing conformation, thus changing the arrangement of the sidechain network.

These results suggest that this Phe aromatic network regulates the opening of the ETM channel pore, either directly or indirectly by allosteric interactions with other residues. This network may be a target for E inhibitors against SARS-CoV-2 and related coronaviruses.

Understanding spatial distribution and crystallization of pharmaceutical cocrystals confined in nanoporous materials using solid-state NMR

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An ordered, size tailored pore network and high surface area attracted considerable attention of the pharmacy and drug development world to mesoporous silicas as carriers for drug delivery. Encapsulation of pharmaceuticals inside the pores of silica scaffolds may stabilize amorphous state of the drug or lead to the formation of new polymorphs due to the differences in nucleation pathways of nanoconfined molecules. This study aims to understand spatial distribution and crystallisation mechanism of flufenamic acid (FA) and 15N-nicotinamide (NIC) assembling into a nanosize cocrystal confined inside mesoporous silicas (MCM; SBA; MCF, pore size diameter 3-30 nm).

1H-15N CP/MAS and 19F MAS solid-state NMR spectroscopy (20 T, and 59 kHz MAS) demonstrated that the FA and NIC are present inside the pores of mesoporous silica in three different environments, i.e. as highly mobile molecules at the silica surface, confined amorphous species and confined cocrystal. The temperature induced FA:NIC co-crystallization inside the nanopores was achieved in situ using variable temperature 19F MAS NMR experiments. The cocrystal was formed via rearrangement of disordered FA/NIC species, while the population of molecules present at the silica surface remained unchanged. The observed cross-peaks in 19F-19F NOESY spectra indicated the FA molecules present in different environments are in close contact. 1H-15N HETCOR correlation enabled us to distinguish between the peaks arising from molecules at the silica surface and molecules present in the pore void.

In contrast, encapsulation of neat FA or neat NIC inside the pores of mesoporous silicas led to stabilisation of both drugs in an amorphous state or to direct crystallisation of FA towards form I or NIC into recently discovered δ-NIC. This example of mechanistic understanding of crystallisation of multicomponent solids in confined space will facilitate the use of mesoporous silicas in pharmaceutical formulations as several examples of FDA approved porous silicas are known.

PO293

Characterization of phosphorus clusters via multiple quantum solid state NMR

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Nonclassical growth pathways have significant potential for development of materials with tunable functional complexity, including calcium phosphate solutions which are of interest to the bone community. However, these growth pathways remain mostly elusive to structural control due in significant part to the intrinsic difficulty of characterizing the highly dynamic structural evolution of prenucleation clusters (PNCs). To identify the structure of PNCs, solid-state NMR can be utilized to analyze various time points in nonclassical growth pathways via sample vitrification. We present ^{31}P NMR spin counting studies of both powdered and vitrified phosphate containing samples, for which we have extracted the minimum number of dipolar coupled spins via the creation of multiple quantum coherence orders (MQCOs).

We focus on discrete and extended ^{31}P dipolar coupled systems for spin counting studies, demonstrated respectively by adenosine triphosphate (ATP) and crystalline hydroxyapatite. Utilizing a modified R2$_1^8$ pulse program at 10 kHz MAS, we find for hydroxyapatite MQCOs up to 18 at room temperature with an 8 ms excitation time. When dissolved into dynamic nuclear polarization (DNP) solution and vitrified at 100 K, we find comparable MQCOs of 14 for the same excitation time, indicating vitrification does not drastically affect MQCO extraction. When spin counting experiments were completed on ATP under the same conditions as hydroxyapatite at room temperature, MQCOs of 2 and 3 were extracted, consistent with only intramolecular MQCO buildup. We have extended these spin counting techniques to dissolved calcium phosphate solutions vitrified at various time points to gain insight into calcium phosphate cluster formation processes, of which the results are underway.

This exemplification of ^{31}P spin counting at low MAS room temperature and vitrified DNP conditions has yet to be reported. These quantitative measurements of ^{31}P nuclei clustering in vitrified solutions provides a novel basis for the structural analysis of nonclassical growth pathways.

PO294

New methods for methyl resonance assignment in solid proteins at Ultra-Fast MAS

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In nuclear magnetic resonance spectroscopy of proteins, methyl protons play a particular role as extremely sensitive reporters on dynamics, allosteric effects, and protein–protein interactions, accessible even in high-molecular-weight systems approaching 1 MDa. The notorious issue of their chemical shift assignment is addressed here by a joint use of solid-state 1H-detected methods at very fast magic-angle spinning, partial deuteration, and high-magnetic fields. The suitability of a series of RF schemes is evaluated for the efficient coherence transfer across entire 13C side chains of methyl-containing residues, which is key for establishing connection between methyl and backbone 1H resonances. The performance of ten methods for recoupling of either isotropic 13C–13C scalar or anisotropic dipolar interactions (five variants of TOBSY, FLOPSY, DIPSI, WALTZ, RFDR, and DREAM) is evaluated experimentally at two state-of-the-art magic-angle spinning (55 and 94.5 kHz) and static magnetic field conditions (18.8 and 23.5 T). Model isotopically labeled compounds (alanine and Met-Leu-Phe tripeptide) and ILV-methyl and amide-selectively protonated, and otherwise deuterated chicken α-spectrin SH3 protein are used as convenient reference systems. Spin dynamics simulations in SIMPSON are performed to determine optimal parameters of these RF schemes, up to recently experimentally attained spinning frequencies (200 kHz) and B0 field strengths (28.2 T). A resolution enhancement provided by 4D spectroscopy with non-uniform (sparse) sampling is demonstrated to remove ambiguities in simultaneous resonance assignment of methyl proton and carbon chemical shifts.

PO295

Combined use of solution and solid-state NMR data to correctly identify crystal polymorphs

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Accurate discrimination between polymorphs or potential molecular arrangements from crystal structure prediction (CSP) studies remains a challenge for NMR crystallography. Our approach compares NMR chemical shifts measured experimentally (δexp) in both solution- & solid-state to calculated shifts from density functional theory (DFT) (δcalc) to quantitively assess putative crystal structures. Building upon our previous work (Blade et al., 2020), we now extend it to the compound furosemide, which is more challenging with its six rotatable bonds. Differences between the NMR chemical shifts for furosemide in the solid state (δSolid expt) (Widdifield, Robson, & Hodgkinson, 2016) and solution state (δSolution expt) provide a measure of the change in chemical shift due to passing from the solution to the solid state ($\Delta\delta$Experimental). Solid-state NMR chemical shifts for each form (δSolid calc) were calculated by the gauge-included projector augmented wave (GIPAW) method. Solution chemical shifts (δSolution calc) were calculated as the average from an ensemble of 3D-conformations that represents the measured dynamic behaviour in solution (Blundell, Packer, & Almond, 2013). The difference between δSolution calc and δSolid calc was shown as the calculated change in chemical shift ($\Delta\delta$Calculated). Regression analysis and t-tests for $\Delta\delta$Calculated against $\Delta\delta$Experimental provides statistical significance in identifying the correct polymorph. Additionally, it has been found that the averaged chemical shifts from 54 single crystal and co-crystal structures of furosemide (δSolution calc proxy) can be used as an approximate substitute for the shifts calculated from the solution ensemble, identifying the correct polymorph with an equivalent sensitivity to the solution ensemble. This method can reliably be used to identify the crystal structure of a compound with multiple rotatable bonds from a range of possible structures with greater sensitivity than existing methods that solely use solid-state NMR data.

PO296

Elucidating the hydration effect on structure and dynamics of HA-extracellular matrix hydrogels using solid-state NMR

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Hyaluronic acid (HA) is a ubiquitous natural polysaccharide and one of the main components of the extracellular matrix (ECM). Due to its biocompatibility, high water retaining capacity, and non-immunogenicity, HA is an attractive material for various medical, food, and cosmetic applications. The structure and morphology of HA are firmly influenced by water molecules via controlling its conformation and various forms of aggregation. Elucidating the structure of the hydrated HA is of crucial importance to understand its performance in various applications. To gain deeper insights into its dynamical properties, fully 13C labeled native high molecular weight (HMW)HA was produced and, the structural and dynamical properties were investigated by means of ssNMR spectroscopy. Our results show that the hydration level has a significant role in affecting the overall and local mobility of HA. Furthermore, crosslinking of HA with biomimetic ECM components leads to interesting differences in structural and motional ssNMR parameters (i.e., relaxation properties, chemical shift values), indicating the occurrence of structural as well as dynamical changes. We identify different conformations and dynamics in HMWHA, as a function of the hydration level, and as a consequence of ECM-like cross-links. This information is uniquely accessible by the versatility of ssNMR spectroscopy, combined with uniform isotope labeling of HMWHA. The employed approach will provide new molecular-level insights into HA-based hydrogels, biomaterials, and ECM model systems.

PO297

Solid-State NMR Crystallography Analysis of an Active Pharmaceutical Ingredient under varied conditions

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Solid-State NMR is a valuable tool in the characterisation and study of active pharmaceutical ingredients (APIs). Here, an in-depth analysis of an API will be presented for the full crystal form. The assignment of the range of one-dimensional and two-dimensional experimental data was aided by the completion of DFT geometry optimization followed by GIPAW calculations. For the sample, a 1H(DQ)-1H(SQ) spectrum was obtained with BaBa recoupling to probe the interactions between the 1H nuclei that are within 3.5 Å of each other. A 14N-1H HMQC spectrum was also obtained to develop understanding of 14N and 1H interactions within the molecule. Investigation of the obtained data along with crystal structure analysis allows for a broader understanding of the API including hydrogen bonding effects also taking place. Various two-dimensional 1H-13C CP experiments were also recorded in order to probe the one-bond correlations. Further, experiments were recorded using a high field (1 GHz) spectrometer for increased resolution and sensitivity which also assisted in the assignment.

Additionally, data will be shown for variations of the sample itself and when mixed with an excipient, sodium starch glycolate (SSG), before being stressed to high temperature and humidity conditions. High field experiments at 850 MHz were recorded over a wide temperature range to determine the effect on stability of specific 1H and 13C sites of the molecule.

Indirect Detected DNP-Enhanced 195Pt Solid-State NMR Spectroscopy of Catalytic Surfaces

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Platinum is a metal center at the core of many heterogeneous catalysts that can be probed by 195Pt solid-state NMR spectroscopy, providing detailed structural information on its molecular environment. However, until recently, despite the favorable spectroscopic properties of 195Pt, which has a spin quantum number (I) of 1/2 and a relatively high receptivity, platinum NMR has been rarely performed to study catalytic surfaces, as the large CSA experienced by 195Pt spins combined with the low concentration of sites make this technique extremely insensitive. It has been previously shown that this sensitivity limitation could be overcome with DNP. As an alternative approach, we have recently demonstrated that the 195Pt NMR signature of surface metal centers could be indirectly detected through sensitive spy nuclei such as 1H or 31P.

Here, we introduce a novel DNP Surface Enhanced NMR (DNP SENS) methodology under fast MAS frequencies (~40 kHz) to indirectly detect 195Pt NMR parameters using neighboring 13C spins, with high sensitivity, by taking advantage of the strong 13C-195Pt through-bond J-couplings. We demonstrate this approach on a Pt-N-heterocyclic carbene material, a prototypical example of isolated Pt sites on catalytic surfaces. Double resonance 13C{195Pt} experiments are implemented that allow one to detect the full CSA pattern of the Pt surface sites in a site-specific way. Notably, two distinct Pt sites were revealed, whose atomic-scale structure could be determined by comparing the NMR data obtained with DFT calculations performed on an extensive library of molecular compounds mimicking all possible surface coordination geometries.

We will also show how this NMR methodology can be extended to the detection of other low-gamma nuclei and applied on even more challenging systems in terms of structure and Pt loading.

PO299

H-MAS technology and applications update

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H-MAS technology and applications update
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We have developed a sub-mm spinning units which can provide MAS speeds over 200 kHz and improve resolution of 1H in solids. A special care was taken to design a robust and efficient drive geometry and temperature control.

High speeds are also beneficial for suppression of the first order quadrupolar interactions, if the angle setting is accurate.

13C-1H 2D spectra provide unexpected resolution enhancement due to correlated chemical shift dispersions in disordered systems like cellulose.

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PO300

Solid-state NMR spectroscopy of a pre-fibrillar Ð-Synuclein aggregation intermediate

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Oligomeric α-Synuclein (αS) aggregation intermediates are the primary suspects for initiating aberrant biological responses in neurodegenerative diseases. Their ability to nucleate on lipid membranes and disrupt them has long been proposed to be the mechanism for toxicity. However, structural characteristics responsible for toxicity remain elusive as the low population, and transient nature of oligomers makes them challenging to study. We have isolated and characterized a pre-fibrillar αS intermediate prepared in the presence of negatively charged phospholipids. We show prolonged stability of the intermediate when free monomer is depleted making three-dimensional solid-state NMR measurements possible. De novo backbone assignments were obtained using proton detected NMR spectroscopy. Proton-proton z-mixing experiments revealed the lipid and water exposure profile along the αS sequence. Measurements at ultra-high field (1.2 GHz) dramatically improved spectral dispersion and allowed for distinguishing several long-range contacts obtained from proton-proton RFDR mixing. Amide contacts obtained with MODIST mixing on protons determine the consistency of cross-β sheet hydrogen bonds between the fibril and the intermediate. We identify several regions with side-chain arrangements, solvent exposure and hydrogen bonding which are drastically different from the fibril. These findings are a step towards understanding the atomic scale characteristics of a misfolded nucleus responsible for rapid aggregation of amyloid fibrils.

PO301

Solid-state NMR and DNP methods for pharmaceuticals

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High resolution structural information, ideally of unmodified drugs in their free form, but also in complex dosage formulations are the key to rational drug design and drug development. Especially methods allowing the detection of different polymorphs and the determination of structural changes upon formulation of the drug with various excipients (e.g. in tablets) are highly valuable to ensure safety, efficacy and bioavailability of the final medication.

Solid-state NMR, particularly when combined with DNP is a powerful method to obtain high resolution structural information of APIs in complex mixtures[1] as well as structural information of delivery systems on the nano-meter lengthscale[2]. Different components and APIs in complex mixtures can be unmodified and distinguished based on different isotopes (e.g. 13C or 15N labelling), different chemical environments (chemical shifts), or, based on specific nuclei (e.g. 19F). In particular, we are developing strategies based on recent developments in 19F NMR[3,4], making use of the fact that fluorinated drugs represent a growing percentage of commercial pharmaceuticals, and today about 30% of APIs contain at least one fluorine atom while hardly any excipients do.

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Characterization of Acid Sites on Supported Ni Catalysts

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Dimerization of butenes is a well-known and widely used process to valorize C4 raffinate to C8 hydrocarbons which are blended to gasoline fuels [1]. Different catalysts supported on silica alumina have historically been used with a high amount (20%) of NiO in industrial processes, while a lower amount of nickel (0.6%) supported on amorphous silica-alumina or zeolites as state of the art in research [2] showed similar performance as the commercial ones. The acidity of silica alumina supports crucially affects the activity and selectivity of butene dimerization processes.

In this study, different NMR methods are combined to elucidate the amount, type and strength of surface acid sites, as well as the lability of the surface protons. Eventually, paramagnetic nickel comes into play. Here, some occupy the Brønsted acid sites while nearby sites are important to stabilize the carbenium ions during the reaction.

In order to investigate the properties of the relevant surface acid sites and their accessibility, deuterium exchange of the labile protons together with variable temperature 2H-NMR studies are performed. Furthermore, surface impregnation with Trimethylphosphineoxide (TMPO) followed by 31P-NMR is a reliable method to probe the Brønsted acidity of catalysts [3]. Comparison with previous studies of acid site characterization via IR spectroscopy after pyridine absorption [2] allows for a more detailed investigation of the local environment of the active and acid sites.

By combining both approaches we aim to elucidate the reaction mechanism in detail and shed light on the role of the acid sites.

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PO303

Conformational Dynamics and Active Site Ionization of Protein-Water Network of a Prototypical "Rigid" Drug Target

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The function of bio-macromolecules is directly connected to the solvent dynamics on various timescales. Protein function involves conformational fluctuations, which can be quantified experimentally. The dynamics of protein-water interactions, particularly on the µs–ms timescale, are less well characterized. Here we have used solution and solid-state NMR relaxation dispersion measurements focusing on the active-site amino acid residues in the human carbonic anhydrase II (hCAII). The active site emanates a well-defined water network extending towards the bulk solvent, which is thought to shuttle protons out of the enzyme. Here, by looking at the H-bonded amide moieties of active-site amino acid residues, we investigate the fast microsecond timescale dynamics in the water network of micro-crystalline hCAII [1]. Such fast microsecond dynamics cease in the presence of an inhibitor, as witnessed by solid-state NMR 15N R1ρ Bloch-McConnell-type and NEar-Rotary-resonance Relaxation Dispersion [1]. The fast µs timescale motion in the active-site H-bond network of hCAII matches the timescale of catalytic turnover (104 s-1). The data point to the presence of bound-water dynamics in the 100 µs timescale, indicating coupling between dynamic structural changes in the protein hydration networks, active site loop motions, and enzymatic reaction. We support these mechanistic insights by solution NMR structure and dynamics, as well as MD simulations [2]. Further, to gain insight into the electrostatics of the active site and how such electrostatics affect the charged states and H bonding of amino acid residues is explored. We probe the protonation state of active site asparagine sidechain residues and measure their pKa in solution in the presence and absence of an inhibitor [3].

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PO304

Novel paramagnetic metal polarizing agents for site-specific DNP

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Dynamic nuclear polarization (DNP) has shown tremendous potential to increase sensitivity in numerous MAS NMR applications [1]. Even though in conventional DNP experiments uniform signal enhancements are typically obtained, DNP itself can act as a source of specificity as well [2]. The hyperfine interaction is mediating the initial step of the complex mechanism of the overall DNP transfer. Therefore, the effects of the distance dependence of the dipolar hyperfine interaction between the electron spin (source) and nuclear spin (target) are of excpetional importance. By microwave irradiation, electron-nuclear coherences are generated which finally result in nuclear hyperpolarization. If subsequent spin diffusion is restricted, this transfer dynamic can act as a measure for hyperfine interaction. However, the competing paramagnetically enhanced spin-lattice relaxation counteracts the creation of a polarization gradient, theoretically leading to uniform, distance-independent DNP enhancement. Nevertheless, the direct-DNP build-up rate can act as a direct measure of this interaction and can thus yield distance information in biomolecules [4].

We present an approach to quantitatively investigate the distance dependence of DNP transfer based on paramagnetic metal complex polarizing such as Gd(III) complexes which are decorated with specifically isotope-labeled functional groups as molecular rulers. We show details about the chemical synthesis as well as EPR and NMR spectroscopic characterization. Finally, we give a perspective how rigid lanthanide labels may be used for site-specific DNP of proteins.

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Posters

Solid-State NMR study of NaGaS2 and Na3GaS3

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Solid-State electrolyte development is a major research topic to answer the growing demand on high density energy and secure batteries. Nevertheless, finding a material with high ionic conductivity remains a high technological and scientific challenge. Sulfur electrolytes have a lower Young's modulus than their oxide counterparts and their increased elasticity participates in maintaining solid-solid contact at the interfaces during variations in electrode volume during charge-discharge cycles. In addition, inorganic vitreous materials, and more specifically sulfur-based glasses, have several advantages such as non-flammability, single ion conduction, and an ionic conductivity generally higher than the one of the corresponding crystal.

Among these materials, we are interested in the Ga_2S_3–Na_2S binary family[1] synthesized using ball mill method . In order to expand the vitreous domain and control the glass ceramic composition we analyzed by NMR how two compositions are re-crystallizing.

The $50(Na_2S)$–$50(Ga_2S_3)$ glass crystallizes into $NaGaS_2$ crystal, while the $75(Na_2S)$–$25(Ga_2S_3)$ glass crystallizes into Na_3GaS_3 after annealing.

We present advanced characterization of these crystals using ^{23}Na, ^{71}Ga and ^{33}S 1D and 2D NMR of these two crystals.

^{71}Ga MQMAS spectrum of Na_3GaS_3 required the use of lp-cos 3QMAS[2] on 1.3mm on 18.8T spectrometer to tackle quadrupolar constants in the order of 10 to 15 MHz.

^{33}S spectrum exhibit large quadrupolar constant in addition to low natural abundance and low gyro-magnetic constant requiring to use static QCPMG techniques[3].

Finally, ^{23}Na $NaGaS_2$ spectrum exhibits a significant temperature dependance: VT measurements from 220K up to 350K shows that the two distinct ^{23}Na sites are merging into one, due to Na dynamics.

Ab initio calculations show a good agreement with the experimental NMR parameters.

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Posters

Investigation of the Structure and Dynamics of Amorphous Calcium Carbonate (ACC) by MAS NMR

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Amorphous calcium carbonate (ACC) is an essential precursor during the controlled crystallisation of calcium carbonate (calcite, vaterite or aragonite), which forms the skeletal parts of marine organisms. This biomineralisation process is affected by temperature, pH, and impurities.[1] ACC has water present in approximately 1:1 ratio. Despite numerous attempts, neither a complete structural model nor the mechanism of ACC stabilisation is fully understood.

Magic-angle spinning (MAS) NMR has proven helpful in characterising the structure and dynamics of ACC. The 2D Frequency-switched Lee-Goldburg heteronuclear correlation (FSLG-HETCOR) experiment allows an excellent resolution in the ^{1}H indirect dimension by removing ^{1}H-^{1}H dipolar coupling to a large extent. The 2D FSLG-HETCOR ^{1}H-^{13}C experiment on synthetic ACC (natural abundance) stabilised by polyaspartic acid at 400 MHz and at 10 kHz spinning frequency shows three distinct water/proton environments and the presence of a relatively uniform local structure. This result contradicts structural models for ACC in the literature.[2]

1D NMR spectra previously suggested that the structure of ACC is affected by the pH of the solution from which it is formed. [3] Two distinct ACCs produced in this way were termed proto-vaterite (pv) and proto-calcite (pc), as their spectral properties resembled those of the two crystalline forms of calcium carbonate. FSLG ^{1}H-^{13}C HETCOR spectra of pvACC and pcACC show that both exhibit uniform but distinct local structure. Cross-polarisation (CP) at 2 kHz and 10 kHz MAS, ^{13}C direct direction, and 2D WISE (wideline separation) experiments indicate mobility[4] and its timescales relative to the NMR experiments.

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PO307

DNP-enhancement for deuterium in studies of protein side-chain dynamics

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Deuterons are spin-1 nuclei, which can serve as sensitive probes of protein dynamics, especially at side chain sites in solid samples. The goal of our work is to assess the suitability of DNP-enhanced deuterium polarization for studies of protein dynamics at low temperatures. We present preliminary results using selectively deuterated samples of a 9-residue disordered peptide as well as globular villin headpiece protein (HP36), with selective labels at single methyl sites in powder samples. We compared direct deuterium excitation to the one resulting from 1H to 2H transfer in the presence of DNP enhancement with AmuPol or AsymPolPok radicals. For the HP36 protein we choose selectively deuterated methyl groups located either at the surface of the protein or inside the hydrophobic core. For the disordered peptide, we investigate one leucine and one methionine methyl group. The radicals are introduced in these systems in solution, while the mixture is lyophilized and, in some cases, hydrated for the NMR measurements. We conduct preliminary investigations of the enhancement efficiency for the two radicals and the effect the cross-polarized/DNP-enhanced detection scheme has on the measurement of longitudinal relaxation times in the 100 to 160 K temperature range.

PO308

Supplementing X-Ray Data of Large Proteins with Solid-State NMR: Case Study of an RNA Helicase

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A number of advances both in sample preparation as well as hardware, such as fast magic-angle spinning and high static magnetic fields, have transformed solid-state NMR spectroscopy in recent years into a valuable technique in the field of structural biology. We use the 671 residue acetyltransferase and RNA helicase TmcA from E. coli to explore opportunities to study large proteins. Our focus is on gaining structural and mechanistic insights that are difficult to obtain by other techniques, in particular X-ray crystallography. Firstly, whereas crystallisation of the protein has only been possible in the presence of enzyme cofactors, sedimentation by ultracentrifugation has enabled the recording of well-resolved solid-state NMR spectra in the presence and absence of various ligands. This has allowed investigating the interaction of TmcA with different binding partners such as ATP analogues by means of carbon- and phosphorus-detected experiments. Secondly, replacement of the magnesium ion in the ATPase active site by paramagnetic Mn(II) and Co(II) has enabled to localise its position within the protein, which has not been possible with the electron density from X-ray diffraction. Making use of both paramagnetic relaxation enhancement and pseudo-contact shifts, the ion was placed near the conserved Walker B motif aspartate, which is known to coordinate magnesium in many helicases.

PO309

Measurement of weak scalar couplings using CPMG like experiments

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Hydrogen bonds are crucial for the three-dimensional fold of proteins and nucleic acids. Scalar couplings across hydrogen bonds proof the covalent nature of such bonds. In the past, it has been shown that J-based correlation spectroscopy is able to measure J-coupling up to a few Hertz in the solid-state [1]. An issue in these experiments in the short T2' relaxation time of coherences. In the solid-state, this is mostly due to the anisotropy of the bulk magnetic susceptibility (ABMS). As a consequence, the sensitivity of this experiment is poor. Here we present the comprehensive study to measure ^3hJ_NC using a combination of a high degrees of deuteration, high MAS frequencies and CPMG pulse schemes to improve the sensitivity of the experiment. We discuss potential applications in which the scheme can be beneficially applied to yield through-bond correlations in other hydrogen bonded systems.

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PO310

Structural Investigations of Liquid-to-Solid Phase Transition by Solid-State NMR Spectroscopy

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Liquid-liquid phase separation allows cells to regulate biochemical reactions in highly concentrated protein sub-compartments. These liquid compartments, also known as membraneless organelles, may maturate over time into fibril-like structures, which have been associated with diseases like Alzheimer's and Parkinson's. We here use solid-state NMR to obtain atomic-level insights into the liquid-to-solid phase transition of mature liquid droplets of simple peptide derivatives designed based on the sticker-and-spacer concept. In these peptide derivatives, two times two amino acids (phenylalanines, tryptophanes or leucines) are linked via a disulphide-bridge [1]. Fibrils were obtained by aging of the liquid droplets and characterized by transmission electron microscopy (TEM), X-ray diffraction and solid-state NMR spectroscopy. They show structural characteristics similar to those observed in amyloid fibrils (e.g. similar intra- and intersheet distances) and are rather homogeneous and composed out of several protofilaments.

Structural modeling of the molecules was performed with CYANA based on solid-state NMR distance restraints. The structural model reveals a parallel top-on-top alignment of the four phenylalanine residues along the fibril axis. ϖ-ϖ interactions between the aromatic side-chains and hydrogen bonds between the molecules along the fibril axis emphasize that they are not only relevant drivers of liquid-liquid phase separation, but they also play an important role in the liquid-to-solid transition.

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Posters

PO311

Disorder in Cesium Lead Halide Nanocrystals

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Cesium lead halide $CsPbX_3$ (X = Cl, Br, I) perovskite semiconductor nanocrystals (NCs) increasingly mesmerize researchers because they exhibit outstanding optoelectronic properties. One of the remaining hurdles is an atomistic structure determination due to their soft structure and their small size. We addressed this challenge by using nuclear magnetic resonance (NMR) spectroscopy. This accessible technique can not only give insights into the elemental composition, but also into structural properties without having to manipulate or even damaging the labile samples during the measurements. The NC cores of colloidal solutions of perovskite semiconductor NCs could be studied in depth with solution NMR for the first time. The determination of surface and core sites could be done by probing various size selected NCs with an edge length between 3 and 12 nm. In doing so, a correlation between the chemical shift and the band gap of the NCs was observed, which was already predicted by theory. Furthermore, the distortions of the core structure induced by the dissimilar monodentate and zwitterionic capping ligands was studied, quantified, and could be correlated with their optical properties. Ab initio molecular dynamics simulations performed on various sizes of $CsPbBr_3$ NCs confirmed our observations. In mixed halide $CsPb(Br/Cl)_3$ NCs, an anion gradient was observed for the first time. These findings showcase the great potential of solution NMR in shedding light on the structure of colloidal semiconductor NCs.

PO313

Monolayer-protected gold nanoparticles as tailorable receptors for the NMR chemosensing of neuroblastoma biomarkers

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The majority of sensing methodologies for the detection of target compounds in complex mixtures exploits the feedback of a sensor to indirectly detect the analytes of interest. The response is then processed using standards, if available, or ensured by the robust selectivity of the sensor itself. On the other hand, NMR chemosensing aims to obtain signals directly from the analytes, in the form of an NMR spectrum, to unequivocally identified the target molecules. This is done by sensing protocols based on diffusion-ordered spectroscopy (DOSY) and magnetization transfer sequences (STD and water-assisted STD). In this context, "nanoparticle-assisted NMR chemosensing" combines these techniques with the recognition abilities of monolayer-protected gold nanoparticles (AuNPs) to push the detection of relevant diagnostic analytes down to the micromolar concentration range. To this aim, an high-power water-assisted STD experiment has been proposed by our group, capable to detect analytes at 10 µM concentration using standard instrumentation. From an NMR point of view, the reduced molecular motion of bulky nanoparticles, affecting also water of its solvation shell, offers a way to transfer magnetization to the interacting analytes and to promote efficient spin diffusion, useful in saturation transfer experiments. In addition to that, AuNP can be easily capped with a variety of thiols to provide tailored binding sites in term of both selectivity and strength, for virtually any class of substrates. In this sense, is crucial to rationalize the different ligand-to-receptor recognition in terms of nanoparticles size, ligand design (i.e., chain length, nature of the thiol hydrophilic groups) and the nature of chemical interactions involved. In this communication our latest results will be reported, aimed at deeply investigate the use of AuNP (such as peptide-coated AuNP) for the detection of neuroblastoma urinary biomarkers at low micromolar concentration.

No more nosey NOE – Fitting of 1H R1ρ in the presence of dipolar relaxation

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Proton R1ρ relaxation dispersion experiments allow the characterization of biomacromolecules undergoing fast conformational exchange (kex > 2 kHz) between a highly populated ground state (GS) and higher energy excited states (ES), which are invisible to conventional structure elucidation methods.[1] However, even in systems with low proton density such as nucleic acids, cross-relaxation due to dipolar couplings can hamper the analysis of exchange parameters. Here, we present a systematic study of how the nuclear Overhauser effect (NOE) contributes to 1H R1ρ in nucleic acid model systems. Imino protons can display both multi-state exchange between different conformers and have NOE contacts, which can be misinterpreted as an additional ES.[2] While the presence of an NOE contribution to R1ρ relaxation dispersion can be easily ascertained using high-resolution NOESY experiments, an appropriate theoretical model for extracting accurate conformational exchange parameters under such circumstances is missing. The simple inclusion of transversal and longitudinal auto- and cross-relaxation rates for a dipolar-coupled proton spin pair into the Bloch–McConnell matrix fails to reproduce experimental data. In this study, a correction factor to the spectral density function is identified that resolves this discrepancy. This correction factor is analogous to the correlation time of the internal motion in the Lipari–Szabo model free spectral density[3] but represents an abstract correlation due to exchange. The corrected numerical model allows reliable fitting of NOE-contaminated 1H R1ρ data and will greatly facilitate the investigation of ES structure parameters in biomacromolecules.

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Posters

Activation of the V2 vasopressin GPCR by combined use of cryoEM, MD and NMR

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Vasopressin receptors are members of the G protein-coupled receptor (GPCR) superfamily. and control water homeostasis. In particular, the V2 type is expressed in renal cells, where it governs water reabsorption. V2R has long resisted to crystallization trials and cryoEM characterization, because of intrinsic flexibility. We had previously used NMR to characterize its different structural compartments [1-3)]. In this work, we analyse the conformational landscape of the V2R in distinct pharmacological conditions using liquid-state NMR spectroscopy by monitoring signals from 13C-methyl-labelled lysines. In particular, we investigate the structure and dynamics changes upon binding to different ligands ranging from agonist to antagonists, non-selective or selective (biased) towards the G-protein signalization pathway. Our results outline common features as well as distinct particularities with other GPCRs. We also used specific NMR restraints, ranging from STD (Saturation Transfer Difference) to paramagnetic ones, to decipher the pose of the endogenous vasopressin ligand onto the low-resolution structures of V2 obtained by cryoEM [4], but also to determine the docking pose of a biased ligand in molecular dynamic simulations, revealing the mechanistic details of V2R biased activation [5]. We will discuss the generalization potential of our approach to other receptors and how V2R biased activation mechanism elicited in this study compares to that of the mu receptor to opioid which we recently studied [6].

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PO316

Quantitative band-selective pure shift NMR

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NMR spectroscopy is often described as a quantitative analytical technique. Typically, with some notable exceptions, this is only the case for a simple pulse-acquire experiment.(1) Even then, the small chemical shift dispersion and prominent signal multiplicity in ^{1}H NMR often results in signal overlap which complicates quantitative analysis. Although pure shift NMR techniques simplify spectra by removing signal multiplicity, reducing all signals to singlets, they are susceptible to a variety of sources of site-dependent signal loss. These include spin relaxation during delays, diffusional and convectional attenuation when applying pulsed field gradients (PFGs), losses due to the non-uniformity of radiofrequency and field gradient pulses, and scalar coupling dependent signal losses (e.g. as a result of "chunked" data acquisition).

The EXQUISITE (EXtrapolating QUantitative Integrals by Successive ITEration) method seeks to correct for signal loss from such sources, utilising the principle of repeating pulse sequence elements originally proposed in the $HSQC_0$ experiment.(2) If a given pulse sequence element attenuates a given signal integral by a factor f, it follows that repeating the element n times before detection results in a signal attenuated by a factor of f to the power n if the elements act independently. The integrals from successive iterations, where every element except the initial excitation pulse and any evolution periods is repeated, may then be used to extrapolate the integral to zero attenuation.

Here we apply the EXQUISTE method to the band-selective pure shift experiment in both interferogram and semi-real-time acquisition modes.(3,4) Relative signal integrals within 1 % of those obtained from an equivalent pulse-acquire spectrum have been obtained for a model system with a three-iteration extrapolation.

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PO317

Study of supramolecular drug delivery assemblies in β-cyclodextrin using singlet states

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Supramolecular drug delivery is an emerging field of medicinal chemistry. Its prominent features include target-specific and water-insoluble drug delivery. A supramolecule host that interacts with drugs, β-cyclodextrin, is reviewed here. The impressive half-coned structure of β-cyclodextrin, hydrophobic inside and hydrophilic outside, allows some parts of the drugs to interact and get inside it. NOESY, ROESY, and relaxation properties were used to extract structural information for such drug binding compounds.[1]

Here, a unique relaxation property of singlet states is used. Immunity toward the dipole-dipole relaxation makes the relaxation lifetime of singlet states quite long. Also, it can be used to extract structural information out of complex systems.[2][3][4] We demonstrate the use of singlet states to find structural details of specific host-guest complexes involving β-cyclodextrin.

Indomethacin (a) and CI-933 (precursor 4-methoxy benzamide(b)), and Paracetamol (c) are the chosen drugs. Singlet states are created in AA'XX' type spin-system present in these compounds, which interacts with the β-cyclodextrin ring. A change in singlet state relaxation lifetime is observed as the guest concentration increases. Computational studies (Docking, DFT, and MD) validate the results obtained. The distance between chosen protons in the host and guest is measured, and the theoretical value of singlet relaxation lifetime is calculated.

The change in relaxation lifetime of singlet states is more significant than that of chemical shift or even longitudinal relaxation time (T1), making it a better tool to examine the structural changes when the host-guest complexation occurs.

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PO318

Antisymmetric cross-relaxation in cis-difluorodichloroethene

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An antisymmetric part of the nuclear magnetic shielding tensor (anti-CSA) causes an additional but overlooked mechanism of cross-relaxation. This new effect was investigated in the 19F-19F-13C strongly-coupled three-spin system in cis-difluorodichloroethene. It follows from the conducted quantum chemical computations, i.e., including rovibrational, solvent, and relativistic effects, that the favorable pair of nuclei for observation of anti-CSA cross-relaxation is 19F-19F (the antisymmetry about 30 ppm). The results of the computations are strongly supported by the high reproducibility of tiny NMR experimental spectral features, e.g., shifts of 19F due to the 35/37Cl isotope substitution. Moreover, the choice of cis-difluorodichloroethene is advantageous since the highest rate of the anti-CSA cross-relaxation is predicted for planar molecules. The presence of carbon-13 permits distinguishing fluorine nuclei, i.e., 1J(19F, 13C) = 300 Hz and 2J(19F, 13C) = 35.8 Hz; however, it also causes that the spin dynamics of cis-difluorodichloroethene is not straightforward for analysis since at 14.1 T magnetic field the 19F isotope shift due to 13C is merely 40 Hz. Therefore, the generation of clean zero/double coherences that are required for observation of anti-CSA cross-relaxation in INADEQUATE-like experiments becomes challenging and requires the aid of advanced modeling tools of quantum dynamics such as Spinach used in this work. The effect of anti-CSA on cross-relaxation rates is also pronounced in the results of extensive computational DFT studies of alanine tripeptide (Ala-Ala-Ala) that show the Ramachandran angles derived from cross-correlation rates are noticeably affected by neglection of anti-CSA, which impacts the dihedral angles of the peptide backbone and may influence the determination of the overall three-dimensional structure of selected biomolecules.

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Paramagnetic Guest Exchange Saturation Transfer (ParaGEST) Revealing Hidden Kinetic Features in Supramolecular Host-Guest Systems

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Three-dimensional supramolecular assemblies are characterized by dynamic processes, providing them with unique properties for fundamental research and applicative science. However, studies of such dynamic processes, particularly in host-guest systems, are still rare and limited for specific (and low) dynamic regimes. Contrary to thermodynamic parameters, kinetic properties are poorly described due to the low availability of suitable analytical tools. Moreover, the available tools, mainly fluorescence-based, are limited and fail to detect and characterize systems with relatively fast kinetic features. Thus, accessible and robust approaches, which can be extensively used for the quantitative kinetic evaluation of supramolecular systems and extending their applications in new fields, are greatly needed.

Inspired by the paraCEST approach, where paramagnetic lanthanides (Ln's) induce substantial chemical shifts in nearby atoms, we have expanded our previously established [19]Fluorine guest exchange saturation transfer (^{19}F-GEST) technique, exploited to study slow-to-intermediate exchanging host-guest systems, into paramagnetic ^{19}F-GEST (^{19}F-paraGEST). This method was used to investigate host-guest systems exhibiting intermediate-to-fast exchange rates in the NMR time scale (well above the currently applicable limit), by pseudo-contact shifting (PCS) the resonance frequency of the bound guest, depending on the Ln ion chosen.

Specifically, we modified different cyclodextrins (CDs) macrocycles, characterized by different cavity sizes, with Ln-chelating cradles and Ln chlorides to create isostructural hosts and studied their ^{19}F-paraGEST behavior utilizing the same ^{19}F-guests. Benefiting from the PCS effect of paramagnetic Ln's, we were able to increase ^{19}F-GEST resolution and, therefore, observe different kinetic features for ^{19}F-molecules that are otherwise not detectable with ^{19}F-NMR. These observations imply different cyclodextrins-binding geometries for a given guest, unique for each type of CD.

This work highlights the importance of the ^{19}F-paraGEST approach not only as a tool for studying "NMR-invisible" host-guest systems but also for facilitating the development of future non-fluorescent sensing and imaging tools incorporating cyclodextrins host-guest systems.

Combining Variable Temperature and Field: a new approach to understanding dynamic exchange

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Variable temperature NMR (VTNMR) has long been an established method in small-molecule NMR to study the rate of exchange between nuclei exchanging conformationally or chemically. Despite the ability to quantify rates and activation barriers, several drawbacks limit the use of this technique across many fields. For example, it cannot easily be applied to the study of conformations of biological molecules with a relatively narrow window of temperature activity or stability. In contrast to VTNMR, chemical shift scaling (CSS) allows one to reach a coalescence point, and therefore extract a rate, by only changing the field strength.[1] In addition, application of CSS in concert with VTNMR enables several coalescence points (at different fields and temperatures) to be observed. This can then lead to Eyring plots with smaller errors and thereby more accurate estimations of enthalpies and entropies of activation, $\Delta H\ddagger$ and $\Delta S\ddagger$.

Using N,N-dibenzylnaphthamide, we have demonstrated reliable shift scaling to 2% of the original frequency showing a coalescence point at 25 °C and 100 MHz (compared to VTNMR alone at 50 °C and 500 MHz). By varying both the field and temperature as mentioned above, we have obtained several coalescence points and been able to extract activation energies matching that of the literature.[2] Current work is underway to apply CSS to more complex systems, such as biological molecules or dynamic foldamers, extending the method to other nuclei and improving Eyring-Polanyi plots.

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2D Rheo-NMR of PBLG - impact on RDCs and signal-to-noise

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Since 1963, when Saupe and Englert[1] reported the first spectrum in a nematic liquid-crystalline phase, many liquid crystals were investigated. They can partially align organic molecules, which leads to structural information through residual dipolar couplings (RDCs)[2]. One of the commonly used phases is based on Poly-γ-benzyl-L-glutamate (PBLG) dissolved in CDCl3[3].Recently, we were able to change the director of such liquid crystal alignment by applying shear force inside the sample. With a specifically designed rotatory device, we can switch between different states of alignment between scans and even within a single scan. The method is described and the first examples of 2D Rheo-NMR are presented.

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PO322

Relaxation dispersion on the night-jet: Speeding up to study RNA and DNA dynamics

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The dynamics of nucleic acids play a crucial role for the understanding of their biological function. Relaxation dispersion methods such as CPMG and R1rho enable us to analyze such dynamics. In R1ρ experiments, a spinlock is applied on- and off-resonant, which provides access to dynamic parameters, such as the exchange rate kex and the population of higher energy states, as well as chemical shift information of these low populated excited states. Several experiments for the common nuclei 1H, 13C and 15N have been published. However, multiple off-resonance data sets of different nuclei are required to explain complex dynamics like RNA base-flip or secondary structure rearrangement. This results in a long experimental time of days, which is rate limiting for unstable systems. Here, we present a suite of new fast R1ρ experiments to study dynamics on RNA and DNA, allowing us the acquisition of relaxation dispersion data for the nuclei 13C, 15N as well as 1H in a reduced amount of time by taking advantage of the relaxation enhancement effects of selective excitation. The overall gain on the signal acquisition (reduction of time and gain in signal/noise ratio) for 1H measurements is observed to be around 20x and is similar to 13C/15N nuclei. These fast R1rho experiments can deliver comparable and reliable data (within the estimate of error) on both RNA and DNA. Therefore, fast R1rho experiments can be useful to study crucial dynamics on challenging, unstable systems especially for 1H, which additionally does not require expensive isotope enrichment of the sample.

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Nuclear Spin Singlet Relaxation Mechanisms by NMR Spectroscopy and Molecular Dynamics

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Nuclear spins states have been shown to exceed spin-lattice relaxation times several fold, with impressive demonstrations of singlet lifetimes of more than an hour in organic molecules in solution. Over the years, several relaxation mechanisms have been identified, including dipolar coupling, chemical shift anisotropy, paramagnetic relaxation, spin rotation and spin-internal motion, and the scalar relaxation of the second kind. While in principle, many of the mechanisms are well understood, estimating their size can be difficult. Furthermore, multiple experimental examples have been found that decidedly defy expectations.

We present here work on directly estimating singlet relaxation mechanisms from molecular dynamics simulations. Here we show calculations for intermolecular mechanisms and find good agreement with experiment. It is particularly surprising to see that such mechanisms as intermolecular coupling to 35Cl and 37Cl nuclear spins (of the chloroform solvent) could be rate limiting for singlet states. In addition, we also show work on 31P spin singlets, and compare their lifetimes to those from molecular dynamics trajectories and ab initio calculations of chemical shift anisotropy tensors, which show good agreement.

Calculations of this sort may help in the design of particularly long-lived singlet states, or could be used to identify new probes for dynamics.

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Low power optimal control pulses improve multidimensional bio-molecular NMR experiments at ultrahigh-field (1.2 GHz) spectrometers

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The recent advances in NMR magnet technology have actualized the availability of ultra-high field 1.2 GHz (28.2 T) NMR spectrometers, thus broadening the horizons of bio-molecular NMR studies simply due to the increased resolution and the gain in sensitivity. Unfortunately, the full potential of the above ultrahigh field magnets cannot be exploited at present due to the high power requirement necessary for broadband excitations at 1.2 GHz magnets. These high power requirements are already above the maximum power tolerance possible with the available CryoProbe technology. Hence, at present limiting the maximum volume that one can measure at the available 1.2 GHz magnets to 150 uL i.e. to a 3 mm NMR tube.

Here we design and develop low power optimal control pulses-based bio-molecular NMR experiments to enable large sample volume measurements on biological systems, especially for concentration-limited samples at ultra-high field magnets. We introduce a novel category of low power, B1 inhomogeneity compensated optimal control based band selective pulses for triple resonance experiments. These pulses along with B1 inhomogeneity compensated 1H and 15N optimal control (OC) universal rotation pulses were then used to construct 2D-[1H, 15N]-OC-HSQC and 3D-OC-HNCO NMR experiments. Finally, measurements done at our new 1.2 GHz spectrometer with a 3 mm CryoProbe using the optimal control sequences on a test Ubiquitin protein sample showed an improvement in sensitivity up to 20% for the triple resonance experiment. The optimal control based sequences were also tolerant to B1 inhomogeneity. We expect to see a higher improvement with these sequences on a 5 mm CryoProbe, thus enabling the flexibility to measure on large sample volumes without compromising on the performance.

PO325

Methodological advances for multi-site exchange in Cadherin-11

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Our recent work on Cadherin-11 has revealed that dimerization of Cadherin-11, and probably more generally of type II Cadherins, involves coupled unfolding and strand-swapping [1]. A comprehensive analysis of Cadherin-11-EC1 based on various relaxation dispersion (RD) experiments has delivered a quantitative description of a three-site kinetic connectivity. The three involved conformations, varying in the degree of A-strand exposure, also are coupled to additional conformational states on very fast and very slow timescales.

The very fast exchange process arises from interconversion between ordered and random coil conformations of the BC loop in proximity to the Trp2 binding pocket, with relative populations that depend on the extent of A-strand exposure and dimerization status. The very slow exchange processes link the folded partially and fully strand-exposed conformations with partially unfolded conformational states, corresponding to the states revealed by mutant and high-pressure analysis.

The RD data analysis has applied some of our previous theoretical findings [2-4]. With new tools and new experimental examples, multi-site exchange becomes more accessible due to the improved ability to select appropriate experimental conditions and process the data in the best manner. Herein, we present experimental advances to characterize chemical exchange at the very fast and very slow end of the chemical exchange spectrum. Herein, we discuss our methodical progress on the analysis of multi-site exchange by NMR, mostly in the context of research on Cadherin-11. As an example, we have now explored the scope of a simple pulse sequence which allows us to efficiently record exchange-suppressed Hahn-Echo relaxation, characterizing the previously identified very fast exchange process in more detail by recording Rex at different concentrations and temperatures.

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Intrinsically disordered proteins interacting with membranes: The vesicular SNARE protein Synaptobrevin-2

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Intrinsically disordered proteins (IDPs) are highly dynamic entities making them difficult to study by X-ray crystallography or cryo-EM. However, IDPs are well suited to be studied by NMR spectroscopy. Using solution NMR in the absence and presence of liposomes we have observed a high degree of internal dynamics and IDP-like character of the vesicular SNARE protein Synaptobrevin-2, which plays a key role during neuronal exocytosis, a pre-requisite for neurotransmitter release. Synaptobrevin-2 resides on the vesicular membrane, with its transmembrane region anchored in the vesicular membrane. While the entire protein is highly dynamic, solution NMR relaxation measurements revealed a gradual increase of rigidity and increase in α-helical secondary structure propensity from the N- to the C-terminus, present already in unbound Synpatobrevin-2 in solution (with the TMR being truncated) [1]. This gradual increase in rigidity correlates with an increase of lipid binding affinity in the presence of liposomes, suggesting a conformational selection process upon membrane binding.

While the lipid-bound conformation is invisible to solution NMR because of the size of the liposomes, we observe NMR correlations when performing solid-state NMR experiments (unpublished). Therefore, we follow a combined solution-NMR and solid-state NMR approach to obtain structural and dynamic insights in both the unbound and lipid-bound conformation of Synaptobrevin-2.

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The Ups and Downs of Molecular Interactions by High-Resolution Relaxometry

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NMR relaxation rates are strongly influenced by molecular motions and hence report on both the overall rotational correlation time of the molecule and internal molecular dynamics experienced by the nucleus of interest. We want to obtain optimal atomic resolution information on the dynamics of a molecular system, over a large range of timescales. This requires to fulfill two opposite conditions: on the one hand, the spectral resolution requires high magnetic fields and, on the other hand, relaxation should be measured on a broad range of magnetic fields, down to fields in the milliTesla range, to resolve motions on nanosecond timescales. Here we use high-resolution relaxometry (HRR), a technique that offers the spectral resolution of a high-field magnet and simultaneously samples multiple magnetic fields by moving a sample shuttle out of the magnet for the relaxation delay. We use a new prototype of sample shuttle with enhanced sensitivity and show preliminary results for protein-ligand interactions and protein dynamics.

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Probing the coupled dynamics between lipids and membrane proteins by high-pressure NMR spectroscopy

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Cell membranes represent a complex and variable environment in time and space of lipids and proteins. Their physico-chemical properties are determined by lipid components which can in turn influence the biological function of membranes. Here, we used hydrostatic pressure to study the close dynamic relationships between lipids and membrane proteins in nanodiscs. We demonstrate that nanodisc can reversibly accomodate high pressure, in absence or in presence of embedded membrane protein. We further show that the fluid-gel phase transition triggered by temperature or pressure can be accurately monitored by 1H NMR. Experiments on the beta-barrel OmpX and the alpha-helical BLT2 G Protein-Coupled Receptor in nanodiscs of different lipid compositions reveal conformational landscapes intimately linked to pressure and lipids. Pressure can modify the conformational landscape of the membrane protein per se, but also increases the gelation of lipids, both being monitored simultaneously at high atomic resolution by NMR. Our study also clearly shows that a membrane protein can modulate, at least locally, the fluidity of the bilayer and that high pressure can lead to protein dynamics modulation as revealed by the dramatic x4-5 NMR signal increase in methyl 2D 13C SOFAST-HMQC spectra of the BLT2 GPCR. The strategy proposed herein opens new perspectives to scrutinize the dynamic interplay between membrane proteins and their surrounding lipids.

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Symmetry Theory of Long-Lived States

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The symmetry theory of long-lived states is sketched, including the concepts of: (i) Schur-Weyl duality; (ii) the Commutant; (iii) the Bicommutant; (iv) Spin-Dynamical Lie Algebra. It will be demonstrated how these concepts, which have been implemented in SpinDynamica software, may be used to analyze and predict the long-lived states in any spin system.

Posters

Relaxational signal attenuation during selective refocusing pulses

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In modern NMR spectroscopy, selective pulses are widely used to restrict signal excitation to specified frequency ranges. However, due to the relatively long durations of such pulses, relaxation can affect quantitation. As part of a wider project to investigate quantitation in multiple pulse NMR methods, signal loss during on-resonance selective 180° refocusing pulses of different shapes has been examined.

The practical problem is the lack of analytical results for relaxation during shaped pulses. The full analytical solutions of the Bloch equations for constant radiofrequency irradiation1 reduce on-resonance to single exponential decay with rate constant $(R_1 + R_2)/2$ if the RF amplitude is large compared to $R_1 - R_2$. A heuristic approach to describing relaxation during shaped 180° pulses is thus to use an exponential decay constant which is a pulse shape-dependent linear combination $\alpha R_1 + (1 - \alpha)R_2$.

Selective spin echo experiments on a range of doped water samples, and corresponding numerical simulations using the experimental T_1 and T_2 values, have been used to test this approach for Gaussian,2 rSNOB,3 and REBURP4 shaped pulses. They yield a single parameter α for each shape that satisfactorily describes the relaxational attenuation produced by on-resonance shaped 180° refocusing pulses, and can be used to correct signal loss due to relaxation during selective pulses in experiments such as pure shift NMR.

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PO332

Reaction monitoring with fast and flow-compatible diffusion NMR

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NMR spectroscopy is a ubiquitous tool for the monitoring of organic chemical reactions [1]. Online monitoring by flow NMR makes it possible to study reactions in benchwork-like conditions, thus providing access to the reaction kinetics of interest. Several "flow tubes" have been described [2][3], which allow to loop a small part of the medium to the spectrometer and back to the flask, in a continuous fashion. However, NMR experiments are all affected to some extent by sample flow. This is particularly the case for diffusion-ordered NMR spectroscopy (DOSY). DOSY can provide virtual separation of a mixture's components, and is particularly useful for reaction monitoring. [4][5]

Here we describe the design and implementation of fast DOSY experiments that provide accurate diffusion coefficients and efficient compound separation in flow conditions. First, we show which choice of diffusion-encoding methods provides accurate diffusion coefficients for a sample flowing at up to 3 mL/min, using a commercial flow tube. We then describe a coherence-selection strategy that makes it possible to acquire data with a single scan per gradient increment. We finally illustrate the method by monitoring an organic chemical reactions in flow conditions. DOSY flow NMR will be useful for applications such as the identification of reaction intermediates and progress monitoring of polymerisation reactions.

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Describing transfer RNA dynamics using NMR relaxation

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Transfer RNAs (tRNAs) are essential components of the translation process of genetic information into proteins. tRNAs carry both the anticodon necessary to decode the genetic code and the amino acids that will be added to the nascent peptide chain, making them critical for protein synthesis in the ribosome. To achieve their function, tRNAs undergo a complex maturation process, where several nucleotides are chemically modified[1,2]. Indeed, over 120 post-transcriptional chemical modifications have been observed in tRNAs. These modifications appear in the tRNA core structure and in the anticodon loop region, modulating the decoding during protein synthesis, the folding and stability of tRNA. However, the understanding at atomic level of the impact of those modifications remains elusive.

NMR spectroscopy is a unique tool to probe those atomic-level chemical modifications. In particular, modulation of tRNA dynamic due to post-transcriptional modification can be probed using NMR 15N spin relaxation[3]. Here, longitudinal (R1) and transverse (R2) relaxation rates, along with heteronuclear NOE were measured at 3 different magnetic fields. We are currently analysing this multiple-field dataset using the Lipari-Szabo "model-free" formalism[4,5], describing internal motions on a pico- to nanosecond timescale. This study aims at both characterising tRNA dynamics at atomic level and suggesting optimal parameterisation for model-free analysis on RNA.

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PO334

Observing the permeation of different drugs through an artificial membrane inside an NMR tube

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The conventional way of investigating the passive transport of drug molecules through a membrane is within a diffusion cell.[1] A defined volume from the acceptor chamber is replaced by fresh solution at defined time intervals and the amount of drug is determined via HPLC or UV/Vis spectroscopy.[2] The resulting concentrations can then be used to calculate the amount of drug permeated per surface area over time, which is subsequently referred to as flux.[3] This process either takes very sophisticated hardware or requires much more time to perform manually. Here, we present a method to automate this process by using a 9.4 T NMR spectrometer.

It is traditionally advised to have a homogenous solution inside the NMR tube rather than two different environments given the challenge for the gradient shim algorithms. Even though, here we explore the idea of building an artificial membrane inside an NMR tube and observing the time depended permeation of four different drugs. This is achieved either by slice-selective excitation of the two different compartments or by pulling the inner tube right over the active coil volume and measuring under quantitative conditions. Afterwards, the amount of drug present is determined and complementarily verified via HPLC. The two approaches are compared, including discussion of setup specific problems and workarounds, as well as showcasing that the setup presented here can be an easily accessible tool to complement existing measurement setups for future investigations.

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Can the temperature coefficients support spectral assignment?

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Assignment of NMR spectra is needed to solve various problems e.g. to identify compounds in metabolomic mixtures or to confirm the structures of new compounds after chemical synthesis. 1D and 2D NMR provide information about chemical shifts and correlations. However, some ambiguities in assignment process may be still present. Thus, having more nuclei-specific spectral parameters would be useful.

In protein studies, researchers often measure temperature coefficients (TCs), i.e. the rates of change of chemical shifts with temperature. They indicate the exposure of amide protons to solvent exchange, presence of low-populated excited states and other phenomena. We show, that TCs can be used in the analysis of small molecules, such as metabolites [1], and to support the spectral assignment of groups of similar compounds.

Therefore, we propose simple 1H variable-temperature (VT) measurements whose time can be the same or even shorter than the those of the conventional 2D spectra. We present two approaches: 1) TCs for metabolites identification in natural mixture (plasma) based on serum's TCs and 2) TCs from one assigned molecule transferred to another, very similar molecule. We show, that TCs are consistent and reproducible between samples. For metabolomics studies, combining with Radon transform processing [2] provided extra increase in signal-to-noise ratio. We believe that in future, it might be one of the trustworthy stages of analysis.

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Exchange NMR Spectroscopic Studies on 8-amino-BODIPY Dyes

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A detailed NMR spectroscopic investigation into the rotational barrier of the conjugated C-N bond (ΔG‡, ΔH‡, ΔS‡) for various BODIPY molecules in different solvents using exchange techniques will be presented. Substituents that induce steric hindrance over the BODIPY core destabilize the hemicyanine structure and have a much more pronounced influence when compared to solvent effects on the rate of rotation. In the slow-intermediate exchange regime, selective EXSY was used while in the intermediate exchange regime, relaxation dispersion measurements were applied to probe chemical exchange rates. Very good correlation between rate constants obtained using 1D EXSY and those from dispersion was achieved as evidenced by high-linearity Eyring relationships. Relaxation dispersion measurements are commonly employed for studying dynamic phenomena in biomacromolecules but they are not often used for small molecules applications. This work is expected not only to shine light on the effects that govern the rotation around the C-N bond but clearly demonstrates that the $T1\rho$ relaxation dispersion experiment can be easily applied when measuring chemical exchange in small molecules.

HCP transfers for relaxation dispersion measurements: considerations and improvements for measuring RNA dynamics

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Biomolecules exist in an ensemble of conformations and we are studying higher energy states, excited states, interchanging on a micro-to-millisecond time-scale with the most populated conformational state, the ground state, through base-pair rearrangements. Excited states can be elucidated using R1ρ relaxation dispersion (RD) NMR(1).

The implementation of R1ρ relaxation dispersion in a 1D fashion is timesaving and made possible thanks to the selectivity of the heteronuclear cross-polarisation (HCP) transfer steps(2).

The optimum HCP transfer parameters are generally: a radiofrequency amplitude of ~90-100 Hz(3-4) and a pulse duration of 1/J(XH) which allow to suppress unwanted signals outside a ±100 Hz window of the peak of interest. The challenge when combining the HCP transfer step with RD measurements is that even small contributions of an unwanted signal after the two HCP transfer steps can lead to large relative contributions, hence affecting the accuracy of the fitted decay rate.

We show that spin systems found in oligonucleotides with large heteronuclear coupling constants (>200Hz) constitute a challenge to find HCP parameters which allow an efficient, selective transfer for 13Cs which are less than 200 Hz apart. We propose multiple ways to optimize the HCP transfers; scripts to simulate the transfer efficiency for a specific J-coupled 13C and 1H pair to optimize transfer parameters, the "pollution peak approach" to experimentally optimize the HCP parameters and the 2D HCP pulse sequence to visualize the main peak and polluting peaks in a 2D spectrum.

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Structure determination of high-energy states in a dynamic protein ensemble

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Biomacromolecules often require to change their conformation into high-energy states in order to perform their function. Since these functionally essential high-energy states are lowly populated, it is not trivial to reveal their structure and there is a lack of methods to determine them. In this work, a method for high-resolution structure determination of minorly populated states is presented, which combines pseudocontact shifts (PCSs) with Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion (PCS-CPMG). Beside the high-energy state structure determination, this approach allows to characterize the entire free-energy landscape including the corresponding kinetics and thermodynamics while the protein performs its function. Application of this methodology to the metal binding protein adenylate kinase (Adk) during catalysis provided detailed insight into the conformations relevant for catalysis. As a demonstration of the viability of the new method also for non-metalloproteins we conjugated ubiquitin and the chaperone trigger factor with lanthanide chelating tags (LCTs). The two LCTs DOTA-M8-(4R4S)SSPy and DOTA-M7PyThiazole showed different interference of the tag mobility with the protein motion and in both cases DOTA-M8-(4R4S)SSPy delivered superior paramagnetic dispersion profiles. Investigation of the chaperone trigger factor using DOTA-M8-(4R4S)SSPy conjugated with the substrate-binding domain (SBD) revealed an enhanced paramagnetic dispersion profile in the peptidyl-prolyl isomerase domain (PPD), indicating conformational change between PPD and SBD.

The PCS-CPMG method presented here excels not only in the determination of solution high-energy protein conformations in cases involving domain rearrangements of systems smaller than 60 kDa and populations as low as 0.5%, but also in the determination of the kinetics and thermodynamics of the protein performing its function.[1]

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PO339

Building bridges between Lindblad and Redfield master equations

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The Redfield master equation has been the predominant approach to treat relaxation in NMR for quite a long time[1]. However, this theory may fail if the quantum system is outside the weak-order limit or high-temperature approximation, which was recently illustrated in the case of long-lived states, and a Lindblad theory of dissipative Markovian quantum systems may be used to overcome these difficulties[2]. In particular, it predicts the correct rate of magnetization build-up when the two-spin system is initially in a pure singlet state.

It is a striking fact that the Lindblad approach yields a different form of the relaxation superoperator (the Lindblad dissipator instead of the conventional double commutator), even in the high-temperature approximation. In this work, we showed[3] that the Lindblad master equation also arises from the conventional quantum mechanical theory of NMR relaxation. We discuss the origin of the Kubo relations of the spectral density functions in the relaxation theory and the relations between "classical", "semi-classical" and quantum versions of spectral density functions involved in relaxation. Finally, the contributions of the various terms in the relaxation equations are investigated in a numerical example for the high-temperature approximation.

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Posters

Methodological Advances for the Characterisation of Human GPCRs by NMR Spectroscopy

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G protein-coupled receptors (GPCR) are a pharmacologically important class of transmembrane proteins, responsible for sensing a large set of diverse stimuli in the environment of cells. Structures obtained from X-ray crystallography and cryo-EM have previously improved our understanding of GPCR functioning, however, the behaviour of GPCRs is governed by their highly dynamic nature, which cannot be captured by these static images. NMR spectroscopy can be used to study the dynamic aspects of the receptor, yet most standard NMR techniques cannot be directly applied to GPCRs due to their large size and the necessity to use higher expression hosts.

Here, we present new methods for NMR studies of GPCRs. We show approaches for methyl-[$^{13}CH_3$] labelling of Met, Ile, Leu, Val and Ala, as well as an economic U-[^{15}N] labelling approach in mammalian cell culture to obtain labelled receptor preparations suitable for NMR spectroscopical analysis. The resulting fully protonated and detergent-solubilised high-molecular weight samples (100—150 kDa) are studied with the novel XL-ALSOFAST-HMQC with delayed decoupling. This much improved experiment allows to reduce the necessary measurement time by a factor of about 9 compared to previous state-of-the-art experiments, paving the way for studies of unstable complexes and titration series. The obtained spectra have been assigned by an original strategy exploiting the pseudocontact shifts that are observed on the receptor signals upon binding of a Tm(III) labelled tool protein.

Using these methods, we characterised for the first time a human β_1-adrenergic receptor construct – one of the most important drug targets. The conformational equilibria of this construct were studied in solution while the receptor underwent a full activation cycle involving binding of agonist, Mini-G protein and antagonist.

In conclusion, we can show that our newly developed methods enable NMR studies of difficult human GPCRs and that they can give new insights into receptor functioning.

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Symmetry-based Singlet-Triplet Conversion in Solution Nuclear Magnetic Resonance

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Coupled spin-1/2 pairs support a singlet state $|S_0\rangle$ and three triplet states. Nuclear singlet order - the population difference between $|S_0\rangle$ and the triplet manifold - may display exceptionally long lifetimes that are many multiples of T_1. The field of singlet NMR, since its inception in 2004[1], has evolved to a high degree of sophistication, with a plethora of elegant radiofrequency control methods developed for accessing nuclear singlet order.

We show that a recently described[2][3] pulse sequence called PulsePol – originally invented in the context of NV centre applications – is a highly efficient and robust method for the excitation of nuclear singlet order in the solution state, in some cases surpassing the popular M2S sequence in robustness, speed, and simplicity.

We show that the behavior of PulsePol can be understood using a framework of symmetry-based recoupling[4], a theory which emerged in the context of MAS NMR and which has hitherto only been applied to solid-state NMR. This theory allows the form of the average Hamiltonian to be predicted from the values of three symmetry numbers. Sets of symmetry numbers are identified which lead to desirable properties for singlet-triplet conversion in solution NMR. For example, a common implementation of PulsePol has $R4_3^1$ symmetry. We predict an infinite family of R-symmetry sequences suitable for singlet-triplet conversion. We demonstrate experimentally that at least one such symmetry, $R8_7^3$ has superior compensation against off-resonance errors.

We demonstrate that further improvements in robustness may be achieved by adding classic NMR methodology such as supercycles and composite pulses. This demonstration of symmetry-based sequences in solution NMR may have significant implications beyond singlet NMR, leading to novel sequences for applications such as heteronuclear polarization transfer and multiple quantum excitation.

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PO342

A general method for fully homodecoupled 1H-13C HSQC spectra

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Pure shift experiments deliver homodecoupled ^{1}H NMR spectra, boosting spectral resolution by an order of magnitude. Several methods have been proposed, each with specific advantages and restrictions in terms of sensitivity or broadband character, and can also be used for designing high-resolution (selective) 2D J-resolved methods.[1-2] At natural ^{13}C abundance, the BIRD pure shift element is an attractive option to combine with experiments that already are edited for the ^{13}C isotopomers, as it comes with no further sensitivity penalty.[3] Unfortunately, BIRD does not control couplings between geminal protons, severely reducing the usefulness of BIRD pure shift for compounds rich in methylene groups. Proposed solutions have been the use of constant-time, perfect echo, or J-resolved echo processing [4-6], but these respectively require knowledge and near-uniformity of the geminal couplings, cause a doubling of the natural ^{1}H line width, or do not refocus coupling evolution during the experiment. These are important shortcomings when working under molecular alignment conditions, for medium to large size molecules, or for selective 2DJ experimental design.

Here, we present CYBORG (CYcling through Bilinear rotation Operators to Regulate Geminal couplings), a generally applicable pure shift method compatible with ^{1}H-^{13}C HSQC experiments that circumvents all aforementioned shortcomings at a reasonable price in sensitivity relative to the parent HSQC experiment. The method allows maximum pure shift resolution for ^{1}H-^{13}C HSQC experiments, and holds promise for the study of larger compounds, such as peptides or protein side-chains.

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PO343

Studies of chiral polar molecules in a strong electric field

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In our study, we examined the influence of an externally applied time-varying electric field on the course of a liquid-state NMR experiment performed on chiral molecules. In the case of chiral molecules, the perturbation of the molecular motions by the electric field oscillating at the radiofrequency may give direct information about the molecular handedness, i.e., information on which enantiomer is present in the sample without introducing any other chiral molecules [1]. However, due to the inherently small degree of the sample polarization by the electric fields of the magnitudes accessible in the laboratory, observation of such effects requires as strong fields as possible [2]. Therefore, we investigated using computer modeling and experimentally how strong electric field can be applied to a sample of (R)-1,1,1-trifluoropropan-2-ol without significant loss of the NMR spectrum features. First, we calibrated the sample temperature using the temperature dependence of the ^{1}H NMR peaks (CH, OH, CH_3) of trifluoropropanol at temperatures ranging from -20 to 60 °C. The sample was then subjected to an electric field oscillating at 29 MHz which was generated by a capacitor surrounding the transceiver saddle coil. We found that the ^{19}F NMR spectrum of trifluoropropanol, a 6.2 Hz doublet, becomes unresolved if the amplitude of the electric field exceeds a few hundred V/mm due to dielectric heating. The results of finite-elements simulations of the experimental circuits in COMSOL and molecular dynamics computations in GROMACS confirm our findings.

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SCALPEL NMR: performing surgery on spectra of complex mixtures

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Mixtures are ubiquitous in chemistry and biology. Unfortunately, NMR commonly struggles with all but the simplest mixtures. The narrow chemical shift range and prominent signal multiplicity in ^{1}H spectra often cause signals to overlap, hindering analysis. A variety of methods have been developed to aid the analysis of mixtures, such as DOSY[1], relaxation-encoded selective TOCSY (REST)[2], and pure shift experiments.[3] However, even these methods struggle with highly complex mixtures that have numerous components and a high dynamic range.

Here we present SCALPEL[4] (Spectral Component Acquisition by Localized PARAFAC Extraction of Linear components), which allows efficient analysis of such mixtures, reducing the need for laborious physical separation. The SCALPEL family of experiments exploit encoding by multiple parameters such as diffusion, relaxation, TOCSY-t_1 and chemical shift evolution to distinguish between component spectra in complex mixtures. Combining two or more (orthogonal) parameters gives multilinear data, allowing tensor decomposition methods (e.g. PARAFAC) to decompose the data into physically meaningful components and yield the subspectra of the mixture components. Typically, only a small number of increments in each dimension are needed, making these experiments quicker to acquire compared to conventional multidimensional NMR experiments. For example, SCALPEL has been used to extract the component spectra of the main saccharides present in stout beer in a single experiment. The SCALPEL family is not limited to ^{1}H and can be extended to use heteronuclei. Here we also demonstrate the advantages of a heteronuclear SCALPEL method for extracting the component spectra of a mixture of fluorine-containing corticosteroids.

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Posters

Long-lived states of magnetically inequivalent protons in aliphatic chains of nonchiral molecules

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Long-lived states (LLS) [1] have relaxation times TLLS that can be much longer than the longitudinal relaxation times T1. For a LLS to be accessible, the nuclear spins involved must be inequivalent. Previously, LLS were observed for glycine protons of the dipeptide Ala-Gly, where a distant chiral center induces a small chemical inequivalence between the CH2 protons. [2] Here we exploit the magnetic inequivalence of protons in chemical compounds containing two or more adjacent CH2 groups comprising at least an AA'XX' system, thus circumventing the requirement of a chiral center in the molecule. This allowed us to prepare LLS with lifetimes that are up to 5 times longer than T1 in different organic molecules where such states had not previously been observed: alcohols such as butanol, small biologically active molecules like gamma-aminobutyric acid (GABA), taurine, vitamins B1 and B5, and even common NMR standards such as trimethoxypropylsilane and sodium trimethylsilylpropanesulfonate (DSS). In the latter, it was observed that the lifetime of the LLS is very sensitive to interactions with bovine serum albumin (BSA), with the TLLS dropping by about 40% upon addition of a mere 0.5 µM BSA to a 5 mM solution of DSS. These results show a broad scope of possible substrates for the preparation of LLS and offer new perspectives for drug screening. [3]

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PO346

Selective excitation and detection of long-lived states using only low-amplitude pulses

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Long-lived states (LLS) based on singlet configurations within coupled spin pairs[1] have significantly expanded the lifetimes of magnetization in NMR experiments. The most common type of LLS consists of a population imbalance between the singlet and triplet manifold of an isolated two-proton J-coupled system. Several pulse sequences can be used to excite and read these overall non-magnetic states in various scalar-coupling regimes. Such pulse sequences use hard pulses that also excite other proton species in the sample, which can lead to signal overlapping or crowding that may be difficult to eliminate by phase-cycling or other filters. In addition, the experimental parameters of pulse sequences are dependent on magnetic parameters of the two-spin {I,S} system, as the chemical shift difference $\Delta\nu IS$ or the scalar coupling constant JIS between the I and S spins[2]. This, in turn, may lead to the undesired excitation of multiple-quantum coherences in nuclear spin pairs other than the targeted one. Rather than excite LLS specifically for a certain molecule or amino-acid residue, the known methods tend to produce overlapping, crowded spectra in complex mixtures or large proteins.

We propose and experimentally demonstrate a new pulse sequence that only makes use of selective low-amplitude pulses for the excitation and detection of long-lived states ('SELLS') of a specific spin-pair. It is shown both by experiments and theory that the method effectively excites LLS in spin pairs in either weak-coupling or intermediate-coupling regimes.

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PO348

ULTRAFAST TRANSVERSE RELAXATION EXCHANGE NMR SPECTROSCOPY

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Molecular exchange occurs due to diffusion or chemical transformations. The exchange of molecules plays significant role in many important processes such as breathing, chemical reactions, catalysis, and protein folding [1]. The direct observation of molecular exchange is challenging when it occurs inside a solid matrix. Nuclear magnetic resonance (NMR) spectroscopy offers means to investigate molecular exchange in a noninvasive way without tracers [2]. In so called ultrafast Laplace NMR approach, the data is collected in a single-scan fashion, accelerating the measurement up to three orders of magnitude compared to conventional counterpart. The approach is based on spatial encoding of multidimensional data, originally introduced by Frydman et al. [3]. The single-scan approach facilitates significantly the use of hyperpolarization techniques to boost experimental sensitivity.

In this presentation, we introduce a new two-dimensional ultrafast T2-T2 exchange method and demonstrated the feasibility by following molecular exchange in ionic liquid (IL) consisting of trihexyl(tetradecyl)phosphonium cations and bis(mandelato)borate anions. This study reconfirms the coexistence of aggregates and free ions, and exchange between them. The exchange rate determined by this method is in agreement with the study conducted by Javed et al.[4] The ultrafast approach potentially facilitates the characterization of biological samples as the data is measured in a single-scan fashion, therefore, sample evolution during the experiment is rather insignificant. The experimental time efficiency and better resolution data make the UF T2-T2 sequence attractive. In addition, the ultrafast T2-T2 sequence can also be used in UF diffusion exchange spectroscopy (DEXSY) [5] experiments with small modifications.

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Posters

PO349

Synergy of Time-Resolved NUS and DOSY for the monitoring of photopolymerization of anthracene derivatives

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Currently, photochemistry is experiencing an upswing. This is due to the material sciences and green energy industries' interest in the practical applications of photoactive materials. The photopolymerization of anthracene derivatives is of pivotal interest as functional photoresponsive polymers can be obtained. Unfortunately, the monitoring of photopolymerization is not an easy task, as the composition of the sample changes during the photochemical processes, and the analysis of post-photoreaction products can only indirectly give information about photoreaction. Therefore, comprehensive monitoring has to be carried out in real-time mode.

Here, it is demonstrated that comprehensive monitoring of photopolymerization can be achieved through simultaneous time-resolved Diffusion Ordered Spectroscopy (TR-DOSY) [1, 2] interleaved by time-resolved non-uniform sampling (TR-NUS) [3]. TR-DOSY enabled us to follow the change of the mass of a polymer as time progressed, which can be correlated with the TR-NUS HSQC spectra. Thanks to this, we observed step-by-step the creation and consumption of specific n-mers. The presented approach can significantly improve the methodology of the investigation of various photoreaction processes -in particular, photopolymerization.

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PO350

Improved frequency-swept pulse sequences

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Frequency-swept pulses [1] have found a wide range of applications in magnetic resonance thanks to their large bandwidth and their robustness to B_1 field variation, allowing the design of broadband sequences tolerant to hardware errors.

In this presentation, we show that most frequency-swept pulse sequences [2-4] can be explained using the instantaneous flip approximation and pulse sequence element blocks. These techniques are then applied to create several new broadband and B_1-tolerant frequency-swept pulse sequences.

First, we illustrate how a B_1-tolerant CPMG can be constructed. This CHORUS-CPMG uses three-pulse B_1-tolerant excitation sequence, followed by a sequence of refocusing blocks.

We then introduce a Perfect-echo CHORUS sequence, PROCHORUS, that uses sophisticated refocusing conditions to offer broadband excitation with suppressed homonuclear J-modulation during long broadband pulses sequences.

Finally, we demonstrate that 25 to 40% reduction for the duration of frequency-swept pulse sequences can be achieved by utilising overlapped waveforms.

We present these results in the context of NMR and EPR spectroscopy using experimental and simulated data [5-6] but these techniques are applicable to MRI and in vivo MRS too. A MATLAB toolbox, developed and used for simulation and generation of the pulses is available freely on GitHub [7].

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PO351

New insights into the structure – magnetism relationship of lanthanoid complexes

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Lanthanide chelating tags (LCTs) are widely used to study interactions, dynamics and structures of proteins, protein – protein and protein – ligand complexes in solution by paramagnetic NMR spectroscopy. The size of the induced pseudocontact shifts (PCSs) and residual dipolar couplings (RDCs) of a given LCT depend critically on the anisotropy of the magnetic susceptibility tensor. Usually the anisotropy parameters, which describe the anisotropic part of the magnetic susceptibility, are determined from resonances observed on a conjugated protein. Since the LCT is moving relative to the protein and the protein itself being flexible, the anisotropy parameters determined in this manner are inevitably reduced by motional averaging. The intrinsic anisotropy parameters of a LCT remain therefore unknown. Here we present for the first time, the intrinsic anisotropy parameters for the full lanthanoid series determined experimentally from resonances on the ligand itself. Assignment of the strongly shifted proton spectra was no longer possible using conventional 2D-NMR assignment strategies, as they were rendered useless by the extremely short T2 times. Instead, extensive, site-specific 2H and 13C labelling as well as double resonance techniques were combined with combinatorial methods in order to fully assign the 1D proton spectra and to determine the anisotropy parameters. The obtained anisotropy parameters deliver an upper limit for future PCS applications as well as new insights into future LCT designs. Surprisingly, we observed an, at least at room temperature, unprecedented correlation between the oblate or prolate f-electron distribution of the lanthanoid and the orientation of the main magnetic axis. Furthermore, comparison of different ligands revealed that the size of the anisotropy parameters depends critically on the interaction between the ligand and the lanthanoid ion.[1]

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Nuclear/electron magnetic resonance detection of coupled intra- and interdomain protein motion

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Over 80% of eukaryotic proteins include more than one domain. Interactions between these domains alter the conformational dynamics within the domains and serve as inter- and intradomain allosteric pathways. However, no established structure determination method is currently available that can probe the coupling of these motions.

Pin1 is a two-domain cell regulator that isomerizes phospho-peptidyl-prolines. The catalytic domain (PPIase) and the other ligand-binding domain (WW) sample extended and compact conformations. Ligand binding changes the equilibrium of the interdomain conformations, but the conformational changes that lead to the altered domain sampling were unknown.

We developed a novel method to model the coupling between intra- and interdomain dynamics at atomic resolution using multi-state ensembles. The method uses time-averaged NMR restraints and double electron-electron resonance (DEER) data that resolves distance distributions. While the intradomain calculation is primarily driven by exact NOEs (eNOEs), J couplings, and RDCs, the relative domain distribution is driven by PREs, interdomain NOEs and DEER. Our data supports a 70:30 population of the compact and extended states in apo Pin1. A multi-state ensemble describes these conformations simultaneously, with distinct conformational differences located in the interdomain interface stabilizing the compact or extended states. We also describe correlated conformations between the catalytic site and interdomain interface that may explain allostery driven by interdomain contact.

Furtheron, we describe ligand-specific conformational changes in Pin1 that occur upon binding of pCDC25c and FFpSPR. pCDC25c binding doubles the population of the extended states compared to the virtually identical populations of the apo and FFpSPR-bound forms. pCDC25c binding to the WW domain triggers conformational changes to propagate via the interdomain interface to the catalytic site, while FFpSPR binding displaces a helix in the PPIase that leads to repositioning of the PPIase catalytic loop.

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Single-experiment pKa measurements and ion-binding analysis using 1H chemical shift imaging techniques

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NMR-based titrations are an effective if tedious way to study the pH or ion-dependent properties of molecules. In the conventional procedure, the pH or ionic composition of a sample is adjusted manually between successive NMR experiments, with the result that hours of instrument time are required to collect even a small number of spectra. As a more efficient approach, our group is developing methods that allow the titratable properties to be measured in single 1H chemical shift imaging (CSI) experiments using internal concentration gradients. A detailed analysis of systems can thus be performed in a tiny fraction of the time, on standard high-field NMR spectrometers.1-4

In this presentation, I will outline the basis of the technique and demonstrate its application to two problems in solution state-NMR: the determination of the pKa values of small molecules with multiple basic/acidic sites, and the affinity of macromolecules for Ca2+ and Mg2+.

By diffusing oxalic acid into a solution of buffer components, we can create pH gradients spanning seven or more units. By CSI, we record a 1H spectrum every 0.2 mm along the pH gradient and obtain 100 1H spectra across this wide pH range in under 20 minutes.1 This high pH-resolution permits the robust fitting of pKa values. To study the binding of Ca2+ and Mg2+ (M2+) to macromolecules, we diffuse M2+ acetate into a solution of the macromolecule to create concentration gradients of M2+ and acetate. Binding to the macromolecule causes a discrepancy between the concentration of unbound M2+ ions and acetate.3 The solution stability of the macromolecule can be assessed simultaneously by monitoring its 1H resonances.

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GENESIS: Automated Pulse Programme Construction for NMR Supersequences

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One of the most accessible methods for accelerating 2D NMR data collection is NOAH (NMR by Ordered Acquisition using ^{1}H detection).[1–3] In a NOAH experiment, multiple 2D experiments (referred to as "modules") are combined without interleaved recovery delays to form "supersequences", thus achieving speedups of 3–4× compared to conventional acquisition of individual modules. This is made possible through the use of pulse sequence elements which manipulate magnetisation in an isotope-specific fashion, e.g. separating uncoupled protons from protons directly coupled to heteronuclei such as [13]C. Using this principle, many standard 2D experiments such as HMBC, (se)HSQC, HSQC-TOCSY, COSY, TOCSY, NOESY, and ROESY have been successfully incorporated into NOAH supersequences.

The combinatorial nature of NOAH experiments means that there are, in principle, thousands of different possible supersequences, each corresponding to a different subset of modules. In practice, however, we have only ever distributed a few dozen different pulse sequences (either in article SIs or via the Bruker User Library), meaning that users cannot easily obtain customised supersequences. Here, we show that these problems can be overcome through programmatic generation of NOAH pulse programmes, an approach we term GENESIS (GENEration of Supersequences In Silico)[4] and which can be used online at https://nmr-genesis.co.uk.

This approach also allows for newest developments in NOAH supersequences to be rapidly and efficiently disseminated to the broader NMR community. These include the newly described "parallel" supersequences;[5] the addition of new modules including extended heteronuclear correlation experiments and pseudo-2D ("interferogram") pure shift techniques; and improvements to existing modules and supersequences, notably the inclusion of solvent suppression options across a wide range of modules.

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Proton relaxation NMR evidence for pervasive sidechain dynamics in proteins

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We developed a method to measure T_1-rho rates of amide PROTONS in proteins in solution. Our method suppresses / takes into account exchange broadening, scalar couplings and multi-spin dipolar cross correlations. We compare the results with T_1-rho relaxation rates calculated from the crystal structure, taking dipolar interactions with all nuclei within a sphere of 8 Å around the individual amide protons into account, as well as the ^{1}H CSA. The correspondence between computation and experiment is reasonable (R^2=0.43, RMSD 13%) with clear outliers. We proceed to explain these outliers from amide PROTON order parameters computed from MD simulations of the protein. Bringing into account these order parameters improves the correspondence between experimental and computational values to (R^2=0.65, RMSD 9%). This work thus provides new evidence of widespread motions in proteins. This work also presents a new benchmark to help improve the theoretical forcefields underlying the computations.

PO357

Benchtop NMR relaxometry for the follow-up of Cr(III) and Mn(II) removal by ion exchange resin

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Water pollution by heavy metals has become a major public health and environmental concern [1]. The removal of these metals from water is often performed by ion exchange. However, Current techniques to study ion exchange efficiency are indirect and destructive. In this research, the paramagnetic properties of Cr(III) and Mn(II) are used to monitor their removal by an ion exchange resin. Indeed, the presence of paramagnetic ions affect the Nuclear Magnetic Resonance (NMR) relaxation times T1 and T2 of water protons which can be easily measured by benchtop NMR relaxometry [2-3].

In order to study ion exchange kinetics, a NMR tube was filled with a small amount of Dowex Marathon MSC resin and 350 µL of aqueous solutions containing the paramagnetic ion of interest before being shaken by a vortex mixer. The transverse relaxation time was measured at different time intervals which allowed the monitoring of the amount of loaded metal. The same experiment was repeated with different metal concentrations to provide the adsorption isotherms.

The equilibrium isotherm behavior of Cr(III) or Mn(II) are satisfactorily described by the Langmuir model with the maximum adsorption capacity of 21.8 mg g-1 and 58.1 mg g-1 for Cr(III) and Mn(II) respectively. Experimental kinetic data fit well with the pseudo-first and pseudo-second order model.

In the future, it will be interesting to carry out a so-called NMR column experiment in order to follow the loading of adsorbent in real-time through the measurement of the NMR signal of the resin.

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PO358

High resolution spectroscopy at ultra-low magnetic field

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Zero field NMR spectroscopy has demonstrated to be a powerful tool to quantitatively measure scalar couplings between heteronuclear spins with high precision. Most sensitive results can be achieved by exploiting long-lived coherences formed by singlet states. In addition, the observed transitions are often more sensitive to changes in J than in high field spectra of the same molecule.

Unfortunately, zero field spectroscopy relies on a heteronuclear spin system with a large difference in gyromagnetic ratios (e.g. 1H/13C). Consequently, 1H/19F spin systems ar therefore hard to measure at zero field.

Here, we demonstrated a practical and theoretical analysis on determining scalar coupling constants at ultra-low but non-zero magnetic fields. In this intermediate regime where the spins system undergoes the transition between weak and strong coupling we still benefit from the zero field features of narrow lines and highly sensitive transitions regarding changes in J but we avoid some of it's limitations. The recorded data is then being analyzed and compared to simulations to ultimately determine the coupling constants.

We are going to demonstrate the procedure on various fluorinated compounds including trifluorethanol and fluorinated benzenes.

PO360

Approaching Immobilized Polymer Fraction Determination by Low Field NMR Relaxometry

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We aim to study the polymer dynamics in nanocomposites made of poly-2-vinyl pyridine and silica nanoparticles, strongly interacting through multiple hydrogen-bonds. We use low-field NMR relaxometry to quantify the fraction of immobilized polymer, demonstrating that this technique nicely complements more usual dynamical probes such as broadband dielectric spectroscopy (BDS), rheology and differential scanning calorimetry (DSC).[1]

Two approaches have been used to rationalize the mobility drop within the immobilized layer: (i) a model based on a glass-transition temperature (Tg) gradient as a function of the distance from the nanoparticle surface and (ii) a model that considers a single interfacial layer with a distribution of relaxation times. The most interesting findings that emerge from our work are the following: (i) Owing to the narrow range of temperatures where NMR is sensitive to tau_alpha, one cannot reliably assess the expected WLF behavior but just observe an apparent Arrhenius dependence. Further, a systematic shift of the estimated timescale is observed, raising the question of the relevance of additional influencing factors (methodological limitations, but maybe also other dynamic processes). (ii) NMR data treated with the single interfacial layer model allows to quantify the slowing down of ca. 1 decade in the immobilized layer in comparison to the free polymer while BDS was ambiguous on this point (resulting in either one [2] or two decades [3]). (iii) Tg gradient model can be used to reconciliate convincingly DSC and NMR data, providing similar Tg profiles.

These results highlight difficulties and potential model dependencies in quantifying the dynamics of the minority polymer fraction in nanocomposites, hence requiring a multi-technique approach to properly characterize dynamical heterogeneities.

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Towards ultra long-lived singlet states in 103Rh complexes

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Nuclear singlet order may be extremely long-lived in specific circumstances, with lifetimes exceeding 1 hour observed for 13C spin pairs.(1) Even longer lifetimes are anticipated for lower-gamma nuclei, such as 103Rh, which has a gamma value approximately 8 times lower than that of 13C. To explore this possibility, we are investigating singlet lifetimes in dirhodium paddlewheel complexes. Magnetic inequivalence is imposed across rhodium centres via selective 18O labelling of ligands, enabling access to the singlet state. To mitigate rhodium's inherent insensitivity, we utilise an innovative triple resonance pulse sequence to indirectly observe rhodium resonances.(2) In doing so, we are able to present corresponding rhodium 18O shifts. We also utilise computational data to estimate longitudinal and singlet order lifetimes, with a particular emphasis on the field dependence of various relaxation mechanisms. From this, we anticipate extremely long retention of singlet order within rhodium spin pairs at low magnetic fields.

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PO362

HYPERPOLARIZED ULTRAFAST DIFFUSION EXCHANGE SPECTROSCOPY BY A SINGLE SIDED NMR INSTRUMENT

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Single-sided NMR instruments such as the NMR MOUSE (Magritek) are much more affordable and portable than standard high-field NMR spectrometers. Additionally, the single sided design allows for the investigation of samples without size or shape requirements. Laplace NMR (LNMR) methods provide detailed information about molecular rotational and translational motion as well as local physical or chemical environment of nuclei [1,2]. Multidimensional LNMR experiments can be used to correlate relaxation and diffusion parameters and to study molecular exchange via relaxation or diffusion contrast even when the exchange pools are not resolved in spectrum [2]. In this study, ultrafast diffusion exchange spectroscopy (UF DEXSY) and dissolution dynamic nuclear polarization (dDNP) are utilized for the investigation of water exchange in yeast. UF DEXSY [3] experiments rely on spatial encoding of 2D data and shorten the experiment time by two to four orders of magnitude as compared to conventional DEXSY [4]. In addition to time savings for UF DEXSY, dDNP improves the sensitivity by four to five orders of magnitude. Together, these allow for 2D exchange measurements to be performed with a single scan using the low-field, single-sided NMR instrument. The results show water exchange between the intra- and extracellular environments in a yeast cell suspension.

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Solid-state NMR signals in zero-field

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Table-top near zero-field NMR signal detection is becoming viable using currently available compact atomic magnetometers. The NMR signals from solutions in zero external magnetic field have been studied extensively using atomic magnetometers. These predominantly contain information about inter-nuclear J- coupling. We are trying to detect NMR signals in zero field from solid-state samples with atomic magnetometers. Currently, SQUIDS and field cycling experiments are being used to obtain these spectra. SQUIDS require cryogenic cooling for their operation while field cycling experiments are time-consuming. Atomic magnetometers seem to be a good choice as they do not require cryogenics for operation and their sensitivity is comparable to that of SQUIDS. In solid samples, apart from J- coupling, other internal nuclear interactions like dipole-dipole coupling, quadrupole coupling will also be present. Such anisotropic interactions will broaden the spectra in high field for a powdered sample under static conditions. But in zero-field, magnetisation from all crystallites in the sample although randomly oriented will oscillate at the same frequency determined by the internal interactions. This results in narrow spectral features for a low field NMR signal of a powdered sample. Since these internal interactions can range from few Hz to tens of kHz the atomic magnetometer must have high bandwidth. We demonstrate an atomic magnetometer with a bandwidth of a few tens of kHz. We describe our efforts to measure solid-state NMR signals in zero-field using our atomic magnetometer.

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Two-dimensional NMR study of cement materials during sorption cycles

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In this work we present two-dimensional (2D) 1H NMR experiments to measure cement materials during first sorption cycle. 2D correlation measurements can provide a more straightforward way to investigate dynamic transformation of cement paste structures than one-dimensional approach. They allow one to map diffusion, exchange, and molecular motion, and gained popularity in the wetting/drying processes of liquids in porous media [1]. In-situ re-saturation process was investigated with constant water uptake up to 821 hours. T1-T2 and D T2 measurements were performed. 2D data was processed using Upen2D and MUpen2D inversion program [2], a Matlab® [3] script developed at the University of Bologna which computes locally adapted regularization parameters and approximate solutions by solving a sequence of regularized least squares problems. For the T1-T2 map we did not find changes in interlayer water, small and big gel pores components between as-prepared and re-saturated states. We found an increase of capillary pores T1/T2 ratio (from 1.6 to 3.5). With D-T2 experiment we observed the increase of the diffusion coefficient of gel pore and interhydrate components after drying and re-saturation. These results showed that the interaction of water with the surfaces of the pores changed suggesting structural deformation [4]. Further experiments are needed to confirm the results obtained. Nevertheless, the 2D NMR experiment combined with single-sided measurements, is a powerful innovative approach that allows one to follow the changes of the cement structure during sorption cycles.

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Decoupling of spin decoherence paths near zero magnetic field

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Proton-deuterium isotopic substitution is a tactic frequently used in high-field solution-state NMR. At low magnetic fields the advantages of 1H/2H substitution may, however, be outweighed by introduction of additional decoherence mechanisms and splittings due to heteronuclear J coupling. In this work (see DOI: 10.1021/acs.jpclett.1c03714), we theoretically and experimentally investigate the relaxation rates of 1H/2H and other dual-species spin systems near zero magnetic field. By changing the effective symmetry of the spin Hamiltonian by using XY4 decoupling, we demonstrate that different contributions of individual interactions can be switched off. As an example, deuterium spin decoupling near zero field is used to extend the 1H T1 relaxation time by a factor of 2. Using a slightly different pulse sequence, we can decouple both nuclei from an arbitrary background magnetic field but retain the internuclear coupling. We expect these approaches to widen the range of hyperpolarized biomedical contrast-agent compounds and scope of hyperpolarization procedures used near zero field.

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Band-pass pulses for low-, ultralow- and zero-field magnetic resonance

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Pulsed alternating (ac) fields are a staple of atomic, electronic and nuclear spin resonance spectroscopies. Following decades of development in these disciplines and others (e.g., quantum information processing), there exist many pulse species for precise qubit control and compensation of errors inevitably present in experiment. Among error-tolerant pulses engineered are those utilizing phase-shift keying (composite pulses), amplitude-modulation and amplitude-and-phase modulation of the ac fields.

For the most part, the above classification is technological. Spin resonance experiments usually involve studying spins in strong magnets (e.g. superconducting magnets) where magnitude and direction of field are fixed. Thus, only the ac phase and amplitude degrees of freedom remain to exert rotations in the spins' interaction frame, namely rotation axes in the equatorial plane of the Bloch sphere.

In this work we demonstrate pulses that allow for unconstrained orientation of magnetic fields with respect to the laboratory frame of reference. The case includes the emerging area of zero and ultralow-field (ZULF) NMR (DOI: 10.1016/j.fmre.2020.12.007 and 10.1016/j.jmr.2020.106886), which presents an attractive regime for nuclear spin hyperpolarization, relaxometry (DOI: 10.1038/s41467-021-24248-9) and precision spectroscopy in fields of microtesla and nanotesla. As an example, confinement of rotation axes to a single meridian in the Bloch sphere (e.g., the xz meridional plane) may yield spin-selective rotations where excitation with respect to nuclear gyromagnetic ratio follows a band-pass profile.

We illustrate spin-selective, DC pulse sequences analogous to ones used for radiofrequency offset compensation in high-field NMR. Existing offset-tolerant pulses (e.g. BURP, DOI: 10.1016/S0079-6565(97)00024-1) and their analogs produce sharp-edged band-pass profiles with respect to gyromagnetic ratio. The ZULF NMR application is more demanding since out-of-band rejection is a strong requirement in addition to uniform excitation within the passband. Due to the extra selectivity criterion, there is some scope for design of new pulses with improved performance.

Polarization Transfer from Optically Pumped Ensembles of
N-V Centers to Multinuclear Spin Baths

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NV centers in diamond have been recognized as good candidates for nuclear spin hyperpolarization since they can provide very high spin polarization under ambient conditions. However, this possibility has been mainly explored using single defects, limiting the potential applications to the nanoscale. A scale-up of the NV system by means of ensembles is then necessary when signal enhancement of larger sample volumes is the goal, for instance in the case of microscale NV-NMR, bulk NMR or for biomedical applications.

In our work, NV ensembles have been utilized to accomplish spin polarization transfer to nuclei inside and outside the diamond lattice. A sample consisting of a diamond chip implanted with a ~5 nm deep shallow NV-ensemble has been engineered with a thin (1 nm) Al2O3 layer deposited by Atomic Layer Deposition (ALD) and a fluorinated self-assembled monolayer (SAM) grown on the top [1]. NV dressed states generated by spinlock pulses have been utilized to transfer spin polarization to the nuclei matching Hartmann-Hahn condition. Typical spectral features attributed to the use of ensembles are observed in the NV depolarization spectra and are analyzed in detail. In a multinuclear approach, we could address selectively three different spin types (13C, 1H and 19F) in different conditions with respect to their interaction with the NV ensemble and compare the transfer efficiencies by estimating the polarization transfer rates. In particular, direct transfer has been achieved to 19F nuclei unambiguously positioned outside the diamond lattice and over an additional interface [2].

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Magnetic resonance gradient imaging using a "current-focusing device" in a nitrogen-vacancy sensor

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Nitrogen-vacancy (NV) centers are being used for nanoscale magnetic resonance sensing in a myriad of applications. Adding a high gradient magnetic field in the vicinity of the NV gives the advantage of high spatial resolution [1]; thus with a gradient of 1 G/nm one can obtain single-molecule resolution. Yet, the NV signal is sensitive to off-axis fields, which decrease ESR contrast [2], making a constant gradient field impractical for imaging. Using a magnetic field that stems from a current tackles this issue by switching the field off during read-out and achieving high-contrast ESR measurements. To this end, we incorporated a "wire-on-a-tip", in analogy to the MRFM CFFGS [3] in an NV microscope setup. I will show results demonstrating the tip can be controlled and aligned in the vicinity of the NV and that the induced magnetic field is detected by the NV.

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Demonstration of NV-detected NMR at 8.3 Tesla

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Nuclear magnetic resonance (NMR) is an invaluable spectroscopic technique for the characterization of molecular structures. NMR at high magnetic fields is highly advantageous because of its high resolution and improved sensitivity, enabling the resolution of small chemical shifts and offering new insights into the study of complex molecules, such as biological macromolecules. The nitrogen-vacancy (NV) center in diamond, due to its unique properties, has enabled widespread study of nanoscale NMR and electron spin resonance (ESR) at low magnetic fields [1]. However, conventional NV-detected NMR based on AC magnetic field sensing technique is not applicable at high magnetic fields and therefore requires the development of alternate techniques. Furthermore, there have been few studies of NV-detected NMR at high fields due to the technical challenges involved [2]. In this presentation, we explore an NV-detected NMR technique suitable for applications of high-field NMR [3]. We demonstrate optically detected magnetic resonance (ODMR) with the NV Larmor frequency of 230 GHz at 8.3 Tesla, corresponding to a proton NMR frequency of 350 MHz. We demonstrate the first measurement of electron-electron double resonance detected NMR (EDNMR) using the NV center and successfully detect 13C nuclear bath spins. We also discuss the technique's signal sensitivity, spectral resolution, and applications to NV-detected NMR from external spins. This work demonstrates a clear path to nanoscale NMR of external spins and NV-detected NMR at even higher magnetic fields.

References

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