

Agilent Solutions for Proteomics



Christine Miller
Senior Application Scientist
Santa Clara, CA

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Technologies for Proteomics Analysis

Sample preparation

HPLC-Chip

Q-TOF and QQQ Mass Spectrometers



Protein Analysis Workflow

Sample Prep

Extraction of unique proteins or from different samples



Extraction Reagents – FFPE & MS Detergent



Proteases

Standards for Validation & Quantitation

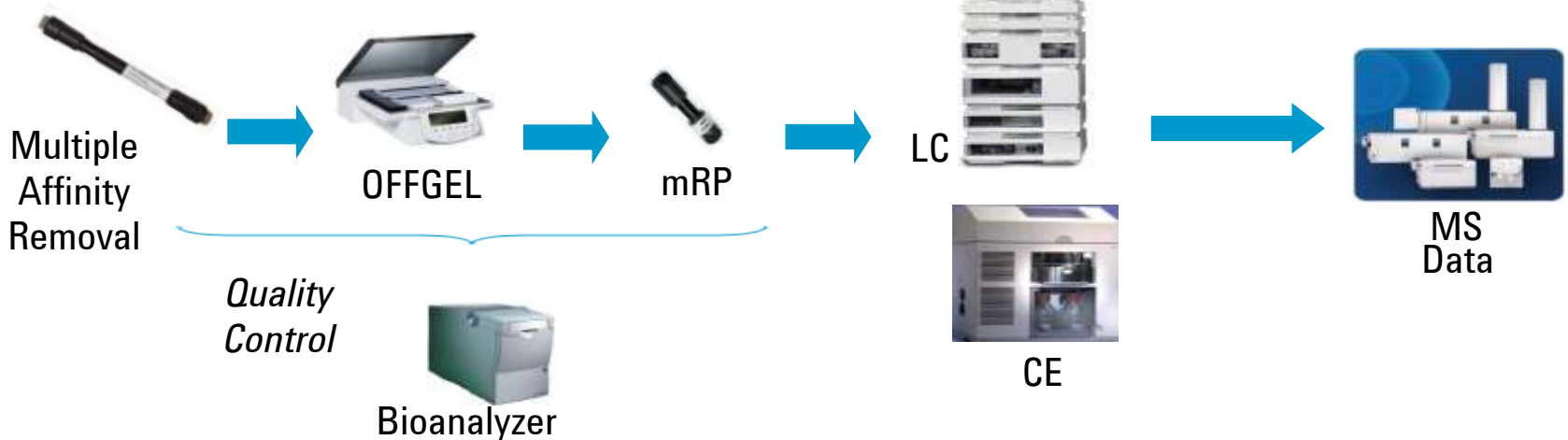
Simplify

Fractionate

Separate

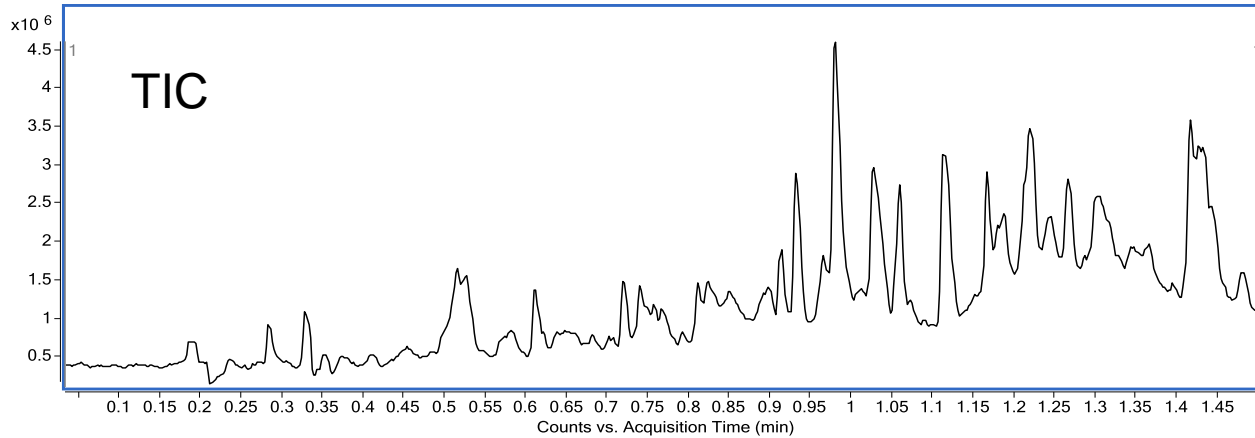
Identify

Reduction of complexity, enrichment



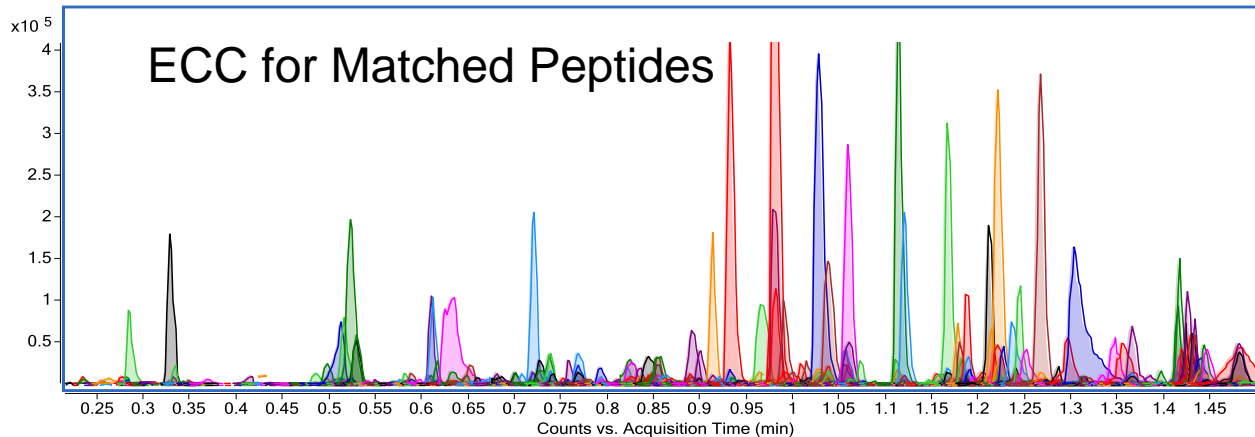
Agilent 1290 + 6530 Accurate Mass Q-TOF: Rapid Peptide Mapping of Proprietary IgG

100% sequence coverage for both light AND heavy chains!



Poroshell 120 column
2.1 x 150 mm, 2.7 μ m

Flow rate 1.2 mL/min



HPLC-Chip/MS Benefits

Max Efficiency & Sensitivity

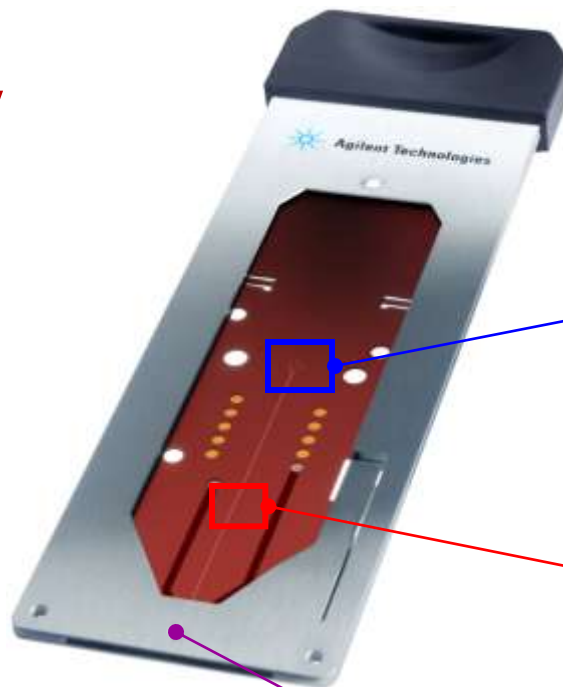
- Zero dead volume for better chromatographic performance
- LC/MS sensitivity

Hassle-free – All-in-One

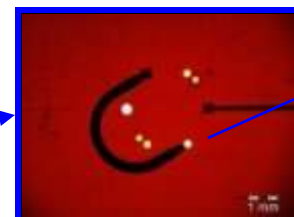
- Laser-ablated channels
- Analytical column
- Enrichment column
- Micro valve connection
- Nanoelectrospray tip
- Micro-filters

Maximum Uptime

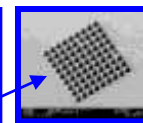
- No clogging of spray needle
- Plug-&-Play replacement



Inert polyimide



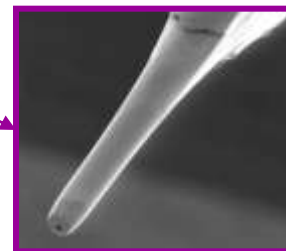
Enrichment column



μ -filter



Analytical column



Sprayer-tip

New HPLC-Chip: Phosphochip

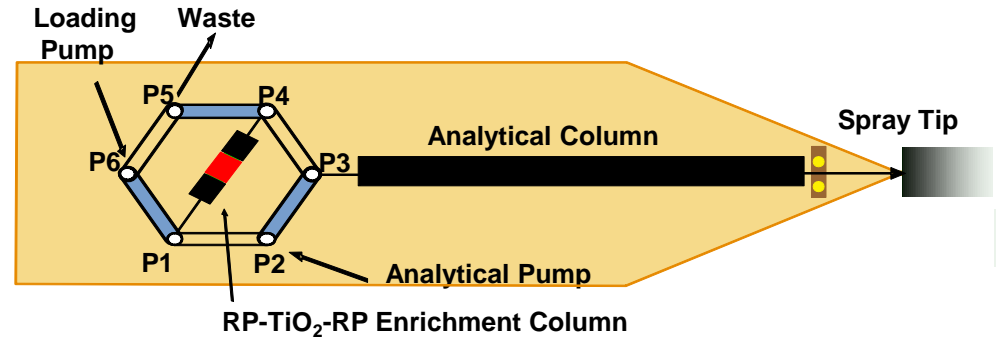
New Product

Phosphochip Features

- Completely integrated microfluidic design
- Simplified enrichment/analysis
- Increased sensitivity for phosphoproteome analysis
- Faster time-to-result
- More routine PTM analysis

Phosphochip Workflow

- Sandwiched RP-TiO₂-RP trapping column for phosphopeptide enrichment
- Dual modes of analysis for both phosphorylated and non-phosphorylated peptides from complex protein digest



Phosphochip Kit: HPLC-Chip, Reagents, Tubing and Manual



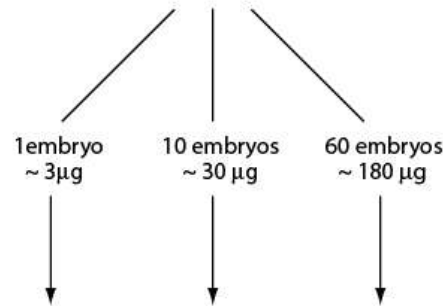
Phosphoproteomics on a single embryo

Zebrafish embryos



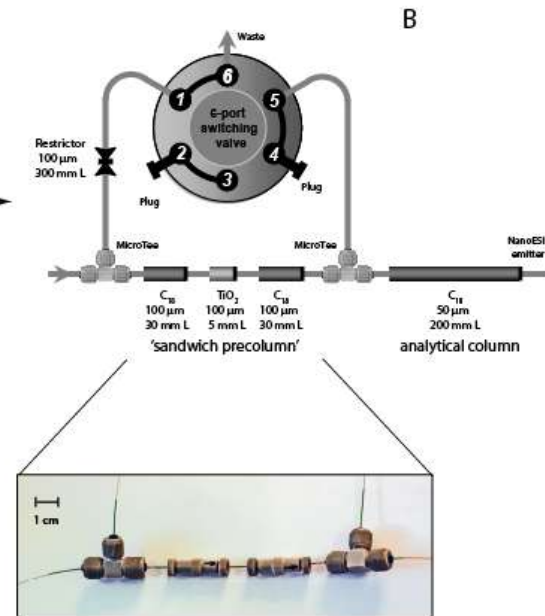
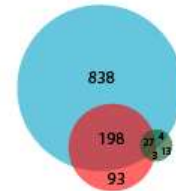
**MASCOT peptide score ≥ 35
p-value ≤ 0.005**

**70% detected in the 10 embryos
also detected in the 60 embryos**



Strong Cation Exchange
Online RP-TiO₂-RP-LC-MS/MS

60 embryos	180 mg	n= 1067
10 embryos	30 mg	n= 321
1 embryo	3 mg	n= 47



On-line automated zebrafish phosphoproteomics; From Large scale analysis to a single embryo
S. M. Lemeer, et al. *J Proteome Res.* 7 (2008) 1555-1564.

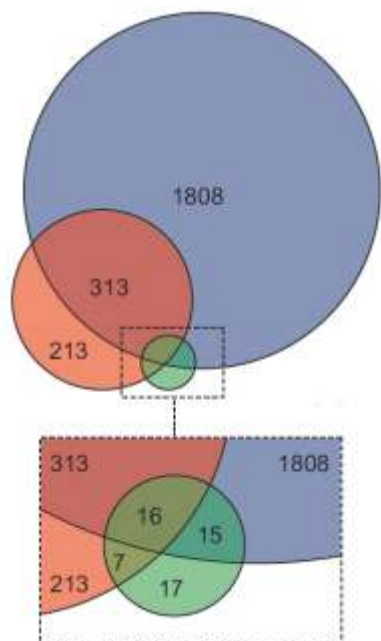
Comparative phosphoproteomics of zebrafish Fyn/Yes morpholino knockdown embryos
S. M. Lemeer, et al. *Mol. Cell. Proteomics* 7 (2008) 2176-2187.



“Harvesting” Phosphopeptides with Phosphochip

Fraction 7

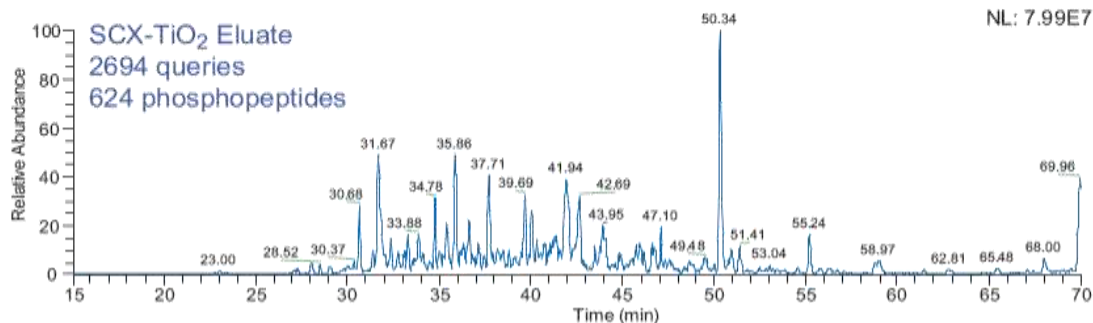
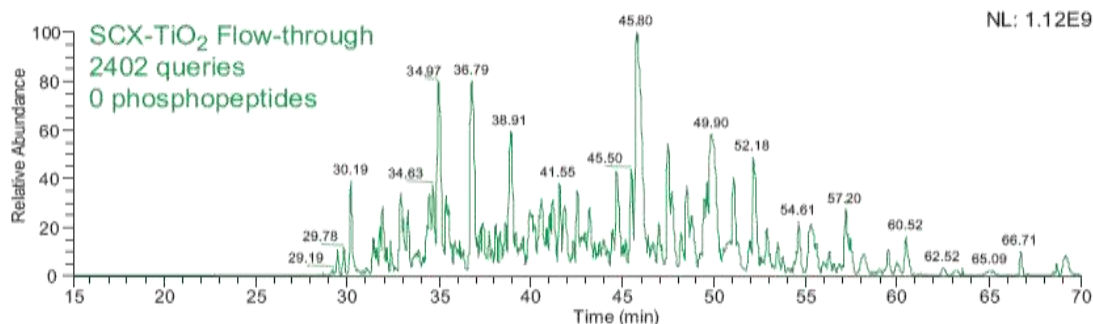
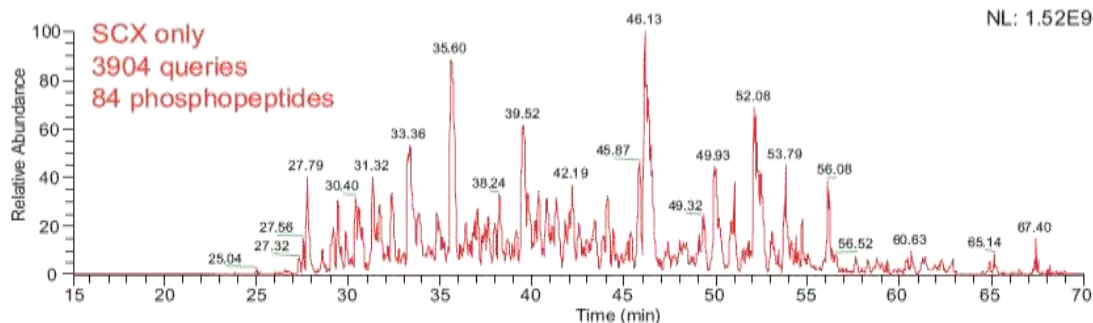
All Fractions



SCX only (n=549)

SCX-TiO₂ Flow-through (n=55)

SCX-TiO₂ eluate (n=2152)



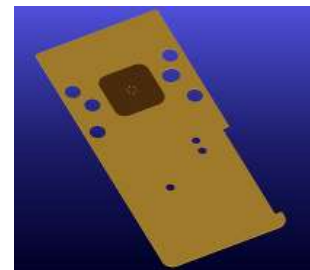
Mohammed, S., Kraiczek, K., Pinkse, M. W., Lemeer, S., Benschop, J.J. and Heck, A. J. R. (2007). *J Proteome Res*

HPLC- Chip II with Ion Implantation Technology

Patent
Pending

New Carbon Ion Implanted Filter

- Improved surface characteristics for optimal contact and sealing
- Reduced friction between rotor and polyimide chip

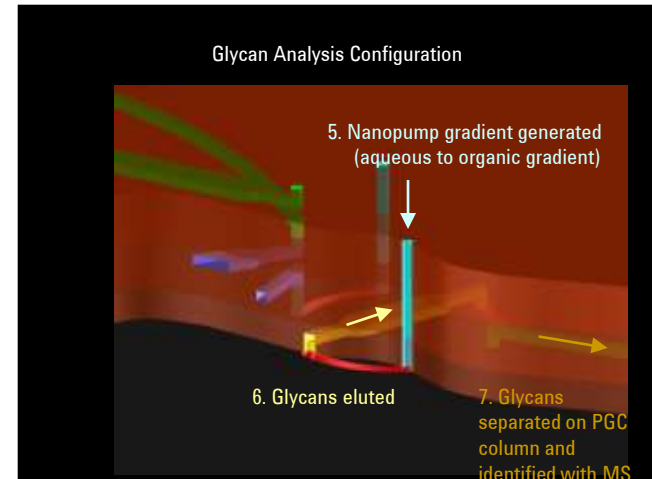
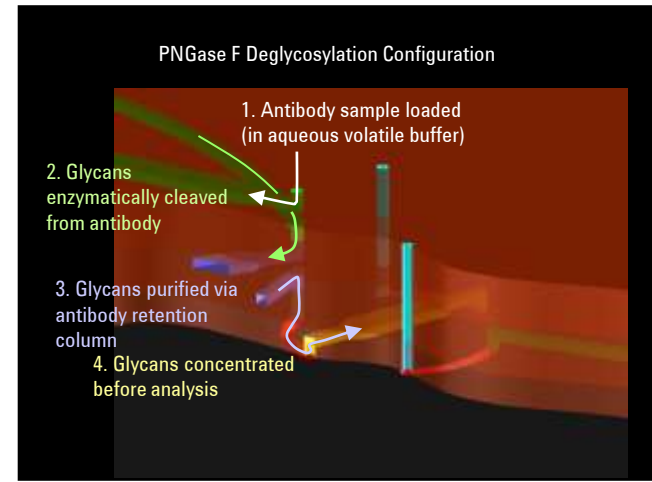
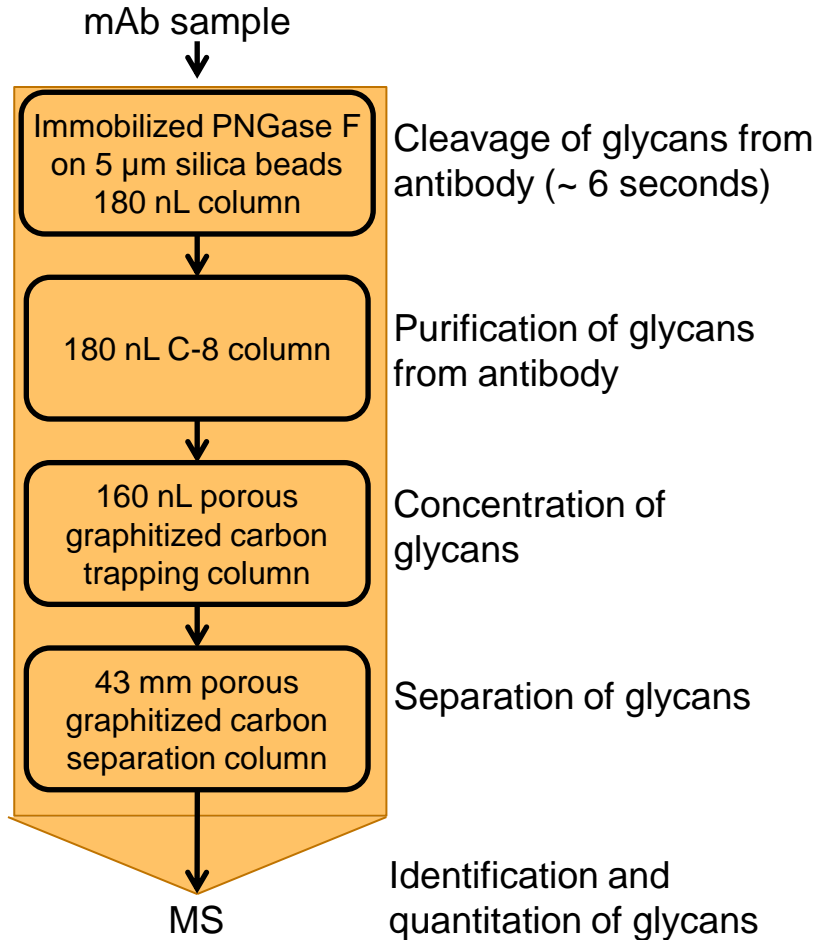


Benefits

- 2X Longer chip lifetime resulting in lower cost per analysis
- Improved chip to chip and run-to-run reproducibility



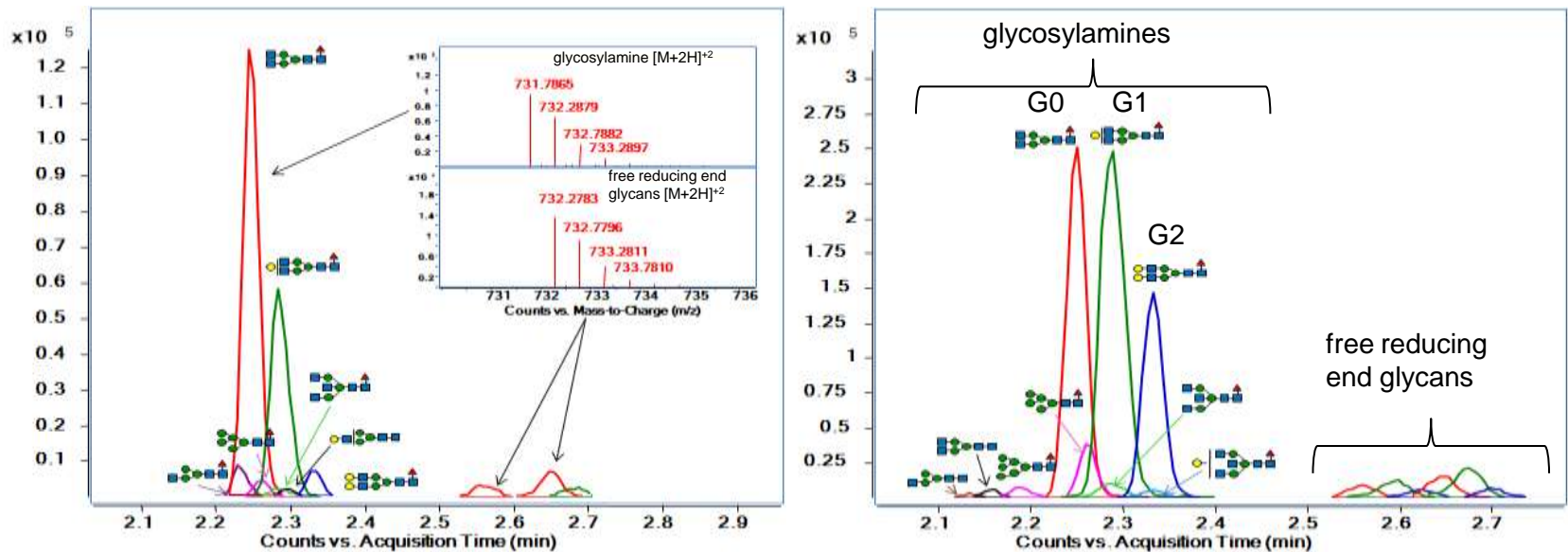
HPLC-Chip Research: Glycan Chip for Antibody Characterization



ASMS 2009: Bynum et al, An Integrated Microfluidic LC/MS Chip for Rapid On-line Deglycosylation and Characterization of N-glycans from Recombinant IgG Antibodies

HPLC-Chip Research: Glycan Chip for Antibody Characterization

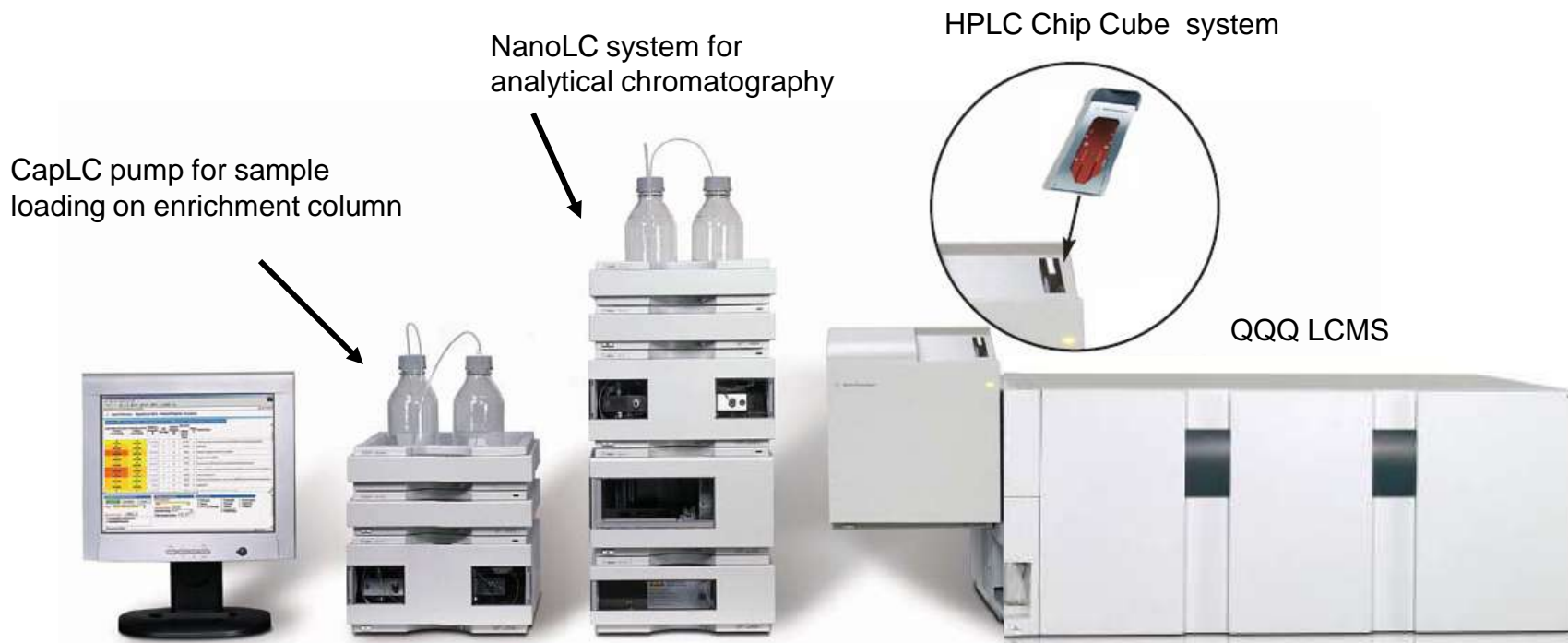
Glycan Profiles from 100 ng of Ab1 and Ab2



ASMS 2009: Bynum et al, An Integrated Microfluidic LC/MS Chip for Rapid On-line Deglycosylation and Characterization of N-glycans from Recombinant IgG Antibodies

Agilent HPLC-Chip/MS/MS Technology

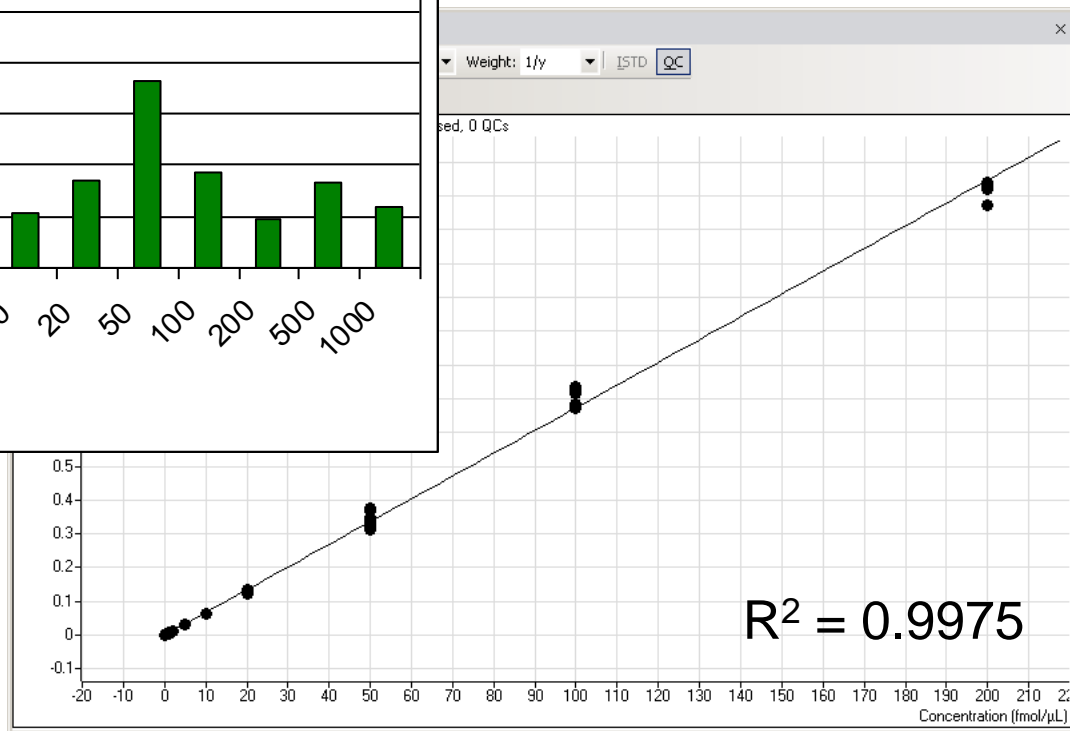
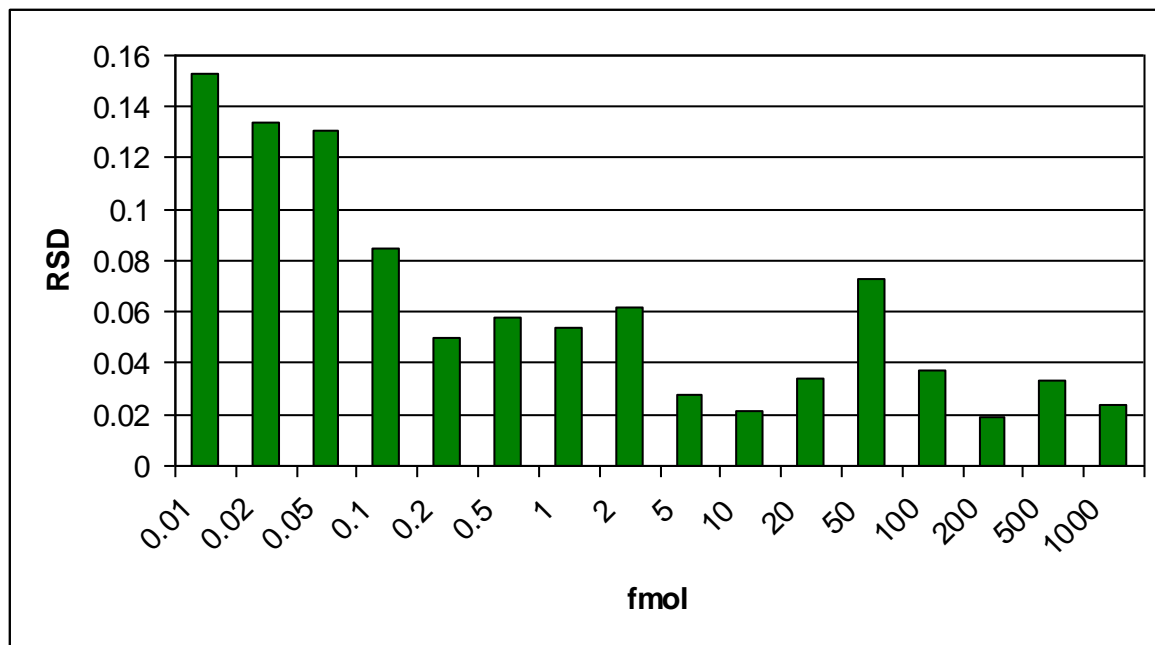
Nanospray chip configuration brings new era in high sensitivity quantitation



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

Excellent Reproducibility on the 6400 QQQ LC/MS/MS

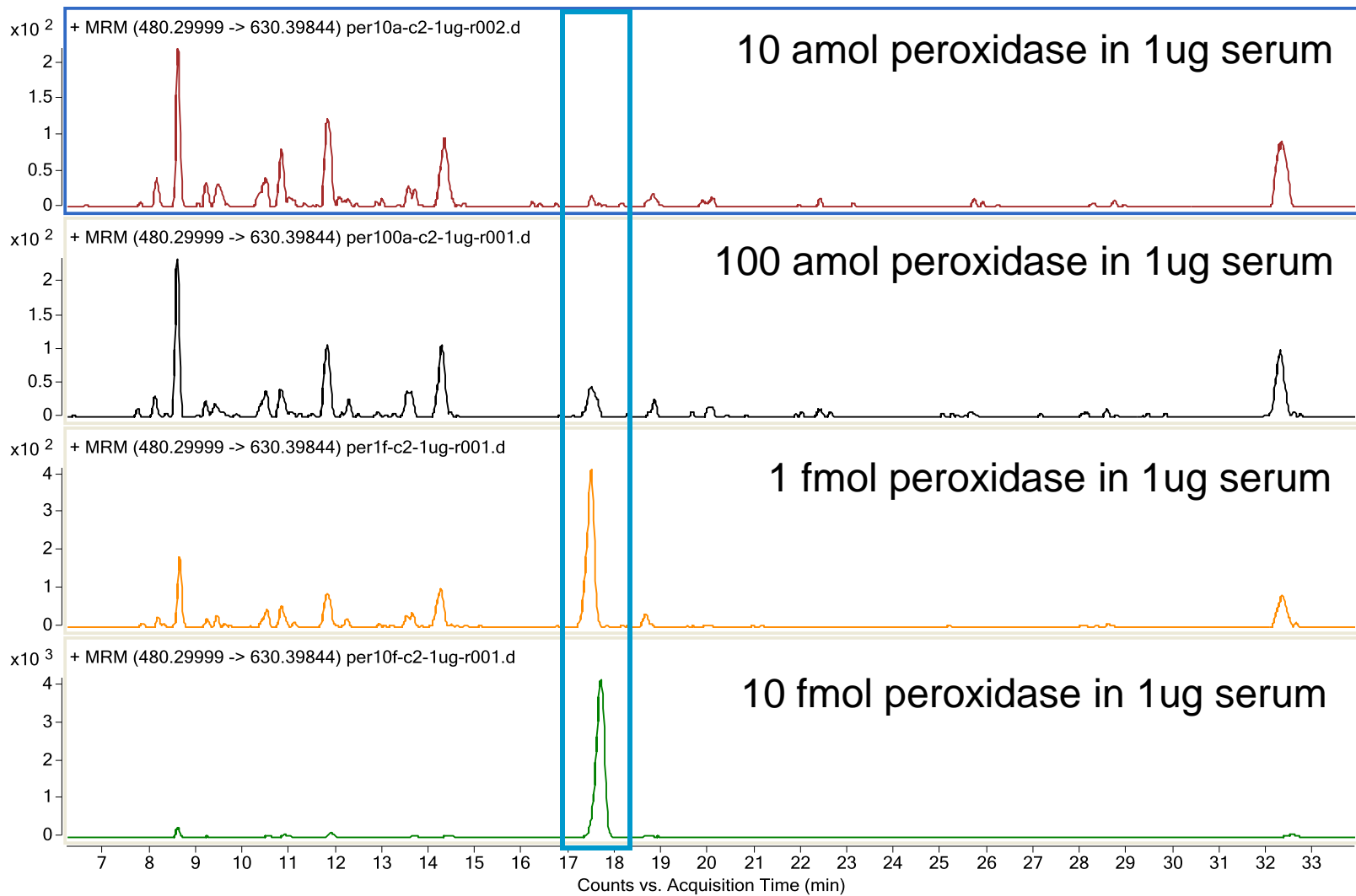
MRM of HSA Peptide LVNEVTEFAK from 10 amol to 1 pmol (n=6)



All RSDs are within 15%

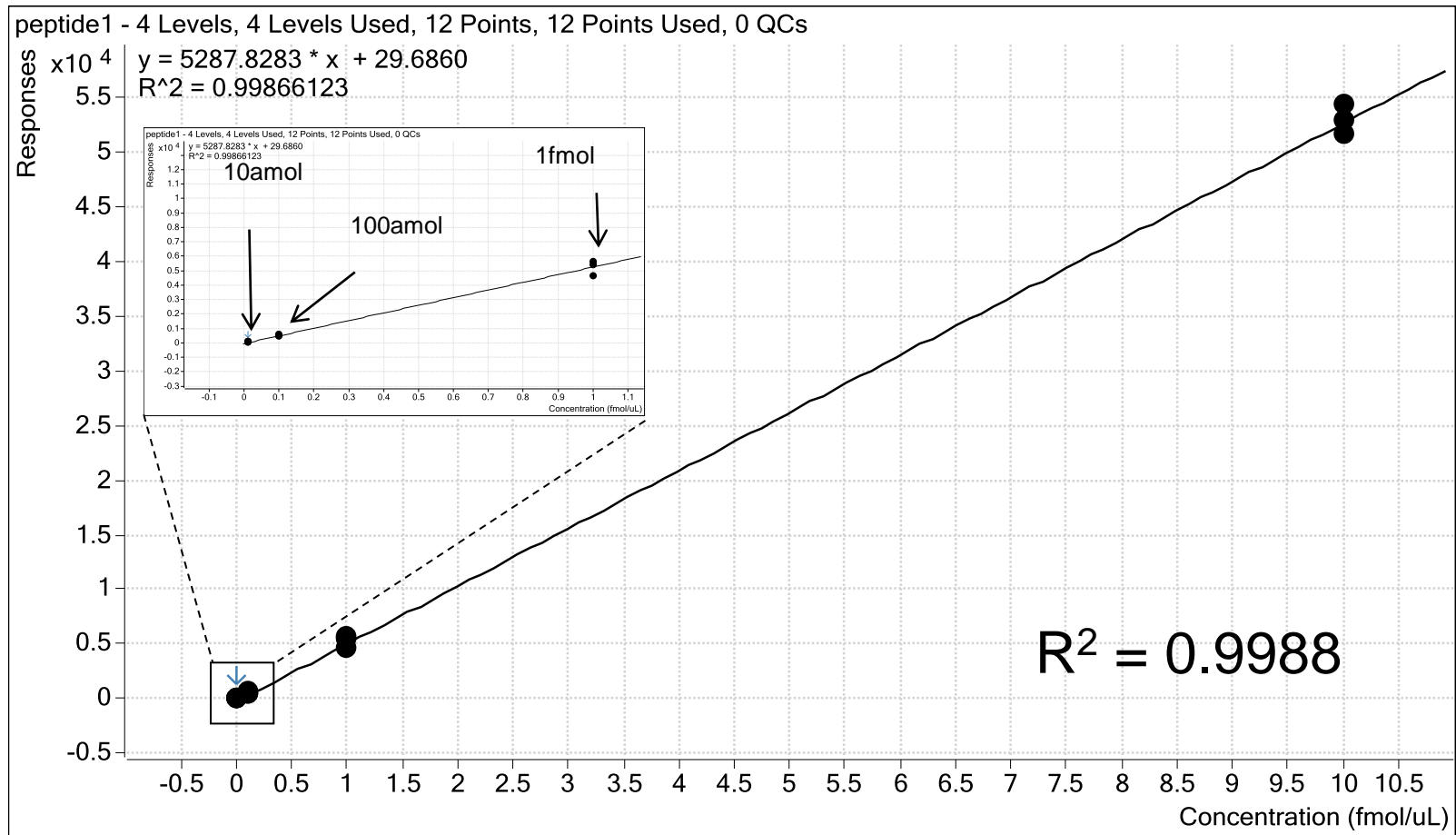
6400 series LC/MS/MS LOQ in the Low Amol Range

Peroxidase 10 amol to 10 fmol spiked into 1 μ g human serum



6400 LC/MS/MS Performance

External Quantitation Curve of Peroxidase Peptide DTIVNELR From 10 amol to 10 fmol Spiked into Human Serum



MRM Method Optimizer for Peptides

New
Product

Setup Optimizer: Import peptide sequences, then view predicted b/y fragment ions and select ions for optimization

Optimize CE in MRM mode optionally using CE setting from Q-TOF or user specified formula as a starting point

Advantages:

- Optimize CE in 1 run
- Can optimize on more than 1 charge state for the same peptide
- Pre-select b and y ions (including multiply-charged product ions)
- Only limit is on total number of transitions per optimization run – software will suggest limit based on user-entered chromatographic peak width



Peptide Optimizer: Define Peptide Sequences and Modifications

The screenshot shows the 'Sequence Editor: Define and Match Sequences' window. The 'Sequences' dropdown is set to 'HSA_LVN'. Under 'Sequence details', 'Sequence name' is 'HSA_LVN', 'Sequence type' is 'Synthetic peptide', 'Monoisotopic MW' is '1148.607743', 'Average MW' is '1149.316964', and 'Molecular formula' is 'C52H84N12O17'. The 'Amino acid list' table is visible on the left, and the 'Sequence editor' shows 'Chain: A: Chain A' and the sequence 'LVNEVTEFAK' between positions 1 and 10.

Amino acid	Count
A Ala	1
R Arg	0
N Asn	1
D Asp	0
C Cys	0
E Glu	2
Q Gln	0
G Gly	0
H His	0
I Ile	0
L Leu	1
K Lys	1
M Met	0
F Phe	1
P Pro	0

1. Specify peptide sequence and type

2. Enter peptide sequence

The screenshot shows the 'Method Editor: Define and Match Sequences' window. The 'Sequences' tab is active, displaying a list of saved sequences: HSA_AAF (Synthetic peptide), HSA_YLY (Synthetic peptide), HSA_LVN (Synthetic peptide), HSA_KVP (Synthetic peptide), HSA_RPC (Synthetic peptide), HSA_AVM (Synthetic peptide), and HSA_HPY (Synthetic peptide). Buttons for 'New', 'Edit', 'Import', 'Save as...', and 'Delete' are visible on the right.

3. Save peptide sequences

Peptide Optimizer: Import Peptide Sequences and Select Transitions for Optimization

Peptide Fragment Ion Predictor

Show selected ions

Show only y-ions Show ions > mz

Show only b-ions Show ions > % of precursor

Sequence Name	Sequence	Nominal Mass
DGTRKPVTDAEN	DGTRKPVTDAE	1938.9330

Precursor Ion	Precursor Ion Charge
<input type="checkbox"/> 970.4743	2

Product Ion	Product Ion Charge	Product Ion Name	AA N-term	AA C-term
<input type="checkbox"/> 899.4158	1	y7	A	E
<input type="checkbox"/> 970.4529	1	y8	D	A
<input type="checkbox"/> 1085.4798	1	y9	T	D
<input type="checkbox"/> 1186.5275	1	y10	V	T
<input type="checkbox"/> 1285.5959	1	y11	P	V
<input type="checkbox"/> 1382.6487	1	y12	K	P
<input type="checkbox"/> 1510.7436	1	y13	R	K
<input type="checkbox"/> 1666.8448	1	y14	T	R
<input type="checkbox"/> 833.9263	2	y14	T	R
<input type="checkbox"/> 1767.8924	1	y15	G	T
<input type="checkbox"/> 884.4501	2	y15	G	T
<input type="checkbox"/> 1824.9139	1	y16	D	G
<input type="checkbox"/> 912.9609	2	y16	D	G

Precursor Ion	Precursor Ion Charge
<input type="checkbox"/> 647.3188	3

Sequence Name	Sequence	Nominal Mass
DKPDNFQLFQSP	DKPDNFQLFQS	1756.8533
GLAGVENVTELK	GLAGVENVTELK	1356.7613
HKTGPNLHGIFG	HKTGPNLHGIFG	1276.6677

Add Cancel

Peptide Optimizer: Results Stored in Database for Easy Import into DMRM or MRM Methods

Peptide sequence

Precursor

Optimized product ions

Compound Name	Group	Formula	Nominal Mass	Vial Number
LVNEVTEFAK			1	P1-F1

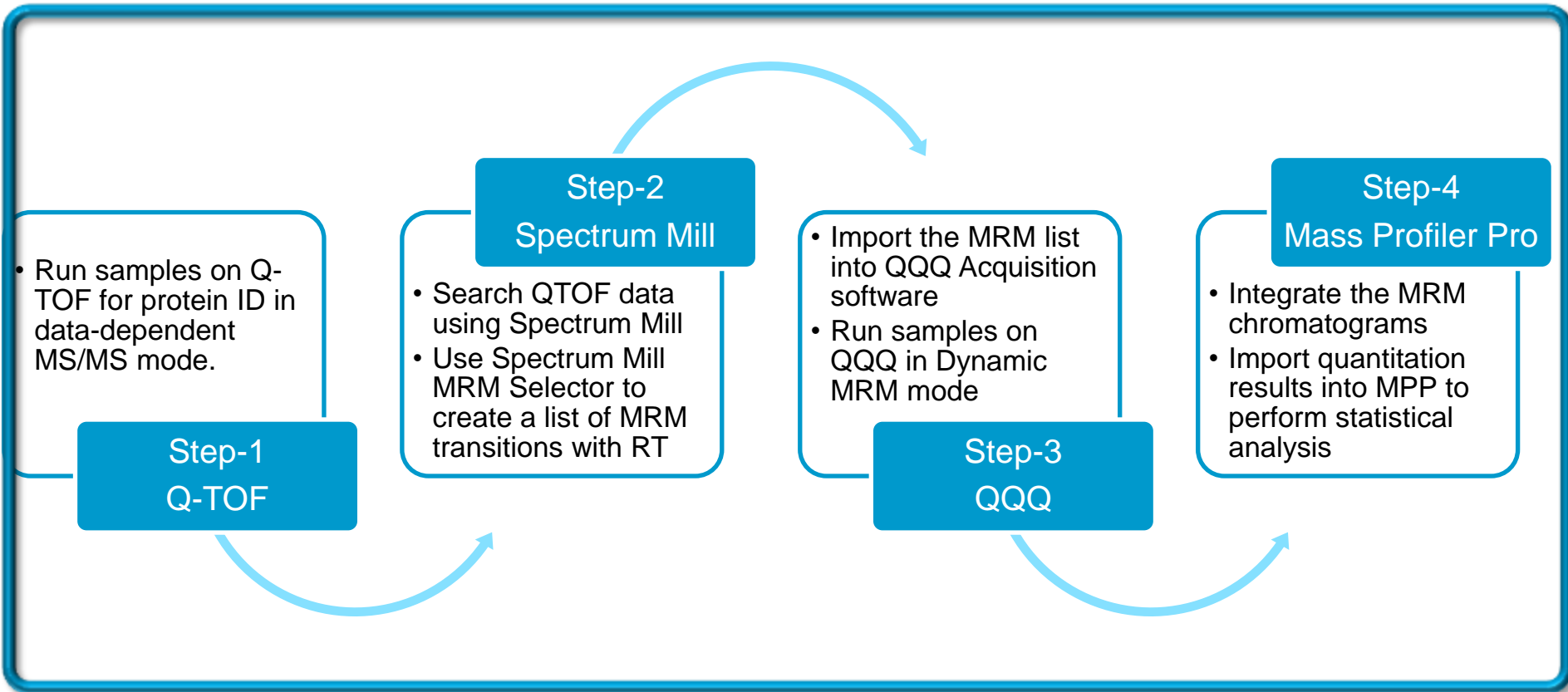
Method	Polarity	Ion Source	Instrument ID	Date Optimized	Flagged
D:\MassHunter\me	Positive	HPLC-Chip		12/18/2008	<input type="checkbox"/>

Precursor Ion	Fragmentor	Abundance
575.31	130	

Product Ion	Collision Energy	Abundance
937.5	16	80384
595.3	16	27268
694.4	16	18548
456.2	16	8572
823.4	16	8508
494.3	24	5845
555.3	12	4998
656.4	12	1309
785.4	12	503
932.5	12	479

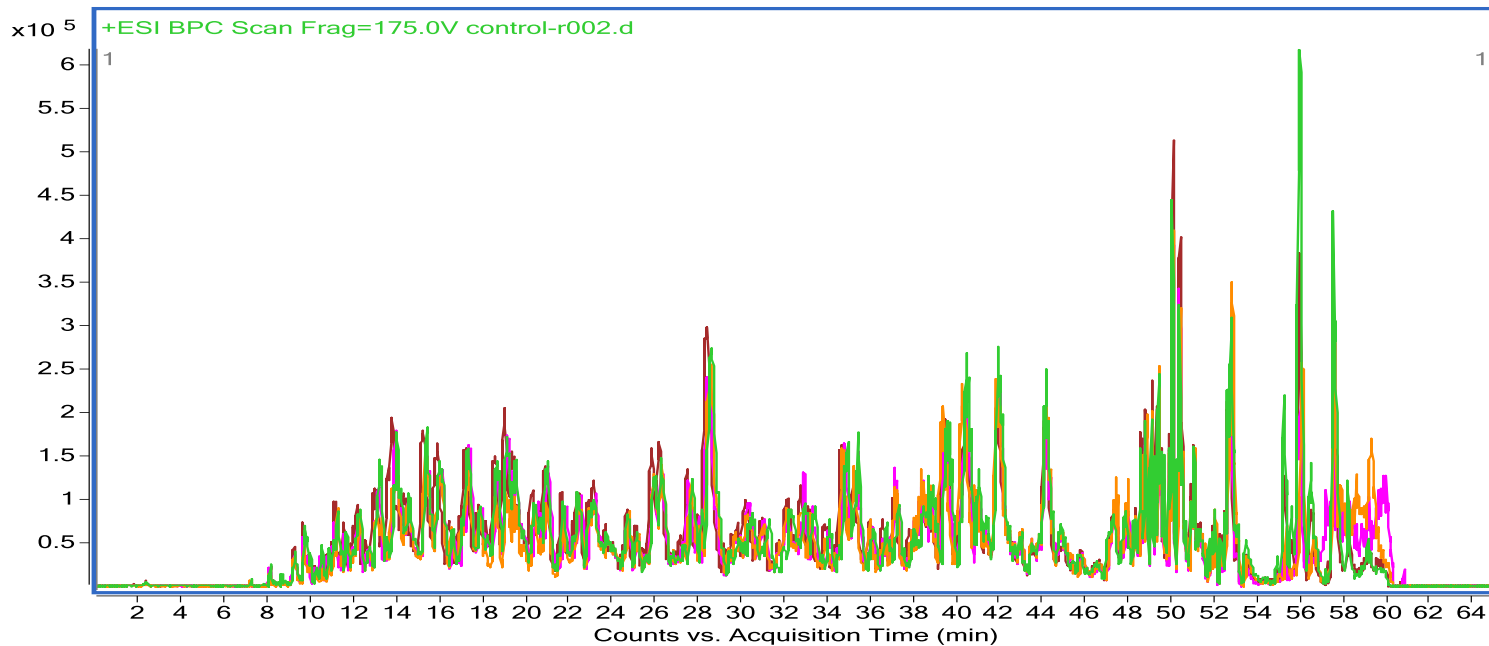
Abundance of each transition allows customer to choose best transitions for the final method

Biomarker Validation Workflow



Step 1: Analysis on Q-TOF for Biomarker Discovery

5 replicate injections of immunodepleted human serum spiked with peroxidase



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Step 2: Use Spectrum Mill MRM Builder for Creating Ion Transition Lists

Spectrum Mill - MRM Builder

Spectrum Mill | P/P Summary | **MRM Builder** | Autovalidation | Build TIC | MS/MS Search | Spectrum Summary | Tool Belt | Help

Initialized MRM Builder
 Results Shown Filtered by Validation Category: valid
 Data Directory: msdata\SM\NT\control
 hit table read - SpecFeatures read
 valid hits read from tagSummary file - Files: 5889 Hits: 5585
 Filtered to only unique peptides: 1182
 beginning to assemble proteins ... proteins assembled 0.228361 sec
 proteins filtered by unique peptides 0.128926 sec
 calculated protein coverage maps 2.876567 sec
 beginning to roll up proteins into groups ... proteins rolled up into groups 2.890582 sec
 protein groups ready for displaySBA: Score

#	Filename	z	Spectrum Intensity	Sequence	RT (min)	Peak Width (sec)	m/z Measured (Da)
1	control-005_12584_12584_3	3	6.42e+005	(R) TFQIPQYTVVWVWVVSPTIERSAFQYVFPK (A)	50.63	36.32	1188.6030
	P04114_TFGIPGYTVVWVWVVSPTIEMSAFGYVFPK_y26(+2)	1188.6	1537.8	10,,38			
	P04114_TFGIPGYTVVWVWVVSPTIEMSAFGYVFPK_y23(+2)	1188.6	1279.2	10,,38			
2	control-001_12332_12336_3	3	3.34e+006	(R) VPSYTLILPSLELPVLR (R)	49.33	16.19	748.4417
	P04114_VPSYTLILPSLELPVLR_y7(+1)	748.4	817.5	10,,22			
	P04114_VPSYTLILPSLELPVLR_y18(+2)	748.4	1024.1	10,,22			

Select Results for MRM Building
 Build MRMs | Save Settings | Reset
 Model: Peptide | Format: Agilent Triple Quad DMRM
 Data directories: Select...
 NT:control
 Search result files: *.apo

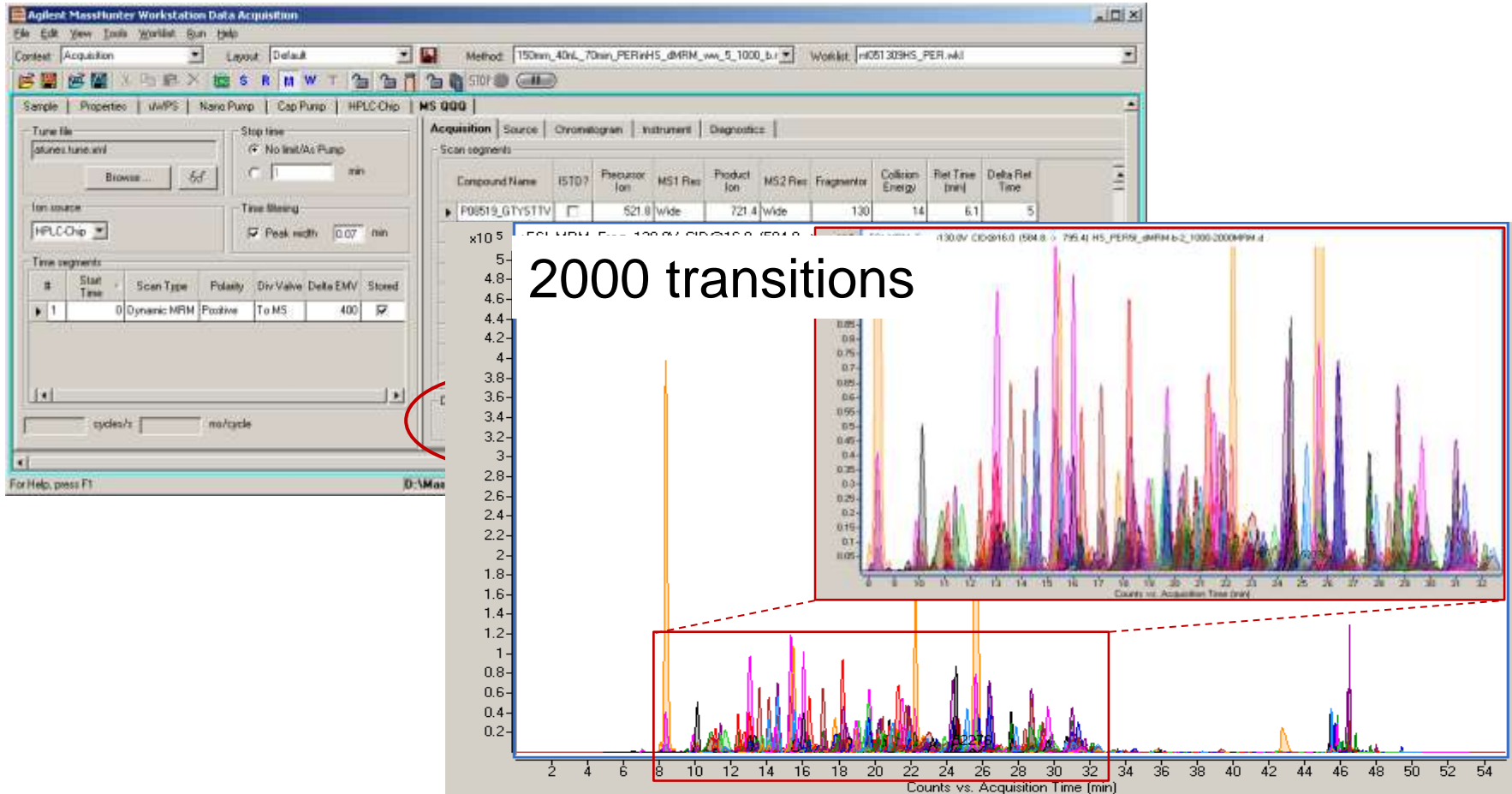
Validation and Sorting
 Filter results by: valid
 Validation preset: none
 Top n peptides for MRM: Limit to 2
 Rank peptides by: Score
 Filter peptides by:
 Score: > 10 % SP: > 50
 Required AAs: any | Disallowed AAs: none
 Peptide pI: from 3.0 to 10.0 All
 Accession #'s:
 Sort MRM List by: Precursor m/z

MRM Parameters
 Destination: Text file export Screen Preview
 Top n Transitions: 2 Above precursor only y-ions only
 Z options: Observed precursor/fragment charge only
 Dwell time (ms): 10
 m/z breakpoint: Potential: Use actual CE if available
 z-state: slope: intercept:
 2 | 0.05 | 5
 3 | 0.044 | 3
 4 | 0.05 | 2

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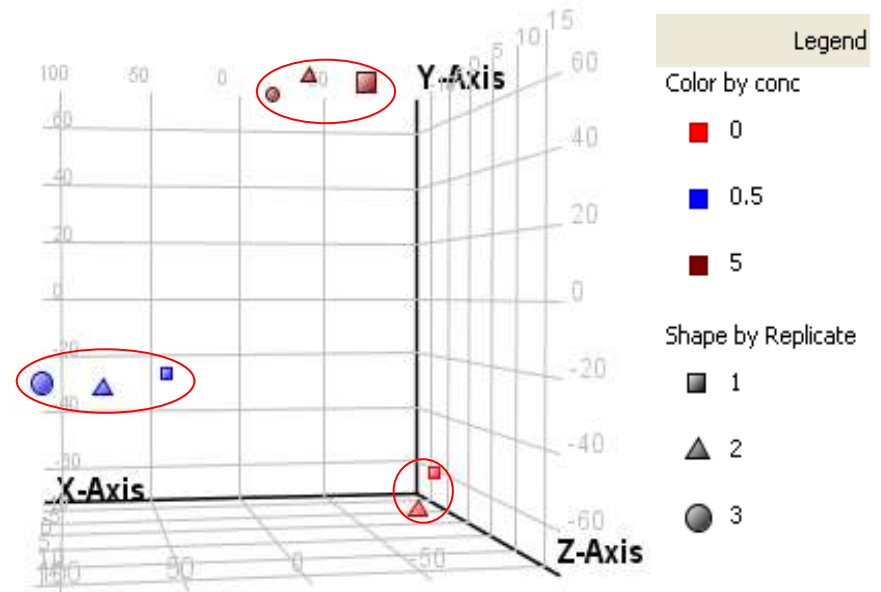
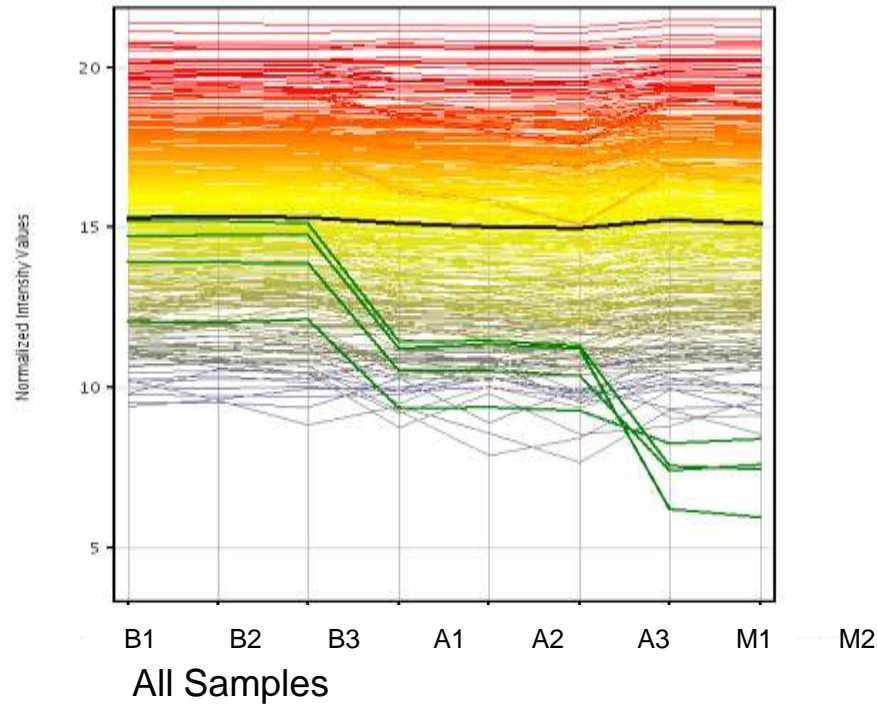


Step 3: Build Dynamic MRM Method for Peptide Quantitation



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Step 4: Import Quantitative Results Into Mass Profiler Professional for Statistical Analysis



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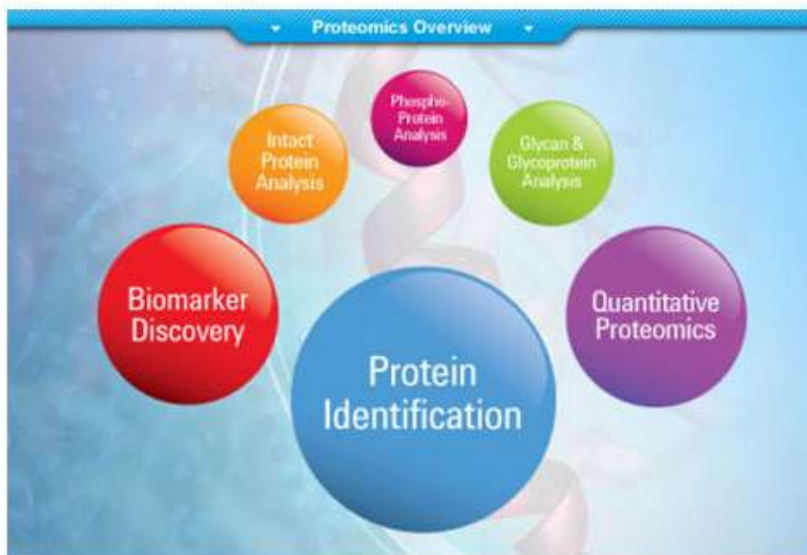
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Proteomics

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**FASTER, EASIER, MORE FLEXIBLE
PROTEOMICS SOLUTION**

HPCL-CHIP / MS TECHNOLOGY

Agilent's protein HPCL-Chip incorporate

Announcements

**Agilent Life Sciences
Announces**



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Acknowledgments

Agilent

- Ning Tang
- Norton Kitagawa
- Keith Waddell
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- Derek Smith
- Michael Kuzyk

