

***IDENTIFY - Identify,  
Characterize and  
Measure Biomolecules  
in a Variety of Sample  
Sources***

Biomarker Discovery and  
Validation by HPLC Chip-  
LC/MS

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LC/MS Applications Manager  
October 22, 2008



# Overview

HPLC-Chip/MS for reproducible, robust nanoflow LC/MS

Biomarker discovery using accurate mass with a mass profiling approach

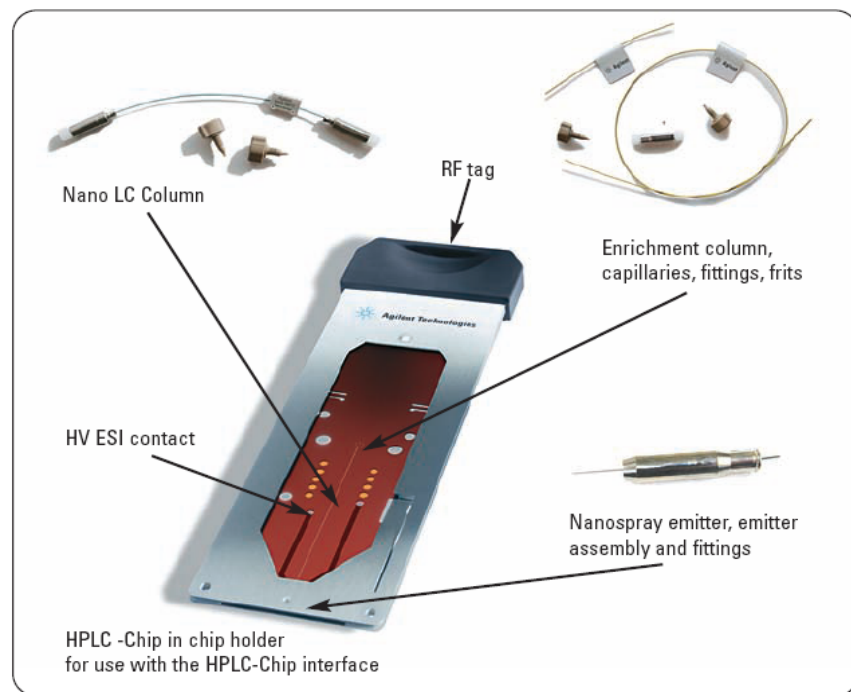
Advanced statistical analysis using GeneSpring MS

Biomarker validation using HPLC-Chip/QQQ for quantitative measurements

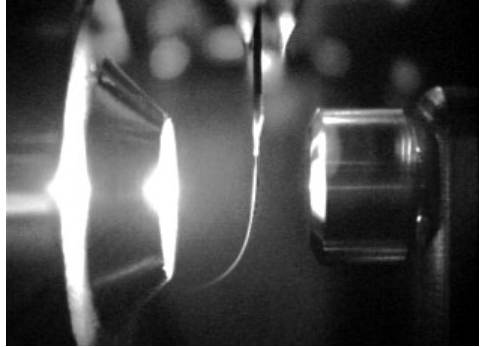
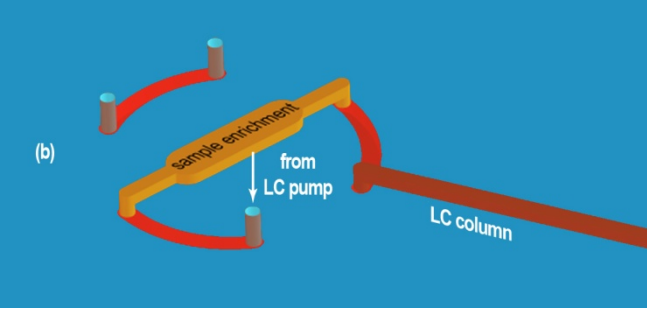
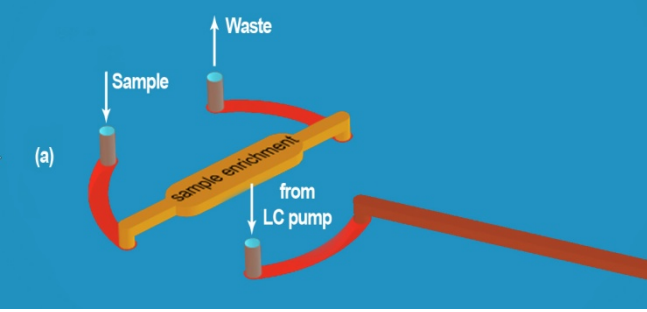
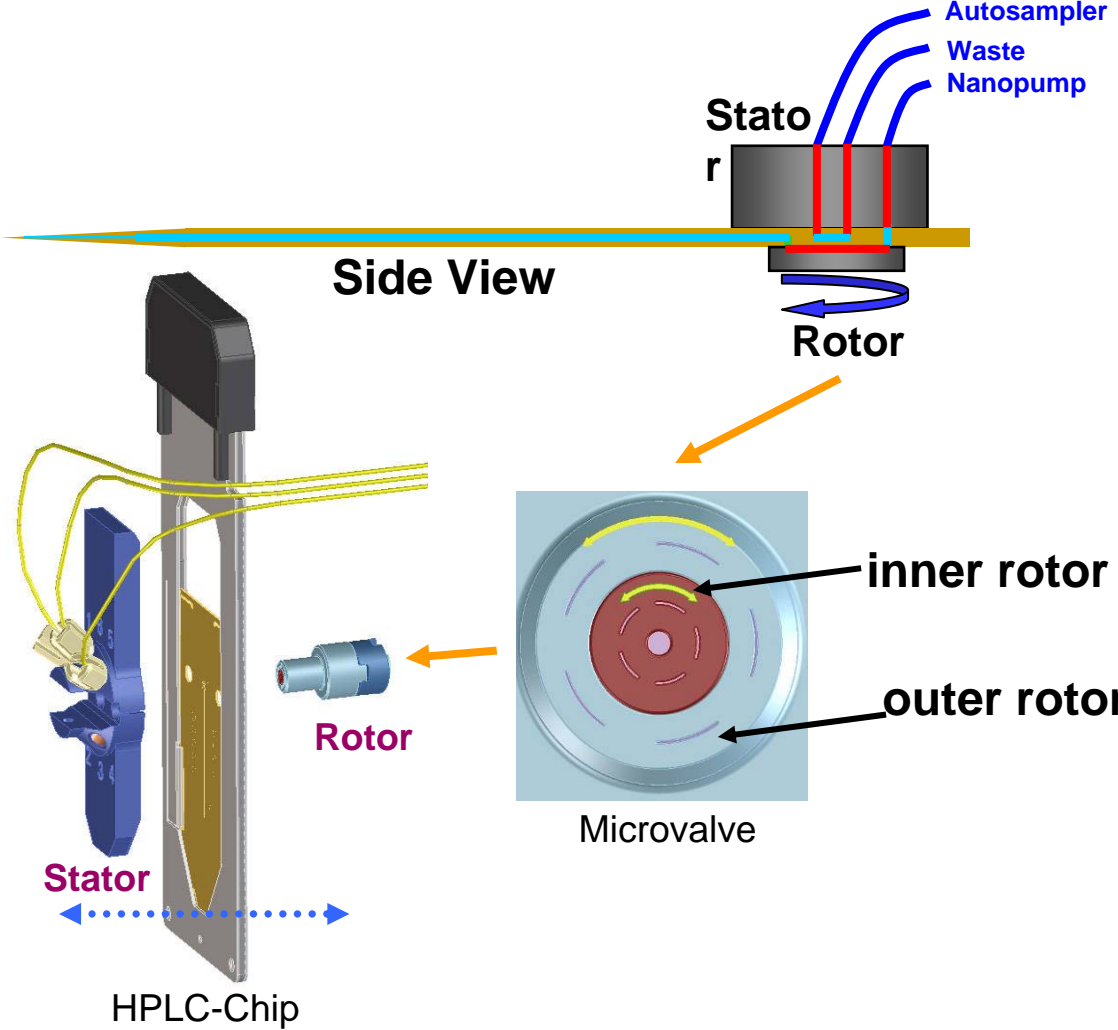
# Why Use Microfluidics For Nanospray LC/MS?

**Integrate functional components onto a reusable, biocompatible chip**

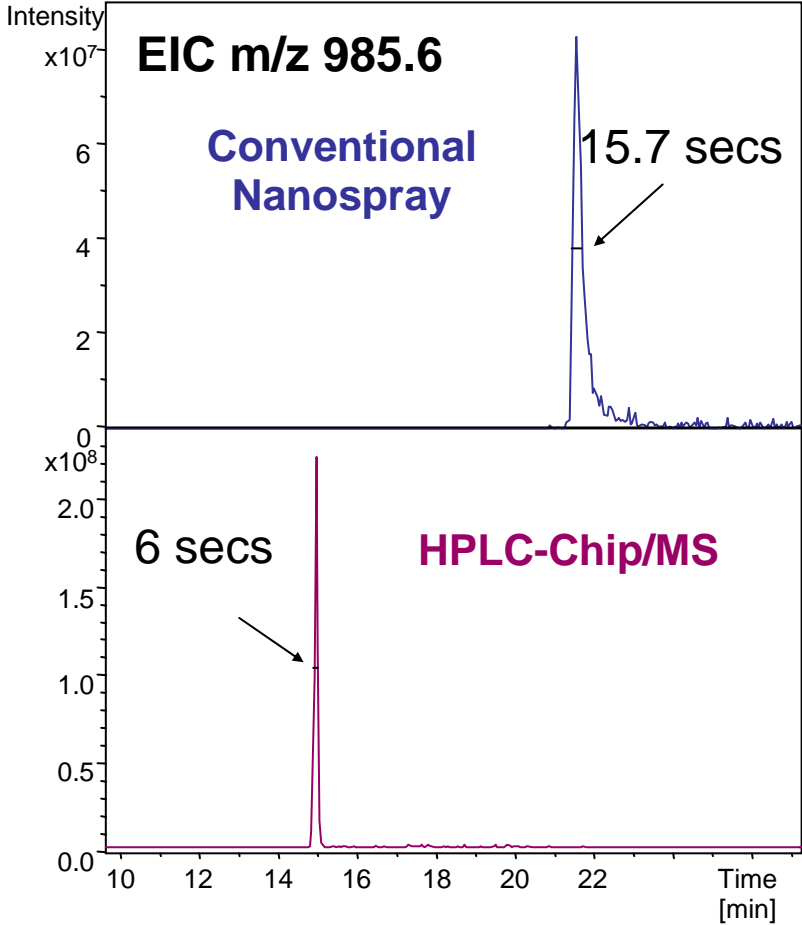
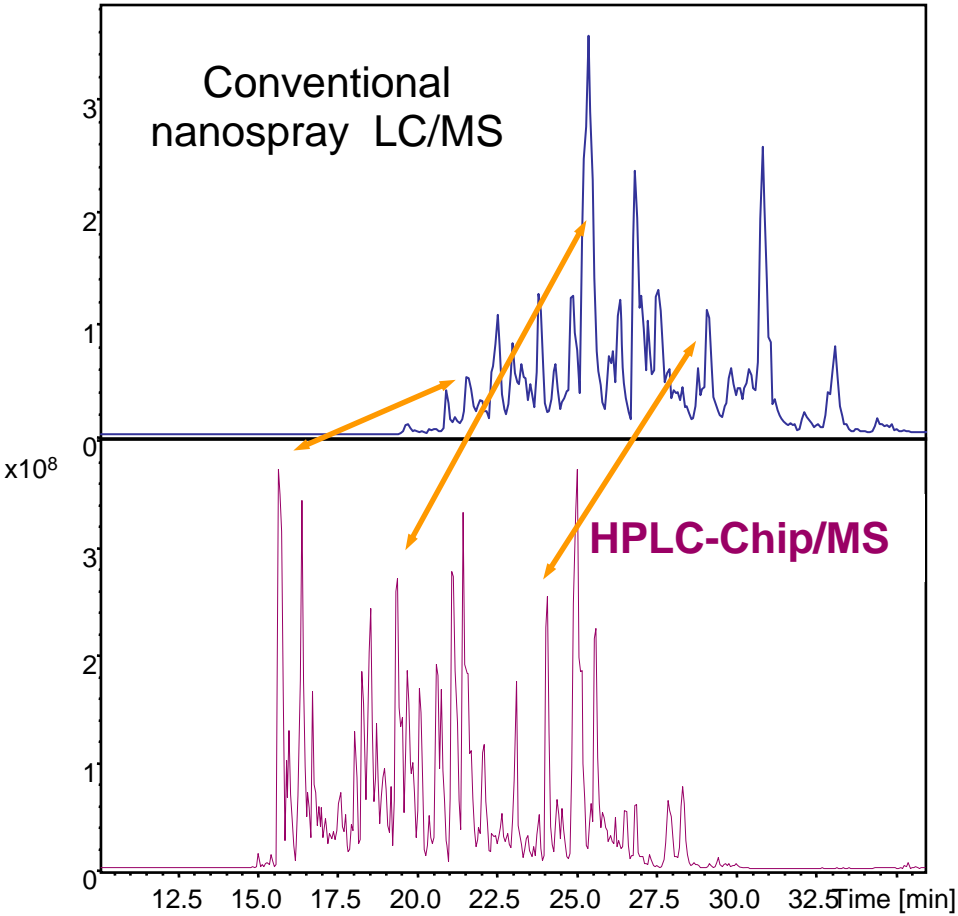
- **enrichment and analytical nanocolumns,**
- **nanospray emitter**
- **fittings and connection capillaries**
- **directly on a reusable biocompatible polymer chip.**



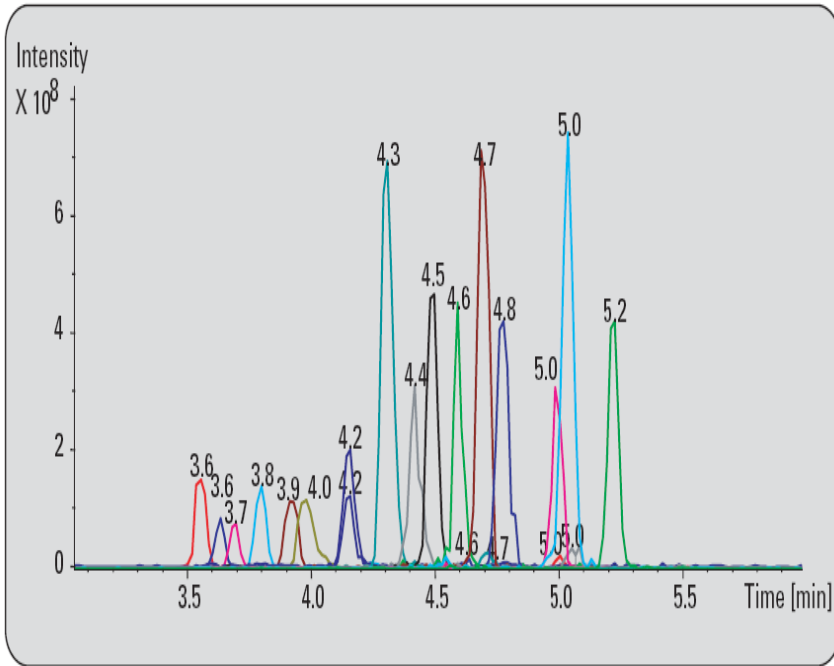
# HPLC-Chip/MS Interface: Fluid Connections to the HPLC-Chip



# Better Chromatography Reduces Interferences in Complex Matrices



# Retention Time Reproducibility



**Extracted ion chromatograms for 17 peaks from a BSA tryptic digest (50 fmol on-column)**

	RT	SD	%RSD
EIC 487.8	3.618	0.014	0.40
EIC 752	3.788	0.011	0.29
EIC 740.6	5.018	0.010	0.20
EIC 874.4	3.968	0.012	0.31
EIC 653.6	4.289	0.012	0.28
EIC 511.7	3.681	0.012	0.31
EIC 722.7	3.547	0.012	0.35
EIC 778	4.143	0.010	0.23
EIC 526.3	4.399	0.015	0.34
EIC 547.5	4.472	0.011	0.25
EIC 746.7	5.196	0.011	0.20
EIC 519.1	4.142	0.011	0.26
EIC 508.2	4.972	0.011	0.23
EIC 582.4	4.679	0.011	0.23
EIC 461.9	3.905	0.012	0.30
EIC 474	4.759	0.011	0.22
EIC 628	4.584	0.010	0.22

**RT reproducibility evaluated using 69 repeat injections**

# Mass Profiling- Find The Differences Between Samples



Molecular Feature: a discrete molecular entity defined by the combination of

- retention time, mass and response in an LC/MS analysis
- retention time, mass spectrum and response in a GC/MS analysis

LC/MS or  
GC/MS  
Analysis

Find  
molecular  
features

Compare  
sample  
sets

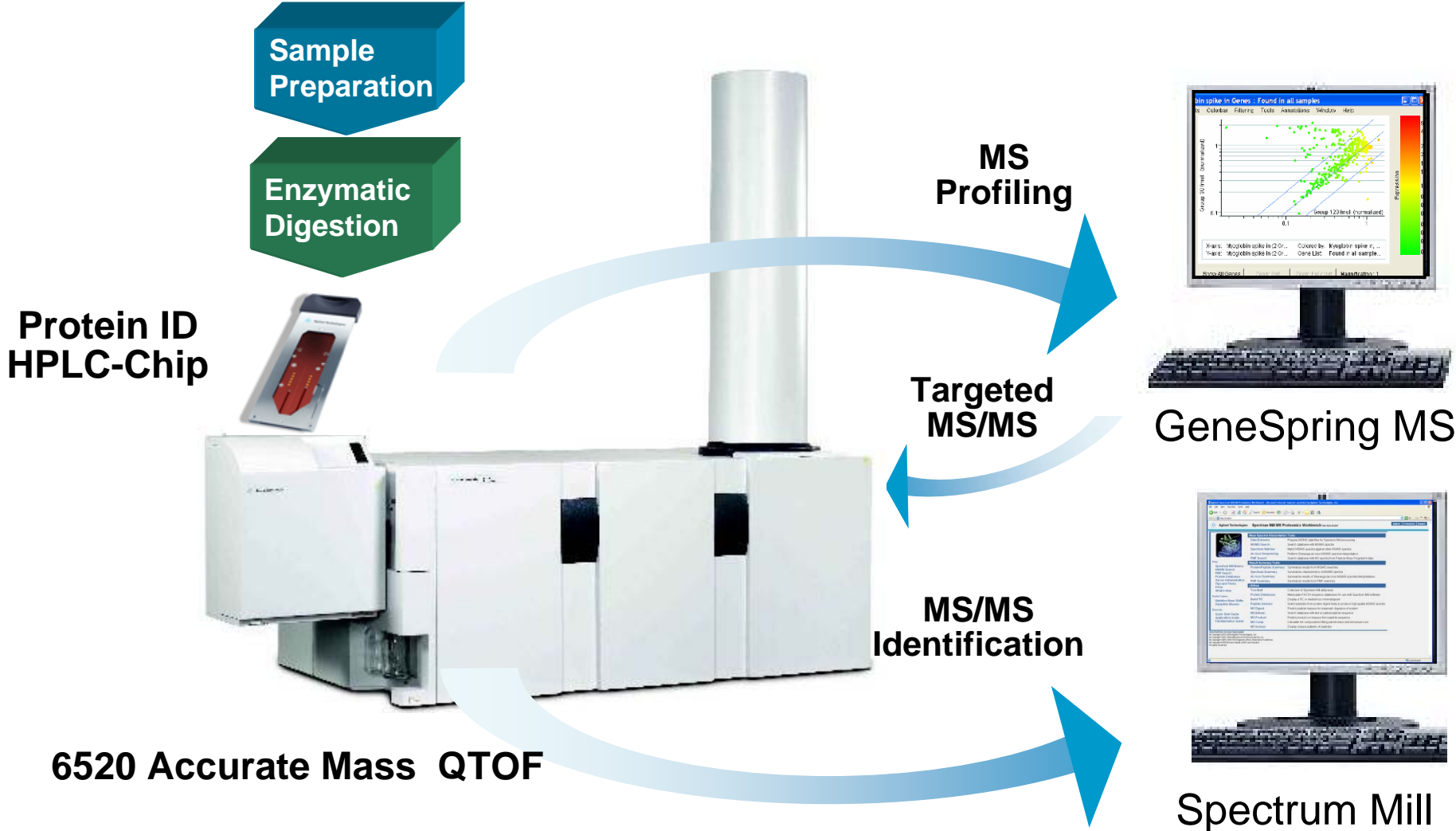
Identification

Validation

- Find real differences in sample sets using statistical analysis
- Reproducible measurements minimize the number of samples!

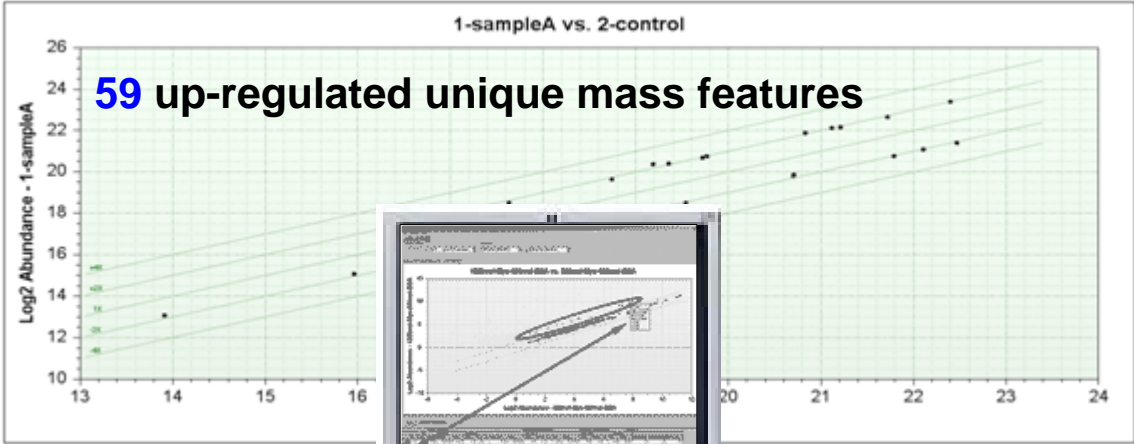
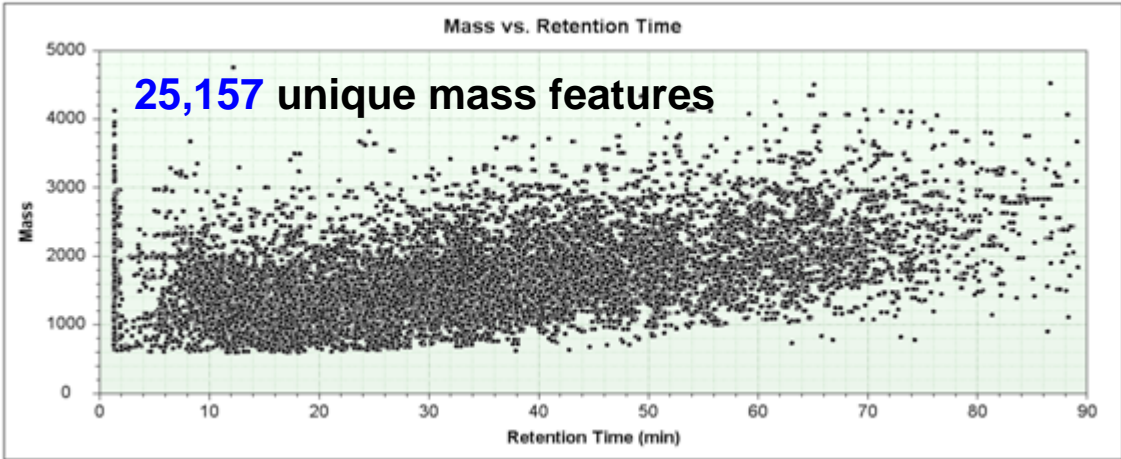


# MS Profiling And MS/MS Identification With The Agilent 6520 Q-TOF And HPLC-Chip



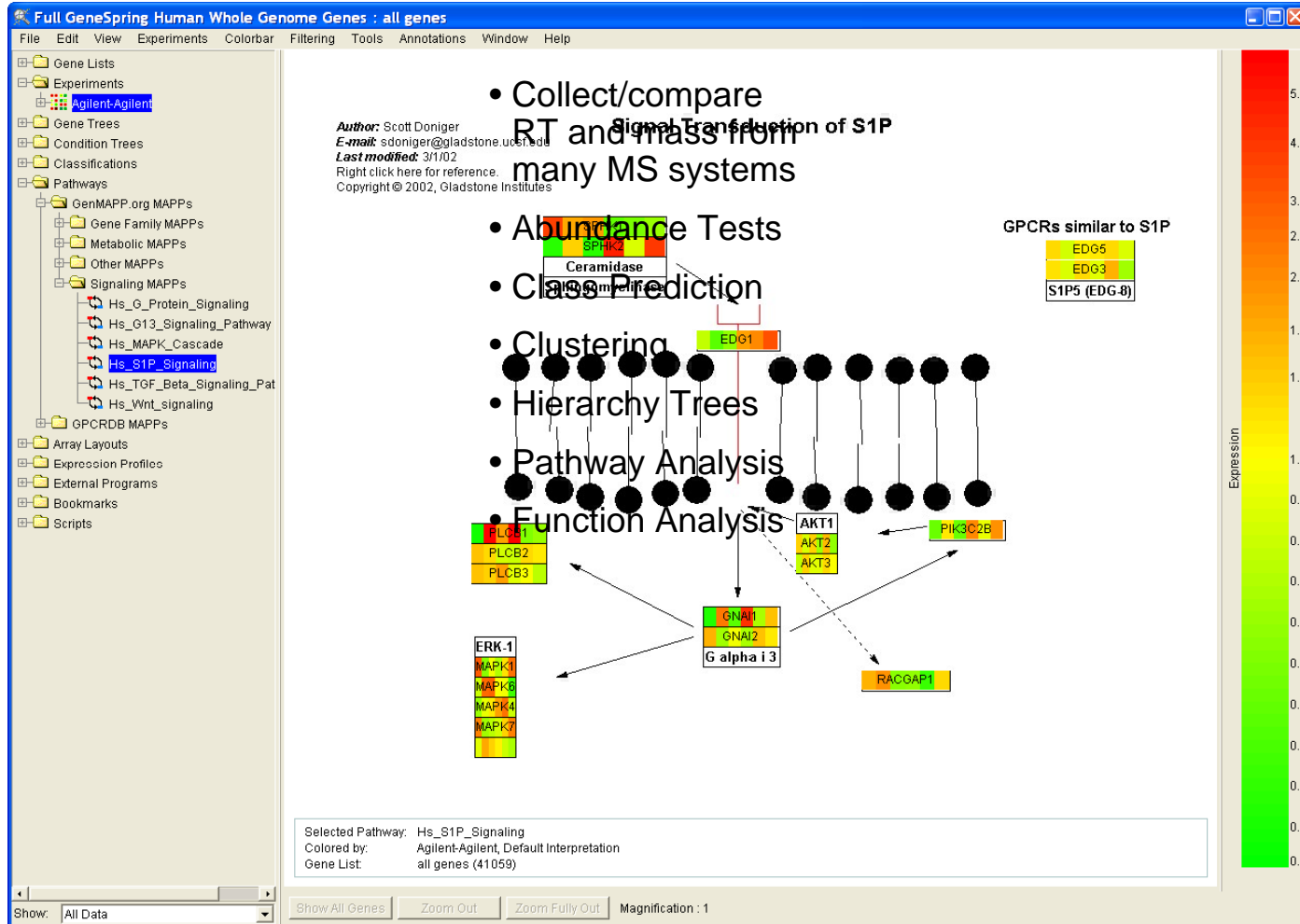


# Mass Profiling Software



Mass Profiler

# GeneSpring MS for Biomarker Discovery



GeneSpring MS

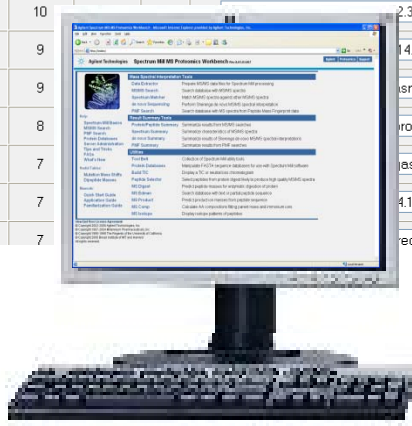
# Candidate Identification From Targeted MS/MS

Protein/Peptide Summary - Agilent Spectrum Mill - Microsoft Internet Explorer provided by Agilent Technologies, Inc.

Address: http://spectrummilletd/millhtml/summaryframe.htm

Agilent Spectrum Mill - Protein/Peptide Summary

30min # spectra mean intensity	60min # spectra mean intensity	Archakov\SCX-ifs # spectra mean intensity	Database Accession #	%AA Coverage	Distinct Peptides (#)	Summed MS/MS Search Score	Group #	Protein Name
4 1.11e+006	12 1.08e+007	28 1.24e+006	P20029	39	21	319.95	1.1	2x 78 kDa glucose-regulated protein precursor (GRP 78) (Immunoglobulin heavy chain-binding protein) (BiP)
9 2.23e+006	14 1.02e+007	22 2.12e+006	P09103	38	18	261.60	2.1	Protein disulfide-isomerase precursor (EC 5.3.4.1) (PDI) (Prolyl 4-hydroxylase beta subunit) (Cellular thyroid hormone-binding protein)
9 3.26e+006	27 1.10e+007	33 1.72e+006	Q64458	39	14	215.05	3.1	3x Cytochrome P450 2C29 (EC 1.14.14.1) (CYPIC29) (P-450 MUT-2) (Aldehyde oxygenase)
6 1.86e+006	16 8.35e+006	12 2.50e+006	Q8VCT4	33	13	201.81	4.1	4x Carboxylesterase 3 precursor (EC 3.1.1.1) (Triacylglycerol hydrolase) (TGH)
2 3.76e+005	11 1.48e+007	19 2.40e+006	Q64459	31	13	196.62	5.1	Cytochrome P450 3A11 (EC 1.14.14.1) (CYP3A11) (P-4503A11) (P-450UT)
10 3.66e+006	11 1.38e+007	20 1.76e+006	Q63880	34	13	195.98	6.1	6.1 Liver carboxylesterase 31 precursor (EC 3.1.1.1) (ES-Male) (Esterase-31)
3 6.26e+005	4 5.82e+006	4 1.23e+006	P37040	22	10	154.70	7.1	7.1 NADPH-cytochrome P450 reductase (EC 1.6.2.4) (CPR) (P450R)
1 7.15e+005	5 1.13e+007	12 8.57e+005	P27773	20	10	142.58	8.1	8.1 Protein disulfide-isomerase A3 precursor (EC 5.3.4.1) (Disulfide isomerase ER-60) (ERp60) (58 kDa microsomal protein) (p58IP7)
5 2.96e+006	10 8.80e+006	9 1.81e+006	P24456	27	9	140.41	9.1	9.1 3x Cytochrome P450 2D10 (EC 1.14.14.1) (CYP2D10) (P450-16-alpha) (P450CB) (Testosterone 16-alpha hydroxylase)
7 4.17e+006	19 1.36e+007	21 1.73e+006	Q63886	25	9	136.88	10.1	10.1 7x UDP-glucuronosyltransferase 1-1 precursor, microsomal (EC 2.4.1.17) (UDPGT) (UGT1*1) (UGT1-01) (UGT1A1)
0 0.00e+000	4 7.76e+006	14 1.21e+006	Q9D379	23	10	136.88	10.1	10.1 2.3) (Microsomal epoxide hydrolase) (Epoxide hydratase)
3 4.57e+006	5 1.14e+007	10 1.68e+006	Q62397	22	9	136.88	10.1	10.1 4.1) (CYP11B10) (Testosterone 16-alpha hydroxylase) (P450-16-alpha) (Clone PF3/46)
3 2.72e+005	6 1.05e+007	8 2.17e+006	P08113	13	9	136.88	10.1	10.1 smic reticulum protein 99) (94 kDa glucose-regulated protein) (GRP94) (ERP99) (Polymorphic stress-inducible protein)
5 7.29e+005	2 6.83e+006	3 1.06e+006	Q08601	11	8	136.88	10.1	10.1 protein large subunit precursor
9 8.90e+005	7 7.21e+006	14 7.13e+005	Q8JZR0	16	7	136.88	10.1	10.1 ase 5 (EC 6.2.1.3) (Long-chain acyl-CoA synthetase 5) (LACS 5)
3 1.12e+006	2 6.06e+006	6 1.35e+006	Q05421	14	7	136.88	10.1	10.1 4.1) (CYP11E1) (P450-J) (P450-ALC)
0 0.00e+000	5 1.10e+007	4 1.23e+006	P08003	15	7	136.88	10.1	10.1 precursor (EC 5.3.4.1) (Protein ERp-72) (ERp72)



Spectrum Mill

# Protein Biomarker Model Study on a Complex Mixture

Five mixtures prepared which contained:

- Tryptic digest of an *E. coli* lysate (complex background)
- Tryptic digests of bovine serotransferrin and BSA at specified levels

Each mixture was prepared 10 times

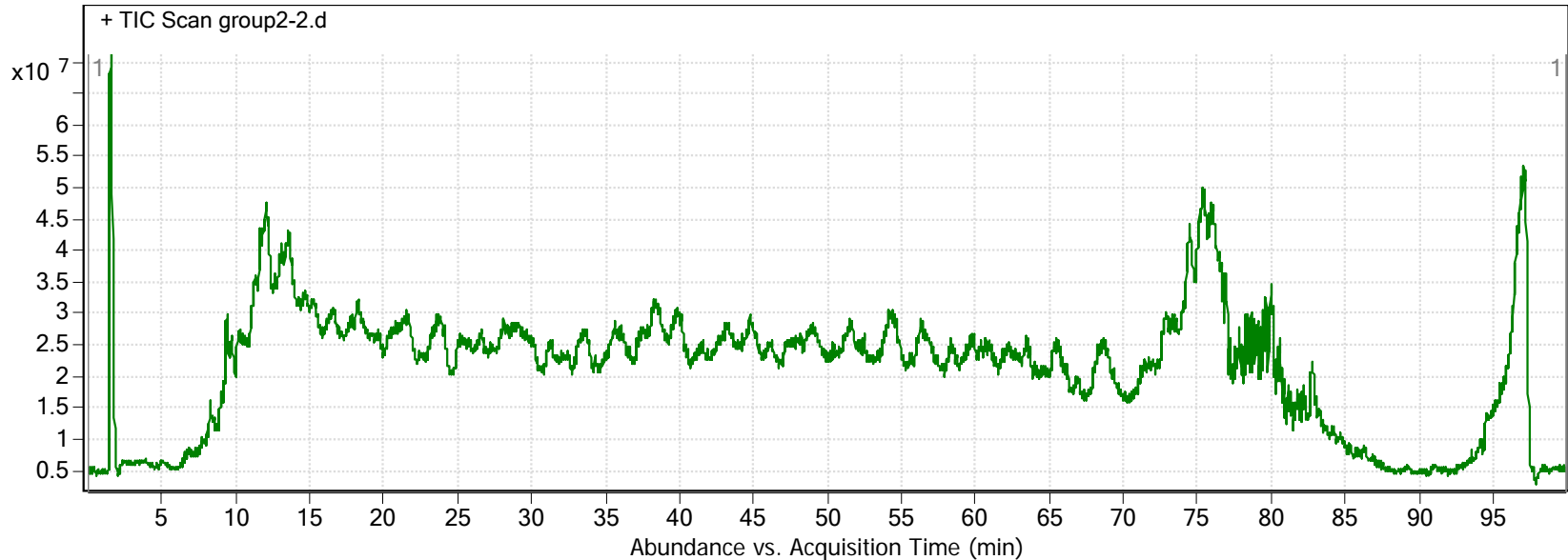
One injection of each sample (1  $\mu$ L injection volume)

Long (100 min injection-to-injection) method used

# Complex Model Study: Spiked *E. coli* Lysate

Sample	<i>E. coli</i> lysate (ng total protein)	BSA (fmol)	Serotransferrin (fmol)
1	400	25	200
2	400	50	100
3	400	100	50
4	400	200	25
5	400	400	5

# Complex Model Study: Spiked *E. coli* Lysate Total Ion Chromatogram

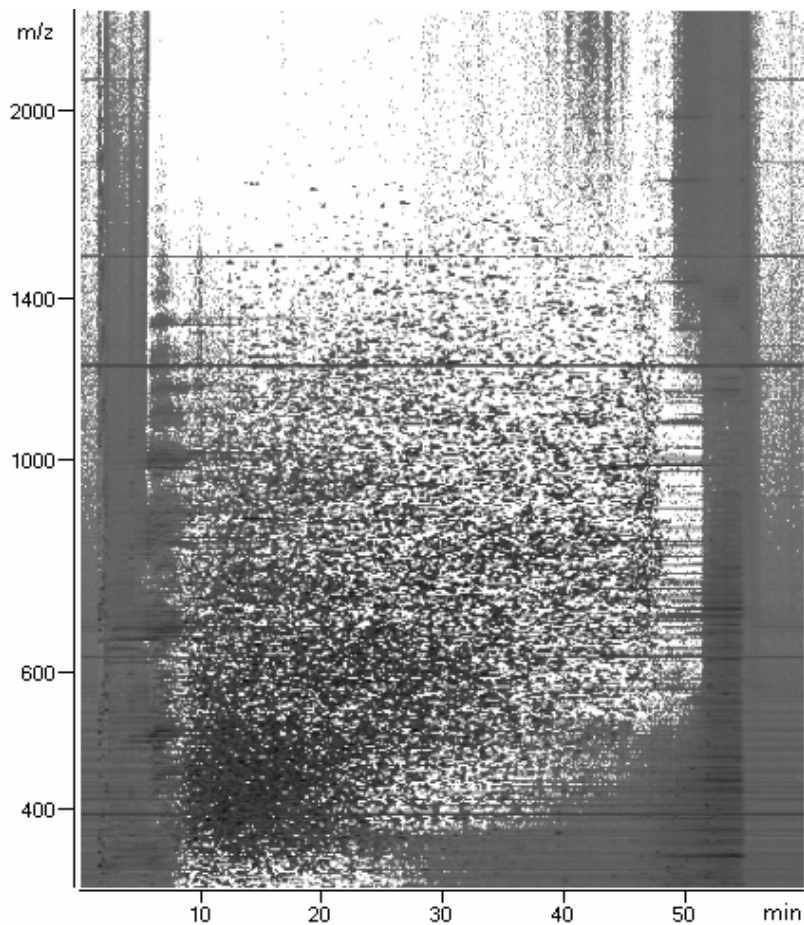


Digest of *E. coli* lysate creates a complex background of thousands of peptides

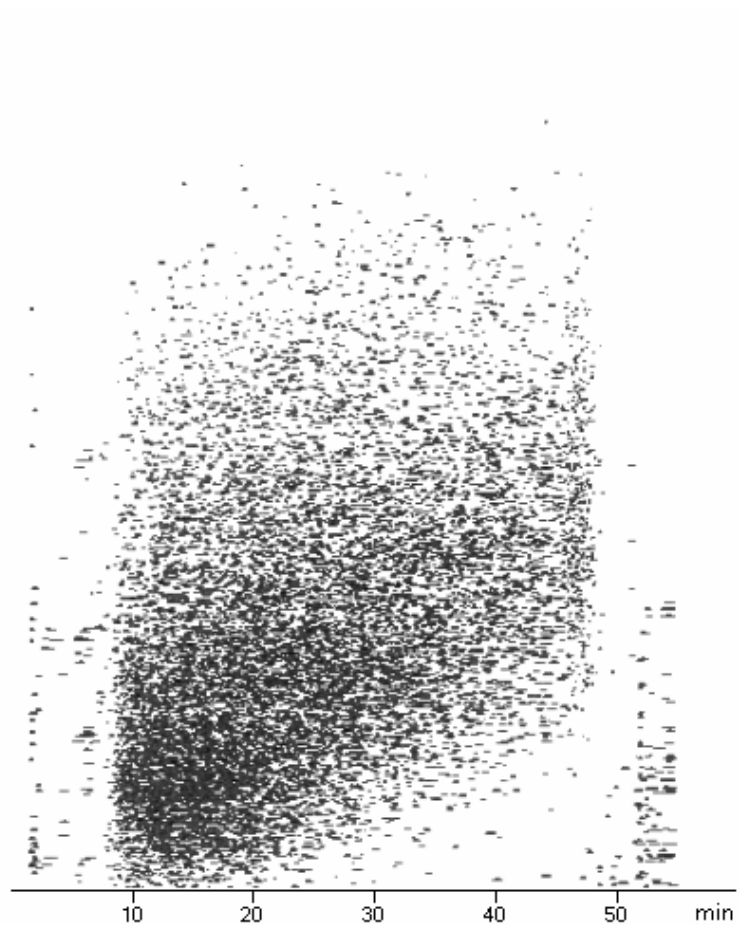
Profiling software must be able to find the bovine peptides that were spiked into the *E. coli* mixture

# Complex Model Study: Molecular Feature Extraction

Raw Data

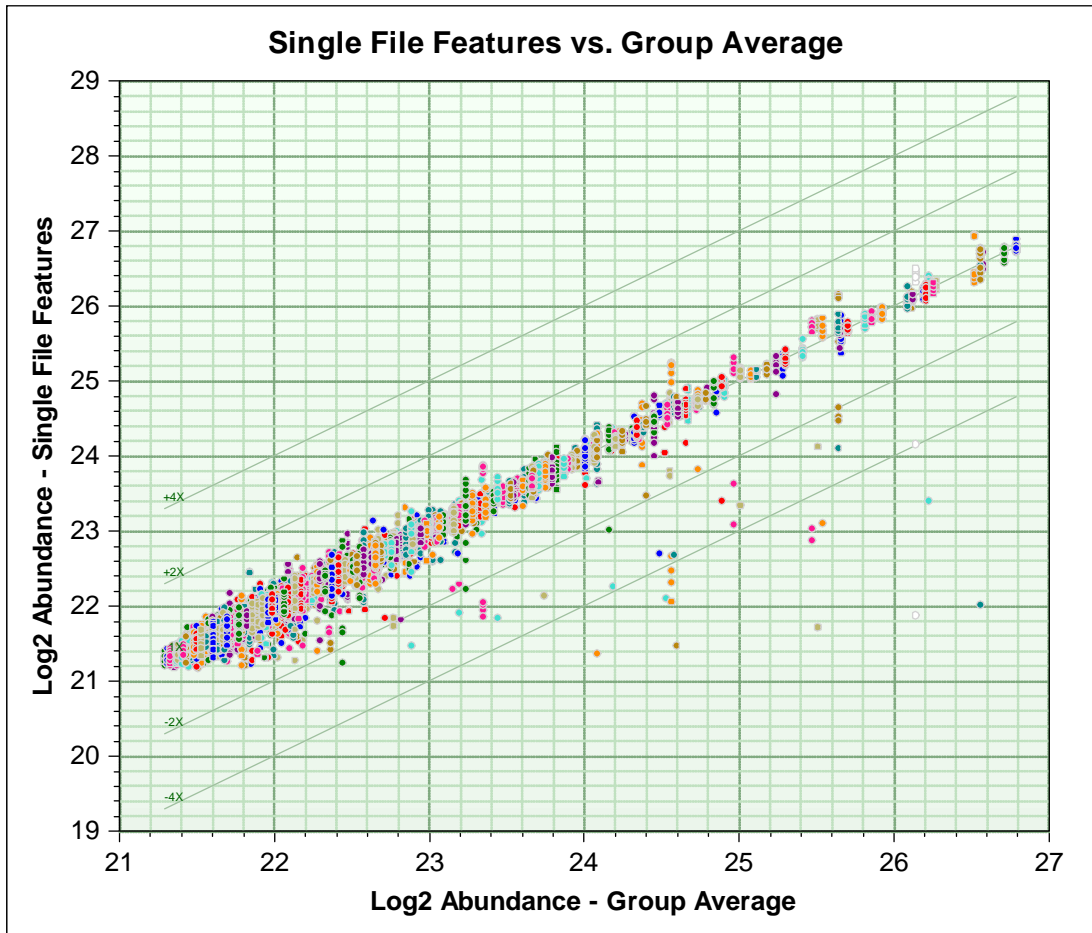


Extracted Features





# Complex Model Study: Reproducibility With Technical Replicates



Color-coding by molecular feature

Total of 762 features shown

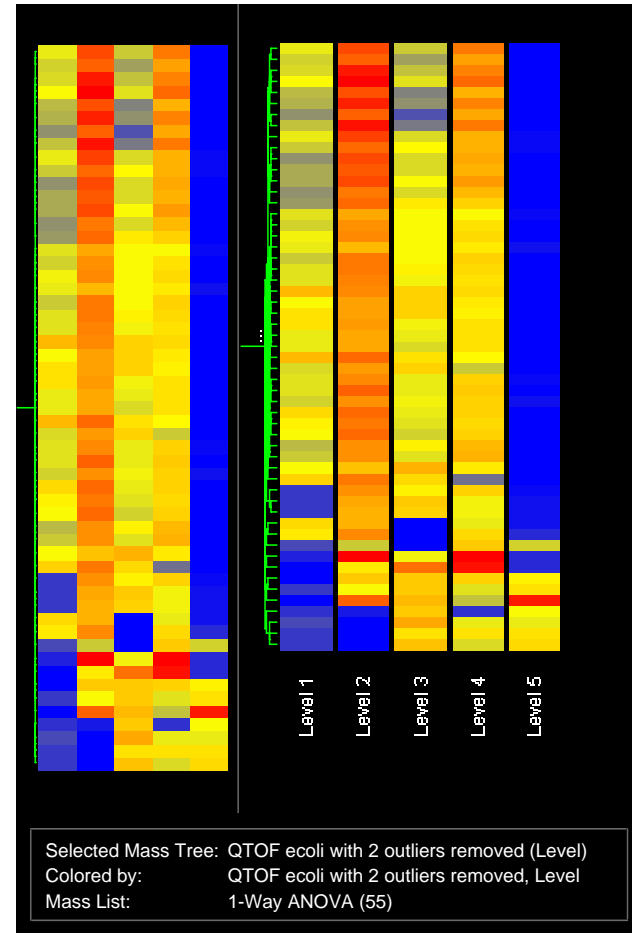
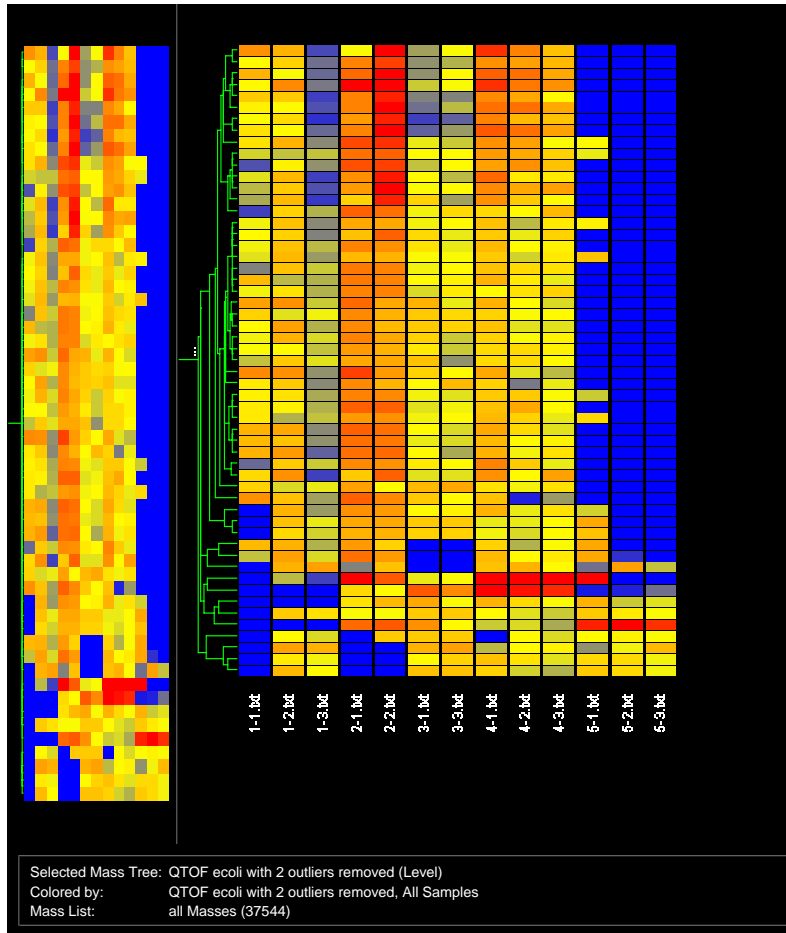
Average SD is  
0.0519 min for RT  
1.6 mDa for mass



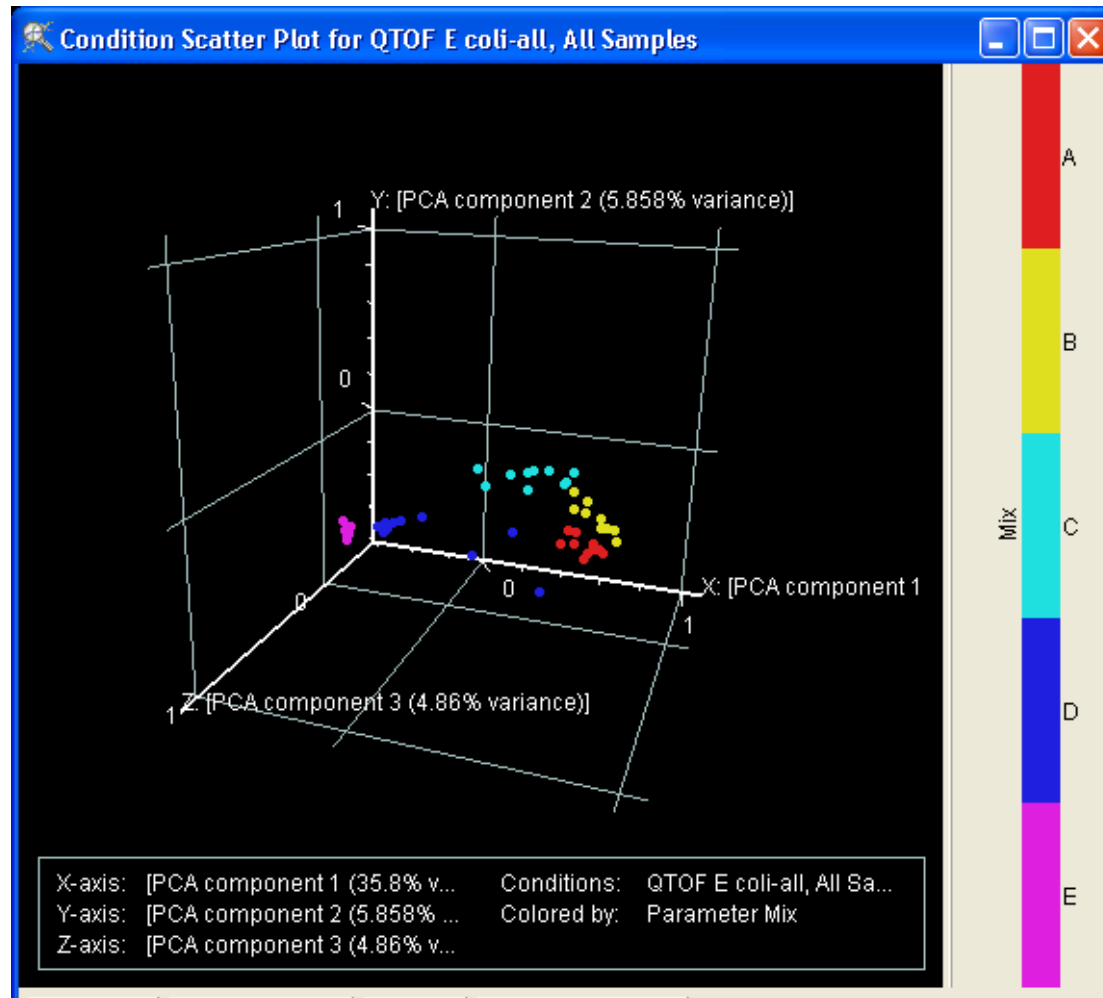
# Complex Model Study: Clustering of Features That Are Significantly Different (ANOVA)

Cluster by sample

Cluster by level



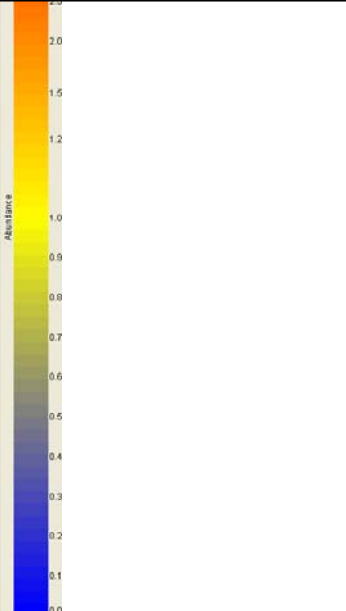
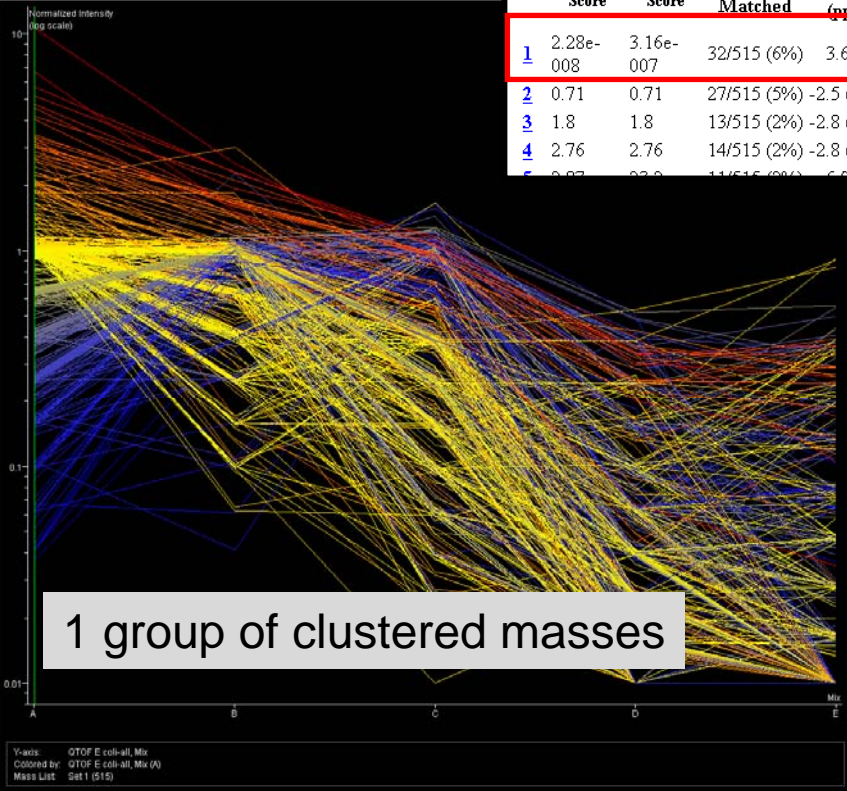
# Complex Model Study: Finding The Differential Features in GeneSpring MS



# Complex Model Study: K-Means Clustering of Differential Features And PMF Search

Sample ID (comment): [Enter Comment](#)  
 Database searched: [SwissProt.mammals](#) [Parameters used in Search](#)  
 Molecular weight search (1000 - 100000 Da) selects 31701 entries.  
 Full pI range: 35824 entries.  
 Combined molecular weight and pI searches select 31701 entries.  
 PMF search selects 25 entries.

Rank	Dynamic Probability Score	Static Probability Score	# (%) Masses Matched	Mass Error Mean (Std Dev) (ppm)	Protein Coverage	Protein MW (Da)/pI	Species	Accession #	Protein Name
1	2.28e-008	3.16e-007	32/515 (6%)	3.6 (6.6)	49%	77753.7/6.75	BOVIN	<a href="#">Q29443</a>	Serotransferrin precursor (Transferrin) (Siderophilin) (Beta-1-metal-binding globulin)
2	0.71	0.71	27/515 (5%)	-2.5 (10.9)	40%	75755.5/5.34	MOUSE	<a href="#">P14824</a>	Annexin A6 (Annexin VI) (Lipocortin VI) (P68) (P70) (Protein III) (Chromobindin-20) (67 kDa cal)
3	1.8	1.8	13/515 (2%)	-2.8 (11.4)	30%	46512.7/6.54	MOUSE	<a href="#">Q91WL8</a>	WW domain-containing oxidoreductase (EC 1.1.1.-)
4	2.76	2.76	14/515 (2%)	-2.8 (10.9)	28%	41450.6/10.65	HUMAN	<a href="#">Q75683</a>	Surfeit locus protein 6



**Create Inclusion List for QTOF** ✖

Select the parameters to create inclusion list

Select RT Tolerance

Narrow  Wide

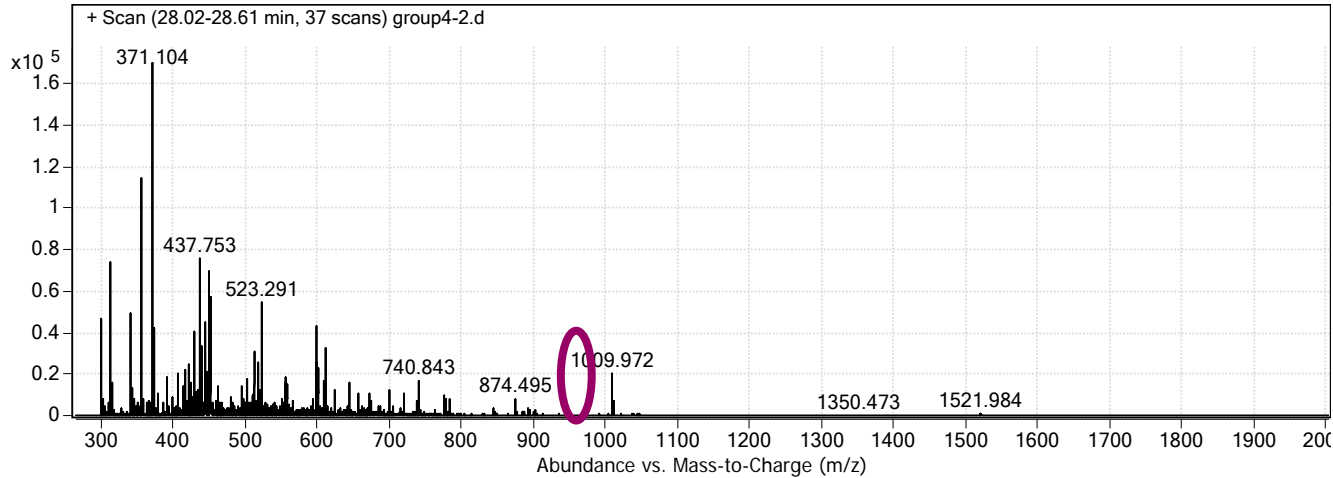
Select criteria for Ion selection

All Z states  
 Most Abundant  
 Prefer 2+ above threshold

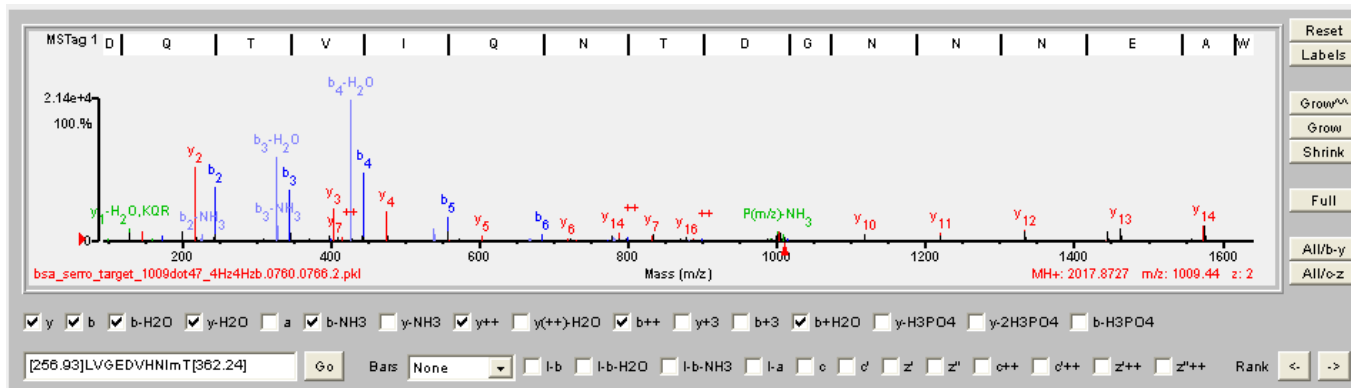
Select output file

Browse

# Complex Model Study: Mass Spectra From a Targeted Peptide



**Apex mass spectrum for a targeted species**



**Fragment ion assignments for targeted peptide (from transferrin)**

# Biomarker Discovery Using Protein Profiling: Summary

- Targeted biomarker discovery can reveal and identify proteins usually missed when using the shotgun data-dependent approach
- Label-free workflow saves cost during discovery
- Biomarker discovery software is essential for identifying candidates
  - Molecular feature extraction algorithm detects low level peaks in complex mixtures
  - MassHunter Profiling software determines differentially expressed features
  - GeneSpring MS software offers a powerful data analysis/visualization tools when analyzing large sample sets
  - Spectrum Mill Protein Identification software provides confident protein results to move onto the next steps of biomarker research

# Jump From Discovery Phase to Validation Phase

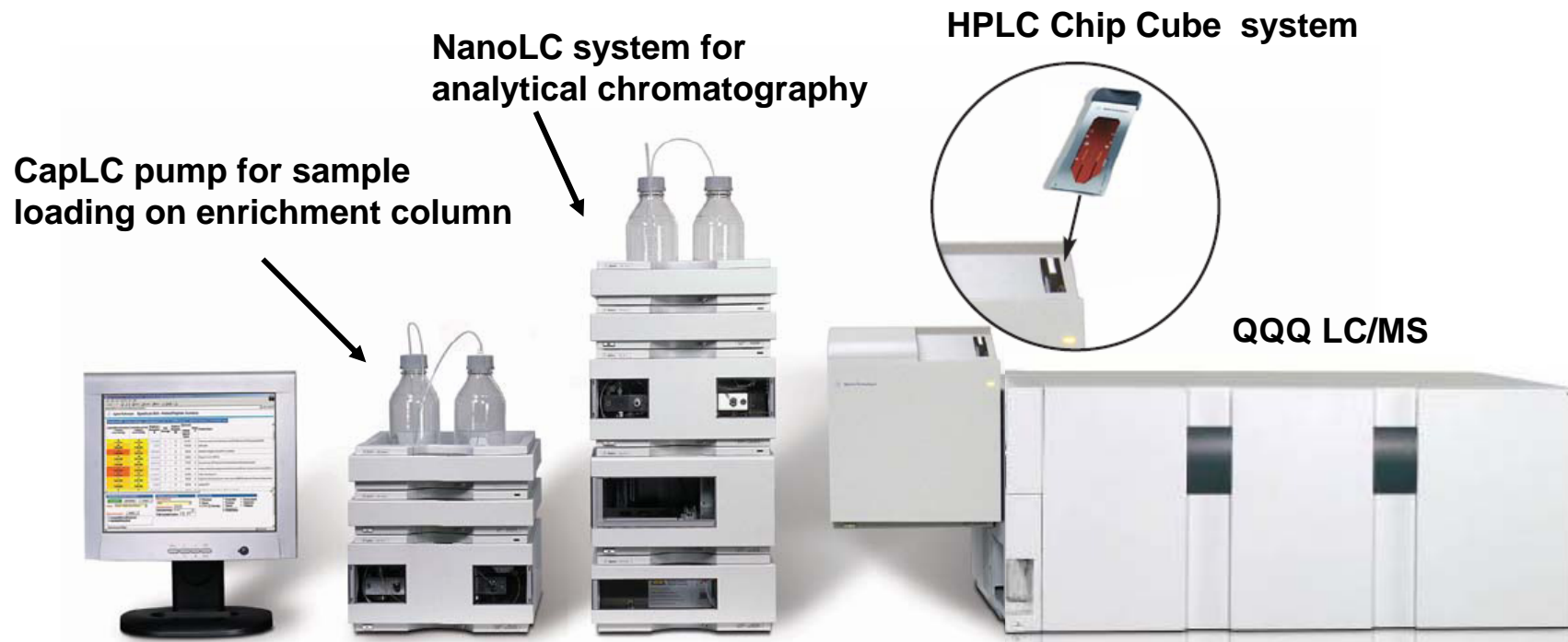
- Reduce the time needed for analysis
- Increase throughput
- Improve CV
- Reduce cost



	Research	Clinical
# of samples	<100	Hundreds - thousands
# of proteins	50-500	1-20
Time	Months - years	minutes - hours
Cost	\$100k-1M	\$10-100
CV	20-50%	3-5%

# Agilent HPLC-Chip/MS Technology

## Nanospray chip configuration brings new era in high sensitivity quantitation



**Sensitivity: down to low amol**  
**Dynamic range: up to  $10^5$**

# Agilent's New *Axial Acceleration* Collision Cell

Overcomes memory or cross-talk effects!

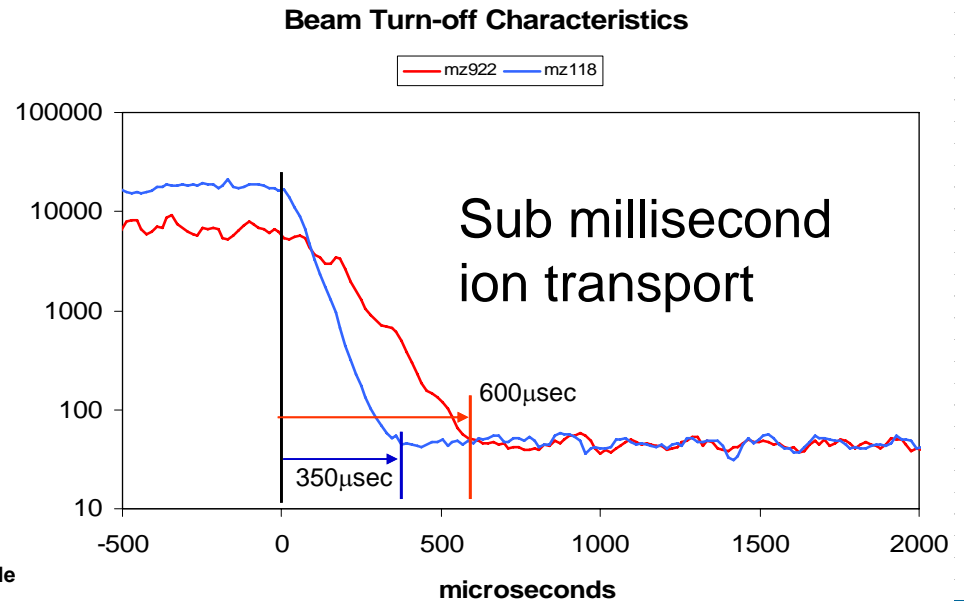
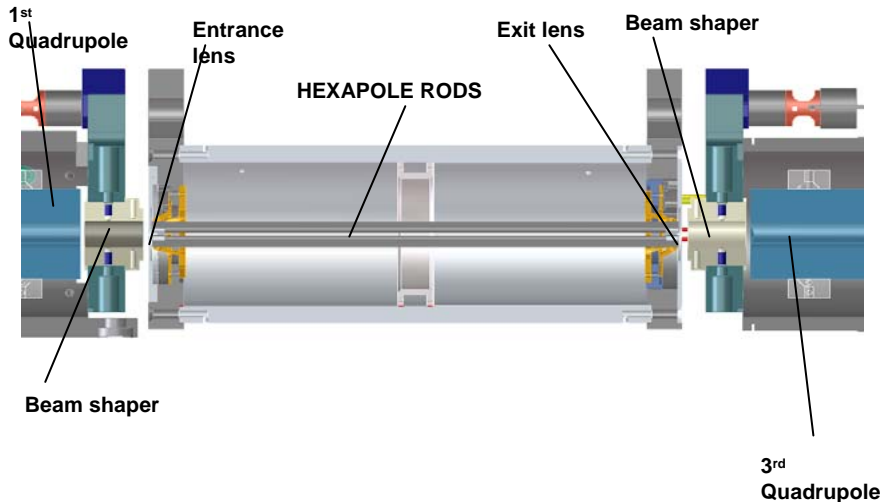
- using high speed ion transport

Maximum sensitivity

- using wide mass range hexapole design

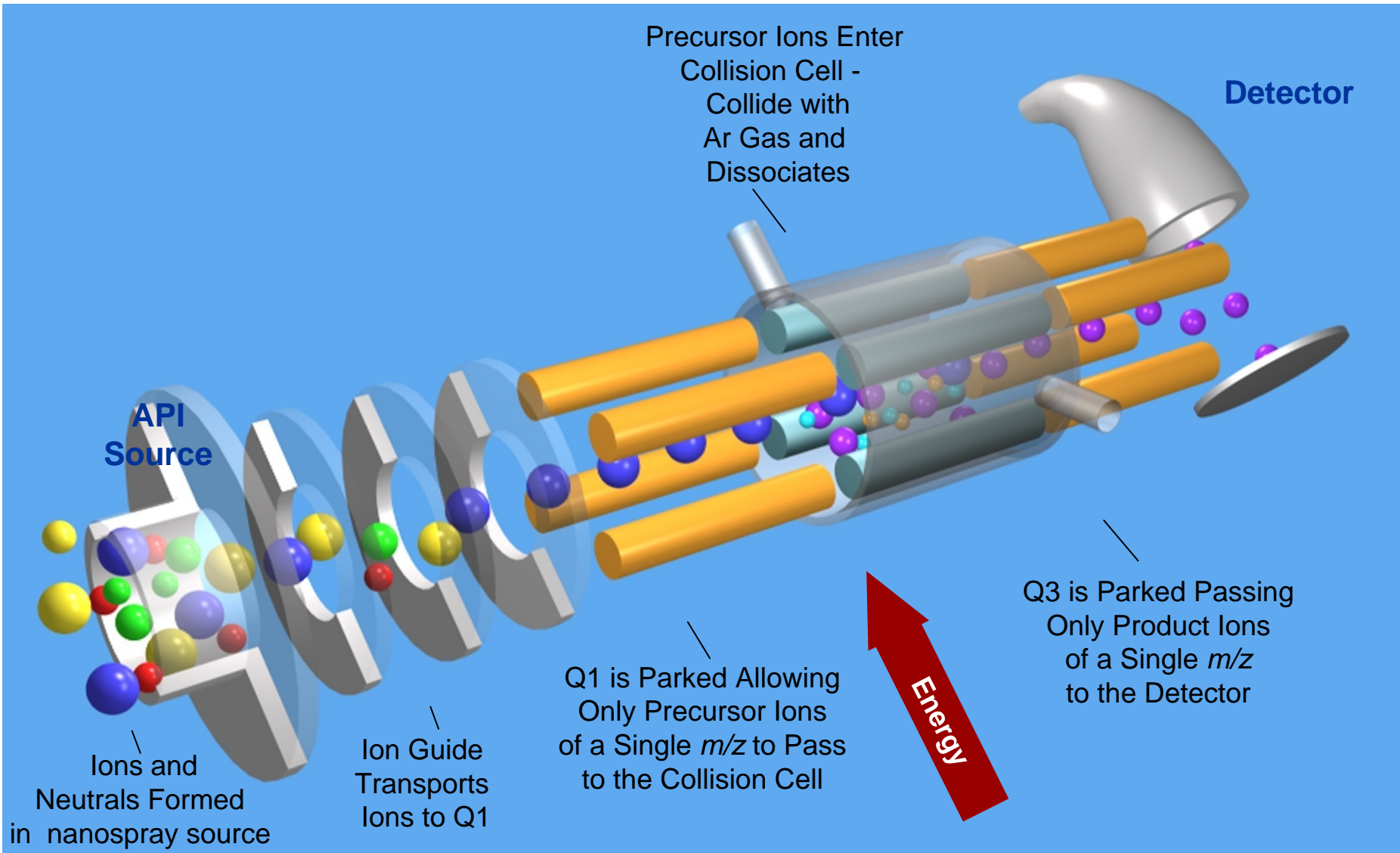
Simple to operate

- no complicated wave forms



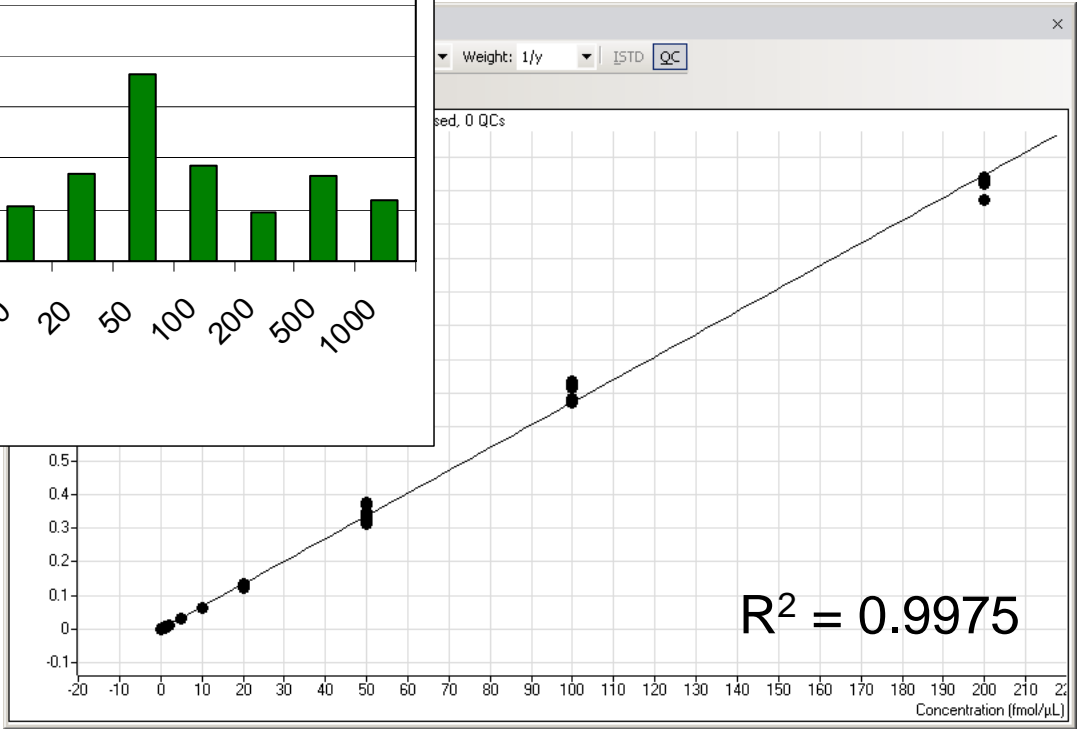
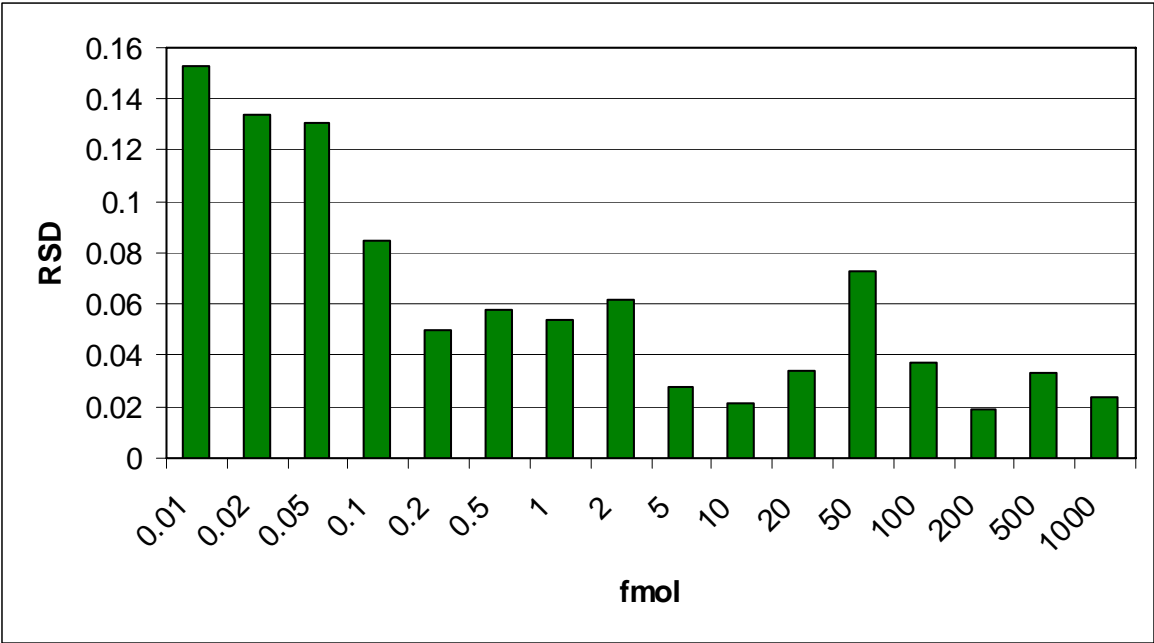


# Triple Quadrupole: SRM



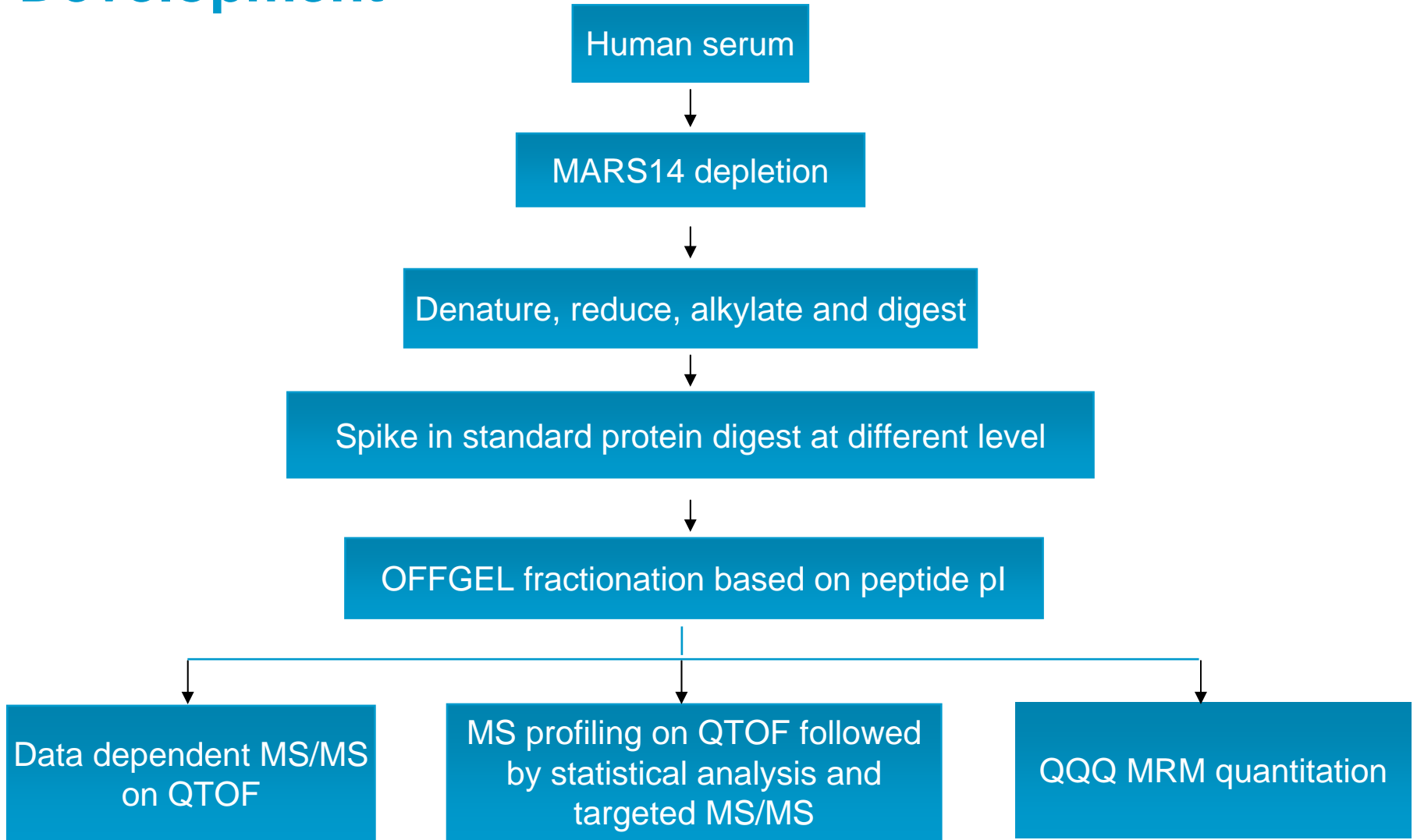
# Excellent Reproducibility of MS Response

## SRM of HSA Peptide LVNEVTEFAK from 10 amol to 1 pmol (n=6)

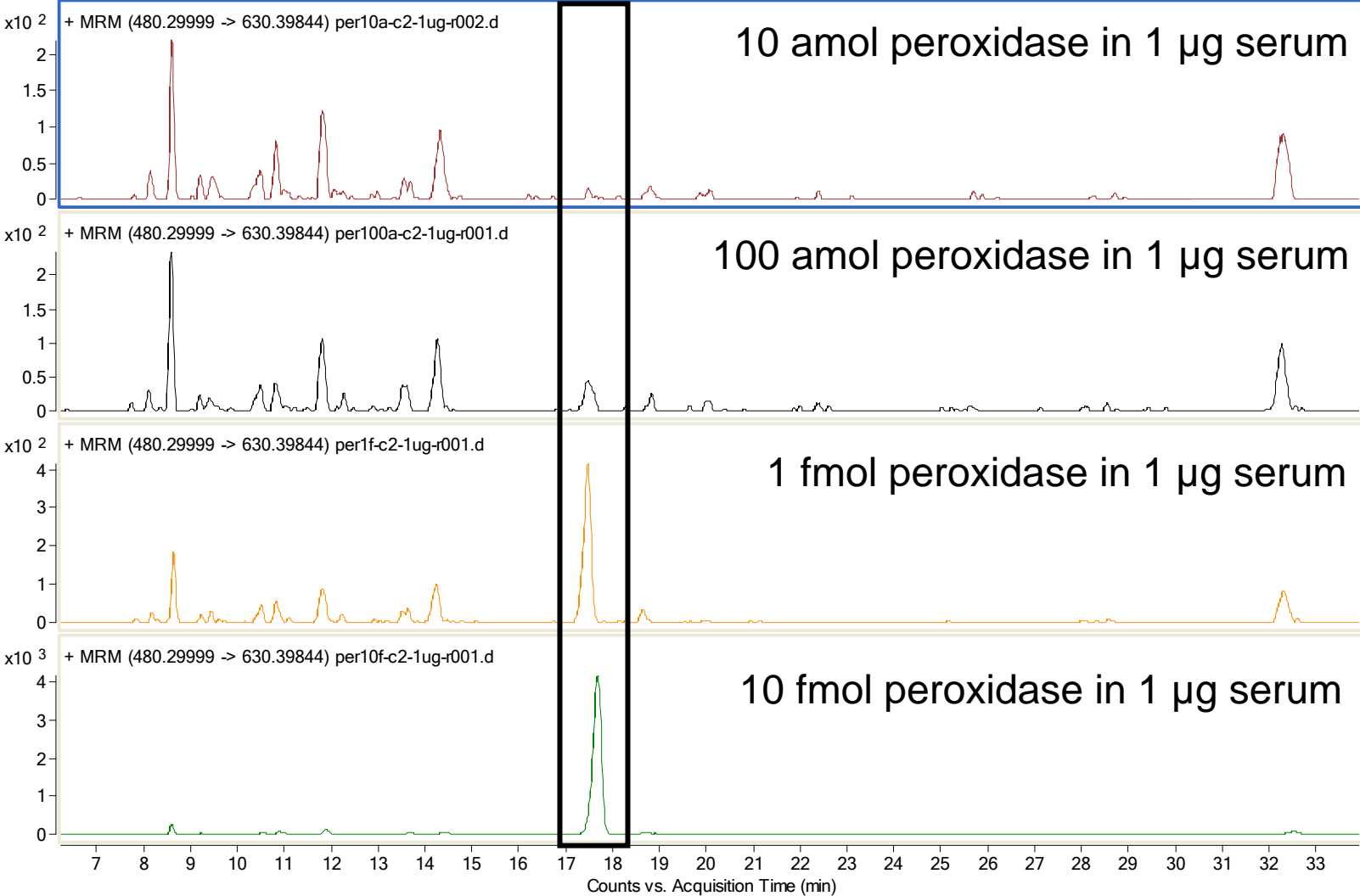


All RSDs are within 15%

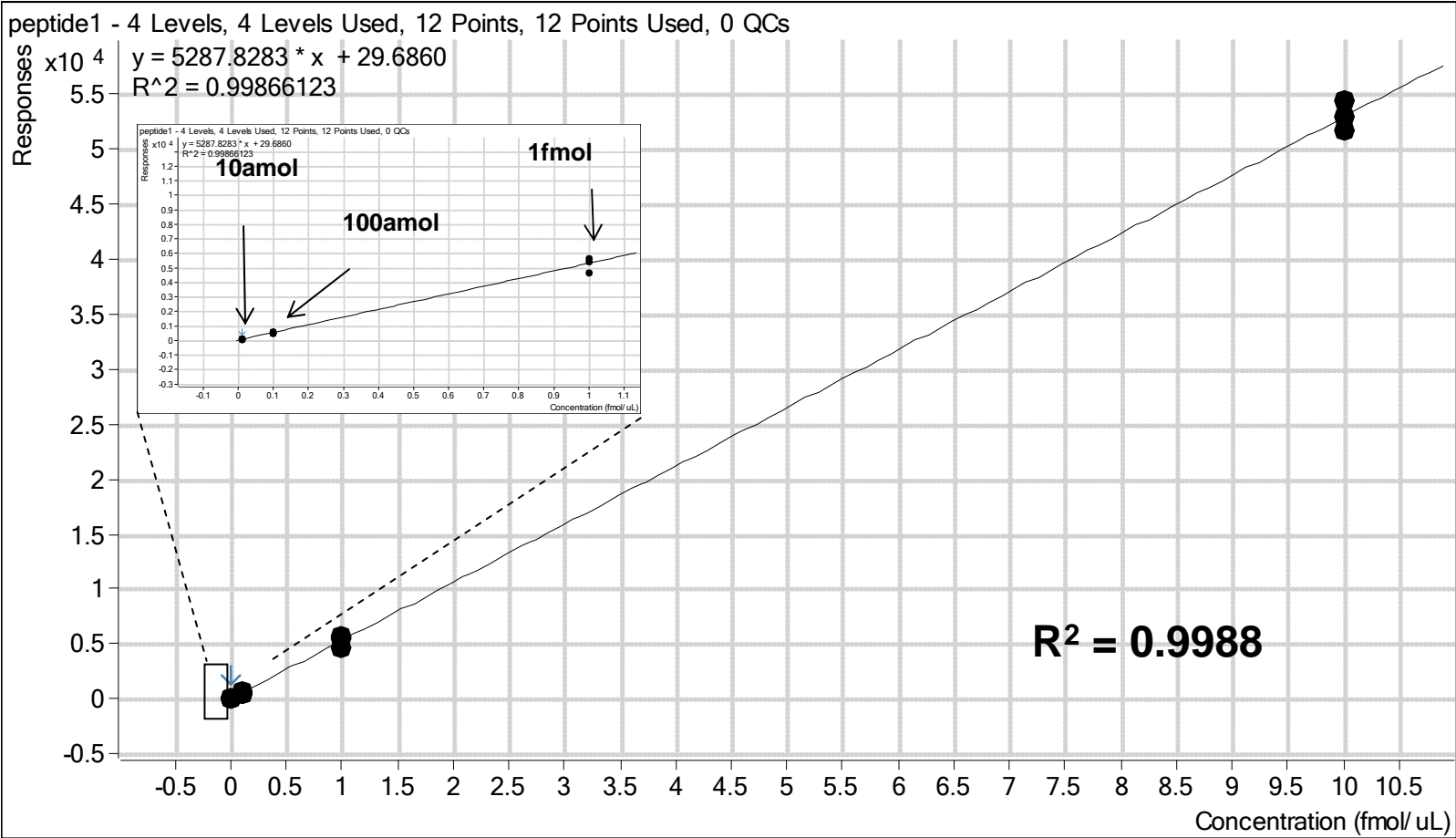
# Biomarker Discovery and Validation Method Development



# Limit of Quantitation for Peroxidase Spiked in Human Serum: 10 amol to 10 fmol



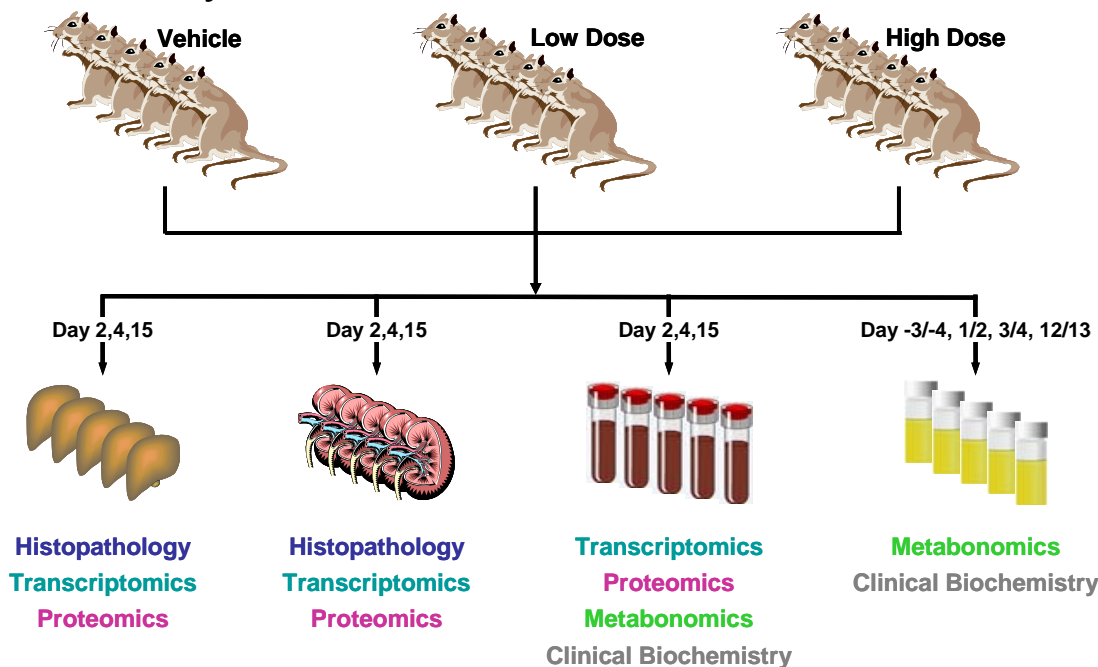
# External Quantitation Curve of Peroxidase Peptide DTIVNELR From 10 amol - 10 fmol Spiked in Human Serum



# Absolute Protein Quantification in the Context of Non-clinical Drug Safety Evaluation

InnoMed PredTox Consortium: 15 industrial and 3 academic partners

Goal: Assess value of combining “omics” data with traditional toxicology data for preclinical safety evaluation



Collins B. C. et al. ASMS 2008 MPQ 477

# Experimental Design

Catalase was selected based on previous 2D-DIGE data



Peptides and MRM transitions were selected using Peptide Selector in Spectrum Mill and  $^{13}\text{C}$ ,  $^{15}\text{N}$  labeled peptides were synthesized



The liver digest were spiked with the isotope-labeled peptides and analyzed by Agilent 6410 QQQ system

Rat liver lysate were prepared from rats treated with troglitazone (hepatotoxicant) or vehicle control



1 mg of soluble protein extract was reduced, alkylated, acetone precipitated and trypsin digested



# Using Spectrum Mill Peptide Selector for Optimizing MRM Transitions

**Chemically reactive residues**  
(Cys = C, Met = M, Trp = W)

**Residues with variable PTM**

**Peptides adjacent to multiple cleavage site**

**Size of the peptides**

**Uniqueness of the sequence in the database**

The screenshot shows the 'Peptide Selector' web application in Microsoft Internet Explorer. The browser title is 'Peptide Selector - Agilent Spectrum Mill - Microsoft Internet Explorer provided by Agilent Technologies, Inc.'. The address bar shows 'http://smapps/millhtml/mssluiuce.htm'. The application has a navigation menu with 'Spectrum Mill', 'MS Edman', 'Multiple Sequence Aligner', 'Databases', 'MS Digest', and 'Help'. The 'Selection' section includes a 'Select' button and a checkbox for 'Hide HTML links (better Excel cut/paste)'. The 'Digest Parameters' section has 'Digest: Trypsin' and 'Maximum # missed cleavages: 0'. The 'Product Ion Parameters' section has a checked 'Show Product Ion Masses' checkbox. The 'Criteria for Excluding Peptides' section includes 'Maximum # basic residues (RHK): 1', 'Minimum peptide MH\*: 900.0', and 'Maximum peptide MH\*: 2800.0'. The 'Peptide exclusion criteria' section has checked boxes for 'Has nearby cleavage site within 3 residues' and 'Contains peptide N-terminal Gln to pyroGlu'. The 'AA Composition Filtering' section has 'Required AAs: KR' and 'Disallowed AAs: CM'. The 'Modifications' section has 'Fixed: Carbamidomethylation (C)'. The 'Protein(s) to Select From' section has 'Database: SwissProt' and a list of protein accession numbers, with 'P00433' selected. The 'Search Mode' section has 'Count Peptide Uniqueness in Database by: None' and 'Species: All'. The bottom right corner of the browser shows 'Local intranet'.



# Peptide Selector – Catalase Results

**Peptide Selector Results**

Number of database entries: 211104  
 Database: SwissProt  
 Exclusion criteria: | Peptide N-terminal Gln to pyroGlu |  
 Required AA's: KR Disallowed AA's: CM  
 Exclude if Nearby Cleavage Sites within 3 residues

Digest Used: Trypsin  
 Max. # Missed Cleavages: 0  
 Fixed Mods: carbamidomethylation  
 Peptide N terminus: Hydrogen (H)  
 Peptide C terminus: Free Acid (O H)  
 Masses are Monoisotopic  
 Uniqueness Count by Sequence

Protein Name: Catalase (EC 1.11.1.6)  
 Species: RAT  
 SwissProt Accession #: P04762  
 MS-Digest Index #: 18675  
 pI of Protein: 7.15  
 Protein MW: 59626.3 Da  
 Amino Acid Composition: A42 C68 D36 E25 F31 G35 H21 I20 K30 L31 M12 N33 P35 Q26 R30 S24 T25 V33 W6 Y21

Protein Name	Acc #	RP-RT	MH+	m/2	# DB peps	Start AA	End AA	Prev. Sequence	Next	b <sub>2</sub>	y <sub>n</sub> - y <sub>1</sub>	C-side of Asp, Glu N-side of Pro						
Catalase (EC 1.11.1.6)	P04762	13.63	984.5109	492.7591	1	243	251	(GIK) <a href="#">NLPVVEAGR</a>	(LAQ)	228.14	870.47	757.38	660.33	561.26	432.22	303.18	232.14	175.1
Catalase (EC 1.11.1.6)	P04762	18.97	1001.5666	501.2869	6	306	314	(PHK) <a href="#">DYPLIPVGK</a>	(LVL)	279.10	886.54	723.48	626.42	513.34	400.26	303.20	204.13	147.1
Catalase (EC 1.11.1.6)	P04762	19.22	1276.6168	638.8120	2	252	262	(AGR) <a href="#">LAQEDPDYGLR</a>	(DLF)	185.13	1163.53	1092.50	964.44	835.39	720.37	623.31	508.29	345.2
Catalase (EC 1.11.1.6)	P04762	22.02	1655.7952	828.4012	2	287	300	(TFK) <a href="#">EAETFPFNPDLTK</a>	(VWP)	201.09	1526.75	1455.72	1326.67	1225.63	1078.56	981.50	834.44	720.3
Catalase (EC 1.11.1.6)	P04762	26.89	2518.2038	1259.6055	7	135	155	(AVK) <a href="#">FYTEDGIVLVGNNTPIFFIR</a>	(DAM)	311.14	2371.14	2208.07	2107.02	1977.98	1862.95	1805.93	1691.89	1505.8
Catalase (EC 1.11.1.6)	<a href="#">P04762</a>				9													

1 ADSRDPASDQ MKQWKEQRAP QKPDVLTIGG GNPIGDKLNI MTAGPRGPLL VQDVVFIDEM AHFDRERIEP RVVHAKGAKA 80  
 81 FGYFEVTHDI TRYSKAKVFE HIGKRTPIAV RFSTVAGESG SADTVRDRPG FAVK**FYTEDG** **NWDLVGNNT**P **IFFIR**DAMLF 160  
 161 PSFIHSQQRN PQTHLKDPDM VWDFWSLQPE SLHQVTFILFS DRGIPDGRHR MNGYGSHTFK LVNANGEAVY CKFHYITDQG 240  
 241 **I****K****N**L**P**V**E**E**A**G **R**L**A**Q**E**D**P**D**Y**G **L**R**D**L**F**N**A**I**A**S GNYPSWTFYI QVMIFK**E**A**E**T **F**P**F**N**P**F**D**L**T**K **V**W**P**H**K**D**Y**P**L**I **P**V**G**K**L**V**L**N**R**N 320  
 321 PANYFAEVEQ MAFDPSNMPP GIEPSPDKML QGRLFAYPDT HRHRLGPNYL QIPVNCYRA RVANYQRDGP MCMHDNQGGA 400  
 401 PNYYPNSFSA PEQQGSALEH HSQCSADV**K**R FNSANED**N**V**T** QVR**T**F**Y**T**K**V**L** NEEER**K**R**L**C**E** NIANHLKDAQ LFIQRKAV**K**N 480  
 481 **F**T**D**V**H**P**D**Y**G**A **R**V**Q**A**L**L**D**Q**Y**N **S**Q**K**P**K**N**A**I**H**T **Y**V**Q**A**G**S**H**I**A**A **K**G**K**A**N**L 526

The matched peptides cover 12% (64/526 AA's) of the protein.

303.18	232.14	175.12					
303.20	204.13	147.11					
623.31	508.29	345.22	288.20	175.12			
981.50	834.44	720.39	623.34	476.27	361.24	248.16	147.11
1805.93	1691.89	1505.81	1390.78	1277.70	1178.63	1121.61	1007.57

Peptide Selector - Agilent Spectrum Mill Rev. 3.3.078

# Catalase Peptide EAE – Peptide Selector

Peptide Sequence: EAETFFNPFDLTK  
 Peptide Mass MH<sup>+</sup>(Average): 1656.84  
 Peptide Mass MH<sup>+</sup>(Monoisotopic): 1655.7952  
 Elemental Composition: [C78 H111 N16 O24](#)  
 Amino Acid Composition: A1 D1 E2 F3 K1 L1 N1 P2 T2

Fixed Modifications: Carbamidomethylation  
 Variable Modifications: PTM - KMQSTY  
 All fragment ion masses below are calculated as: **Monoisotopic** masses  
 (MH)<sup>+</sup>2(Average): 828.9225  
 (MH)<sup>+</sup>2(Monoisotopic): 828.4012

**N-terminal ions**

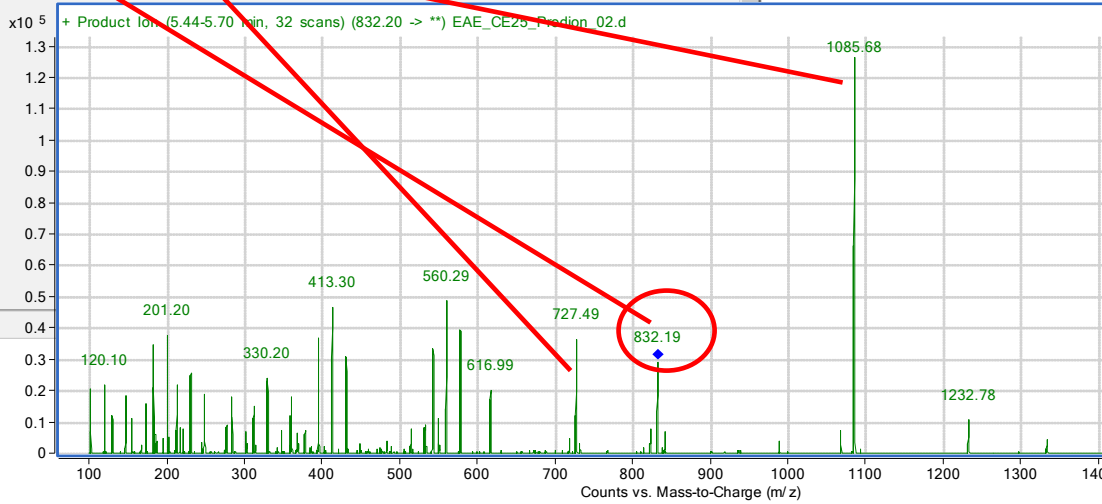
b ions	130.0510	201.0881	330.1307	431.1784	578.2468	675.2995	822.3679	936.4109	1033.4636	1180.5320	1295.5590	1408.6431	1509.6907			
	---	---	---	---	---	---	---	468.7091 <sup>+2</sup>	517.2355 <sup>+2</sup>	590.7697 <sup>+2</sup>	648.2831 <sup>+2</sup>	704.8252 <sup>+2</sup>	755.3490 <sup>+2</sup>			
	1	2	3	4	5	6	7	8	9	10	11	12	13			
	H -	E	A	E	T	F	P	F	N	P	F	D	L	T	K	-OH
	14	13	12	11	10	9	8	7	6	5	4	3	2	1		

**C-terminal ions**

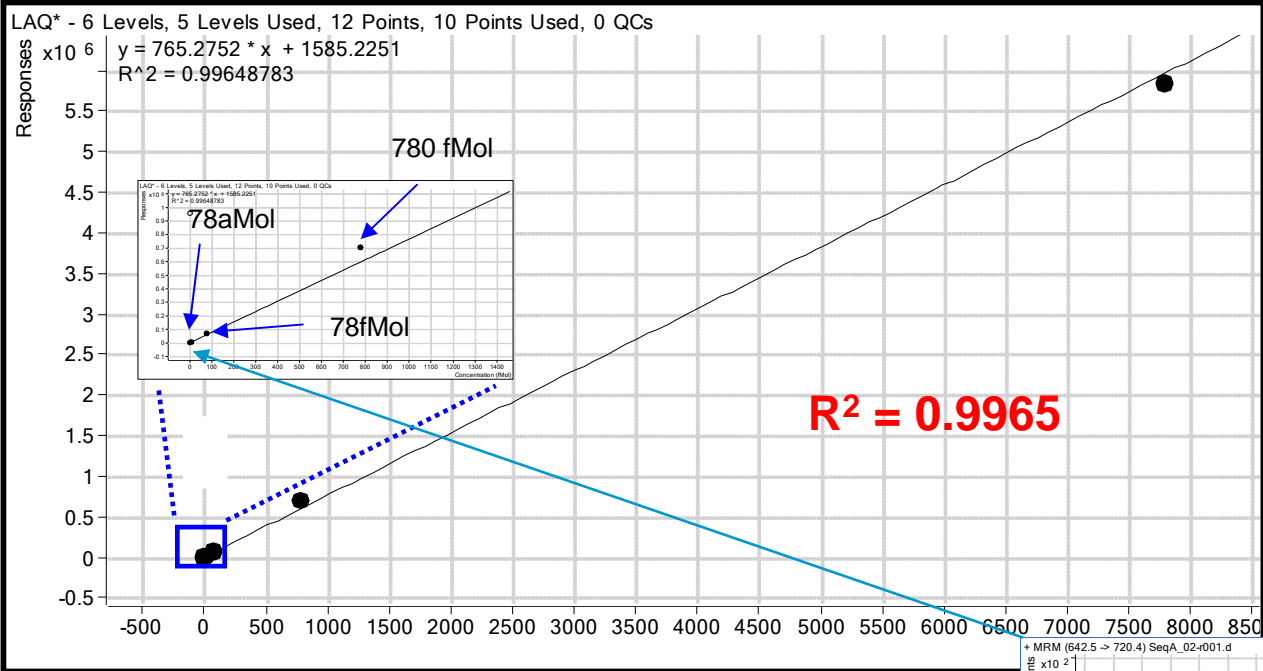
y ions	1526.7526	1455.7155	1326.6729	1225.6252	1078.5568	981.5040	834.4356	720.3927	623.3399	476.2715	361.2445	248.1605	147.1128
	763.8799 <sup>+2</sup>	728.3614 <sup>+2</sup>	663.8401 <sup>+2</sup>	613.3162 <sup>+2</sup>	539.7820 <sup>+2</sup>	491.2556 <sup>+2</sup>	447.7214 <sup>+2</sup>	360.7000 <sup>+2</sup>	312.1736 <sup>+2</sup>	238.6394 <sup>+2</sup>	181.1259 <sup>+2</sup>	124.5839 <sup>+2</sup>	74.0600 <sup>+2</sup>

y <sub>1</sub> <sup>+2</sup>	74.0600 <sup>+2</sup>	b <sub>3</sub>	330.1307	y <sub>9</sub> <sup>+2</sup>	539.7820 <sup>+2</sup>	y <sub>6</sub>	720.3927	y <sub>9</sub>	1078.5568
y <sub>2</sub> <sup>+2</sup>	124.5839 <sup>+2</sup>	y <sub>6</sub> <sup>+2</sup>	360.7000 <sup>+2</sup>	b <sub>5</sub>	578.2468	y <sub>12</sub> <sup>+2</sup>	728.3614 <sup>+2</sup>	b <sub>10</sub>	1180.5320
b <sub>1</sub>	130.0510	y <sub>3</sub>	361.2445	b <sub>10</sub> <sup>+2</sup>	590.7697 <sup>+2</sup>	b <sub>13</sub> <sup>+2</sup>	755.3490 <sup>+2</sup>	y <sub>10</sub>	1225.6252
y <sub>1</sub>	147.1128	y <sub>7</sub> <sup>+2</sup>	417.7214 <sup>+2</sup>	y <sub>10</sub> <sup>+2</sup>	613.3162 <sup>+2</sup>	y <sub>13</sub> <sup>+2</sup>	763.8799 <sup>+2</sup>	b <sub>11</sub>	1295.5590
y <sub>3</sub> <sup>+2</sup>	181.1259 <sup>+2</sup>	b <sub>4</sub>	431.1784	y <sub>5</sub>	623.3399	b <sub>7</sub>	822.3679	y <sub>11</sub>	1326.6729
b <sub>2</sub>	201.0881	b <sub>8</sub> <sup>+2</sup>	468.7091 <sup>+2</sup>	b <sub>11</sub> <sup>+2</sup>	648.2831 <sup>+2</sup>	y <sub>7</sub>	834.4356	b <sub>12</sub>	1408.6431
y <sub>4</sub> <sup>+2</sup>	238.6394 <sup>+2</sup>	y <sub>4</sub>	476.2715	y <sub>11</sub> <sup>+2</sup>	663.8401 <sup>+2</sup>	b <sub>8</sub>	936.4109	y <sub>12</sub>	1455.7155
y <sub>2</sub>	248.1605	y <sub>8</sub> <sup>+2</sup>	491.2556 <sup>+2</sup>	b <sub>6</sub>	675.2995	y <sub>8</sub>	981.5040	b <sub>13</sub>	1509.6907
y <sub>5</sub> <sup>+2</sup>	312.1736 <sup>+2</sup>	b <sub>9</sub> <sup>+2</sup>	517.2355 <sup>+2</sup>	b <sub>12</sub> <sup>+2</sup>	704.8252 <sup>+2</sup>	b <sub>9</sub>	1033.4636	y <sub>13</sub>	1526.7526

MS Product - Agilent Spectrum Mill Rev. 3.3.078

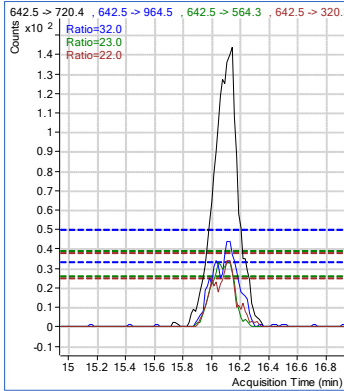
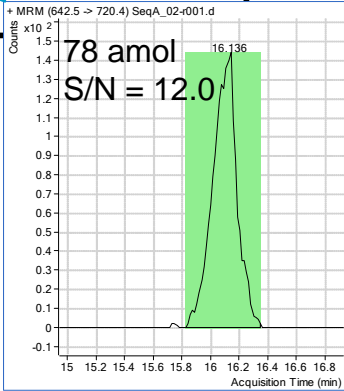


# External Calibration on Catalase Peptide Linearity : five order of magnitude



**RSD < 6%**

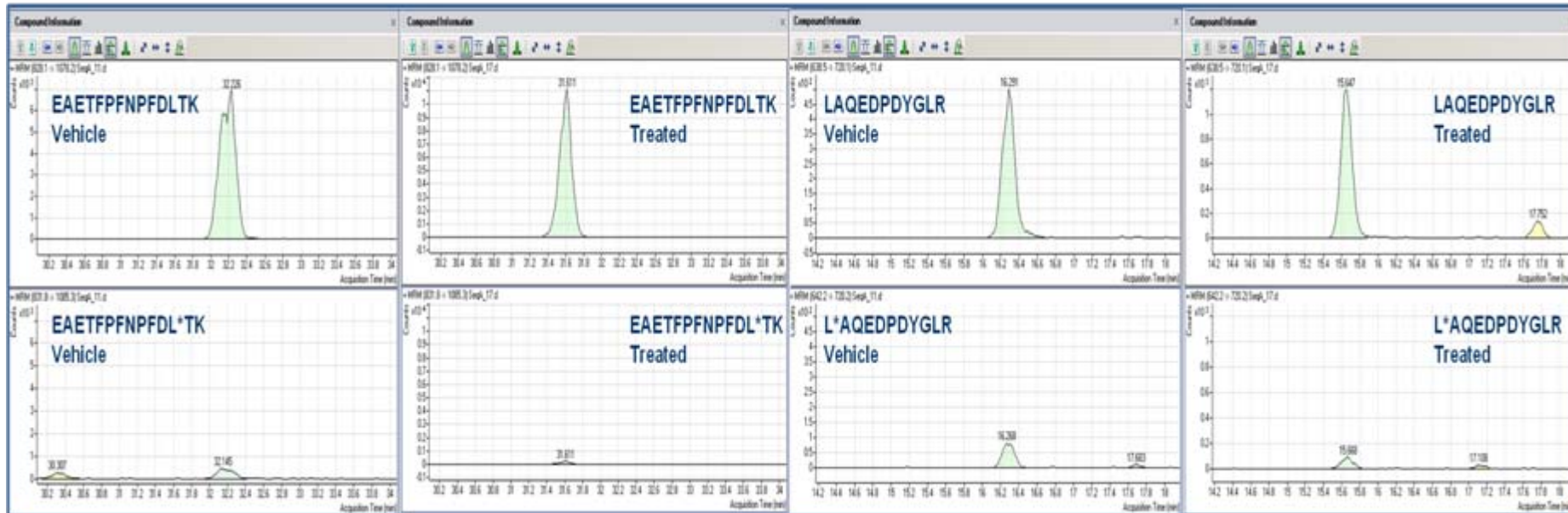
External quantitation curve of catalase peptide L\*AQEDPDYGLR from 78 amol to 7800 fmol



# Catalase Quantitation Results

Sample						EAE Met..	EAE Results							Qualifier (828.1 -> 720.1) Results			EAE* (ISTD) Results		Qualifier (831.8 -> 727.3) Results		
?	∇	Name	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	S/N	MI	RT	Resp.	Ratio	S/N	MI
		RL_Veh_1ppb	Cal	1	2/27/2008 7:49 AM		32.226	85633	169.81		14.6295	14.6295		10.9	44.30		32.145	5853	20.6	2.71	
		RL_HDose_1ppb	Cal	1	2/27/2008 3:09 PM		31.611	104655	298.12		54.3647	54.3647		10.6	71.79		31.611	1925	11.2	1.03	

Sample						LAQ Met..	LAQ Results							Qualifier (638.5 -> 964.2) Results			LAQ* (ISTD) Results		Qualifier (642.2 -> 964.2) Results		
?	∇	Name	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	S/N	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	S/N	MI	RT	Resp.	Ratio	S/N	MI
		RL_Veh_1ppb	Cal	1	2/27/2008 7:49 AM		16.291	4781	71.89		5.8898	5.8898		55.4	35.63		16.268	812	49.9	13.65	
		RL_HDose_1ppb	Cal	1	2/27/2008 3:09 PM		15.647	10560	77.02		12.9958	12.9958		51.2	50.83		15.668	813	44.1	6.34	



# Catalase Quantitation Results

Sample	Peptide	Catalase (fmol/ $\mu$ g protein)	Catalase (pg/ $\mu$ g protein)	Fold Change EAETFPFNPFDLTK	Fold Change LAQEDPDYGLR	Fold Change 2D-DIGE
Vehicle Treated	EAETFPFNPFDLTK	8.84	14.63	1.00	1.00	1.00
	LAQEDPDYGLR	4.61	5.89			
Troglitazone Treated	EAETFPFNPFDLTK	32.69	54.36	3.7	2.2	1.45
	LAQEDPDYGLR	10.13	13.00			

# HPLC-Chip/MS System For Biomarker Validation

Offers high sensitivity and large dynamic range

Provides robust and stable nanoflow with HPLC-Chip

Demonstrates good retention time and MS reproducibility

Peptide Selector helps determine SRM transitions

# Acknowledgements

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Albrecht Gruhler

## **Sanofi-Aventis**

Jean-Charles Gautier

## **Agilent Technologies**

Ning Tang

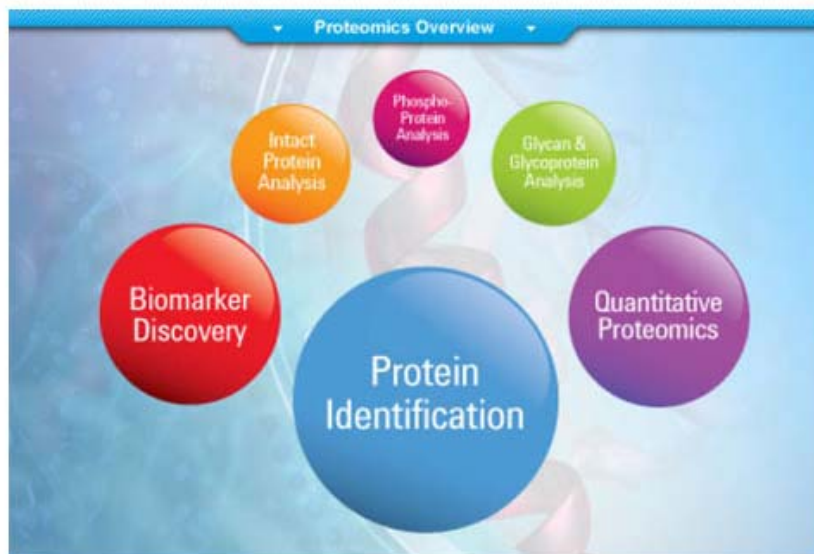
Peter Stone



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