Robert Moritz

Developing a Complete and Reproducible Human SRMAtlas
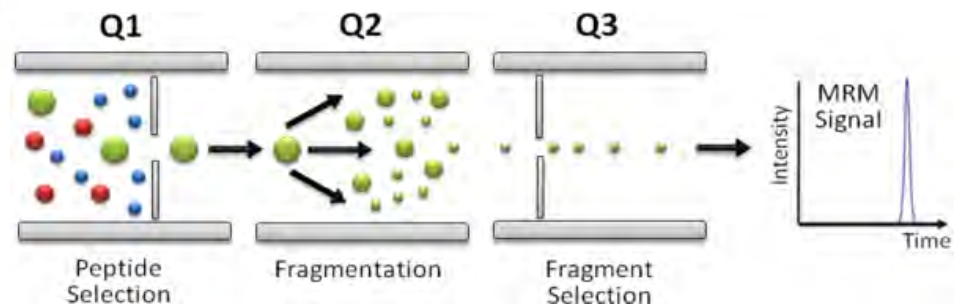

Human SRMAtlas

Duchy of Luxembourg
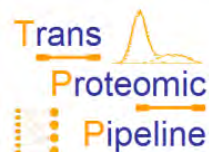
Outline

- What is the SRMAtlas?

- Introduction to the ISB PeptideAtlas: Compendium of peptides and inferred proteins observed by MS/MS

- Development of SRMAtlas: Unified transition resource

- Reproducibility of assay production

- Application tips for extended performance of LC-chips

Human SRMAtlas
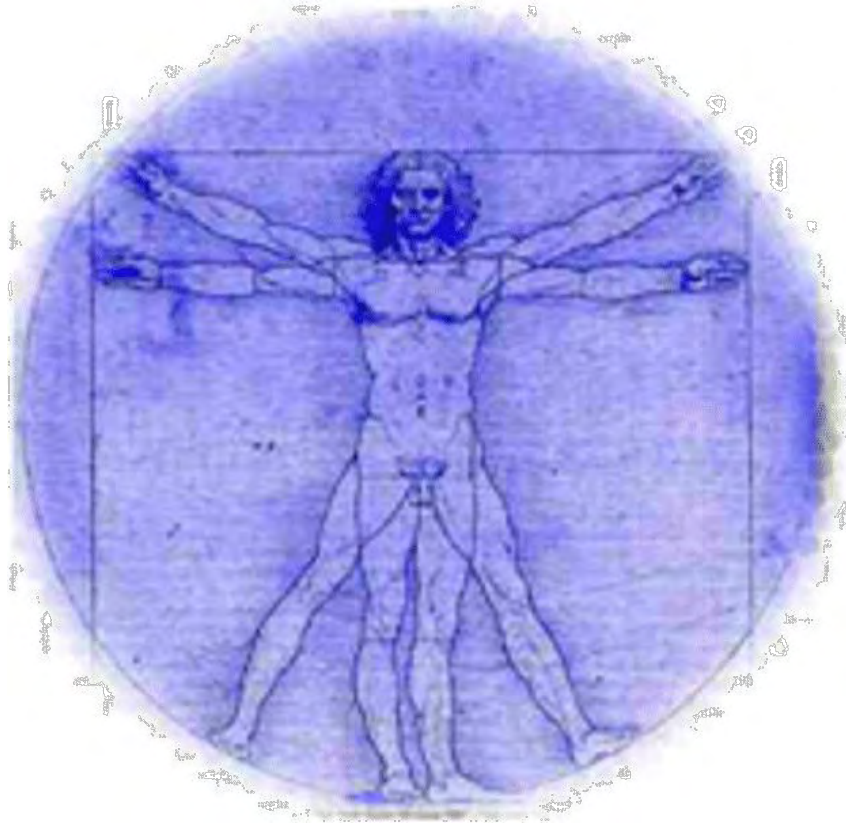
Design multiple quantitative assays for every human protein using a robust high-throughput platform suitable for clinical applications

Understanding the terrain
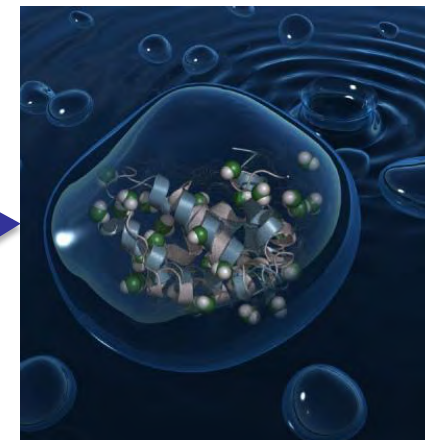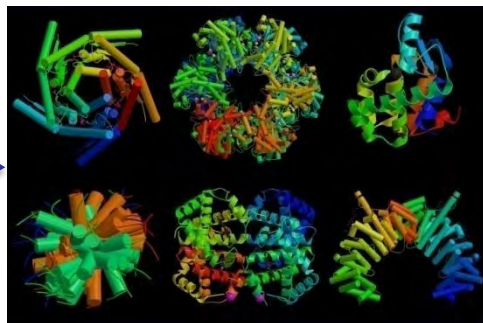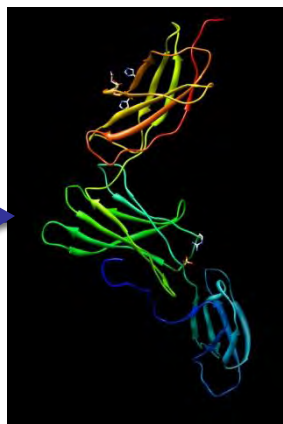
Knowing the components

Surveying to create an Atlas

Navigating the Atlas

Selected Reaction Monitoring (SRM or MRM)

MS/MS profile by segmented or trap mode

SRM-triggered MS2
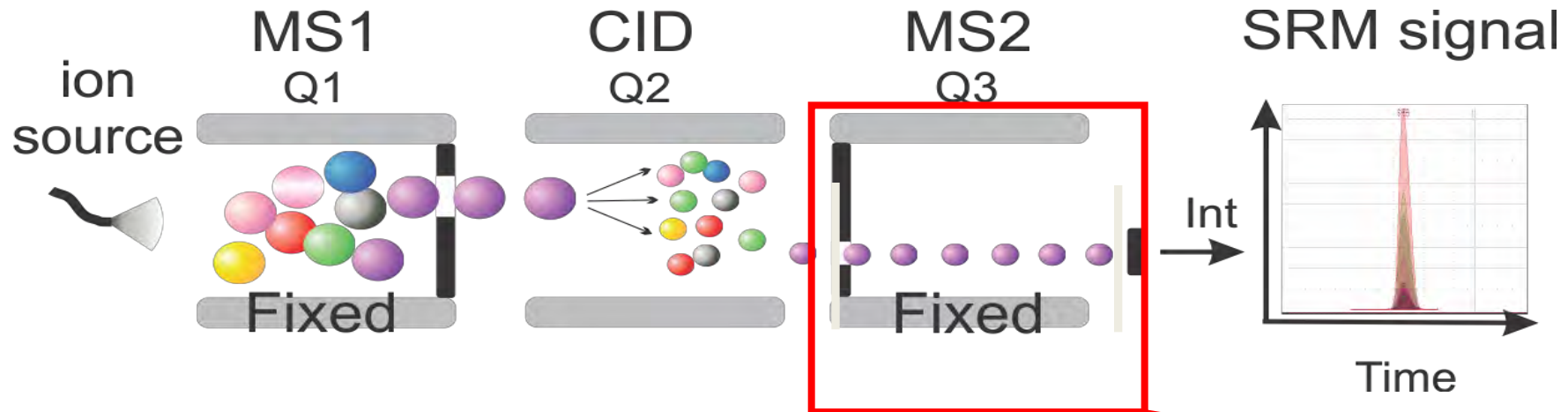
- *two levels of mass selection: high specificity*

- *not scanning (Q1/Q3 static), high duty cycle: high sensitivity*

- *the most sensitive mass spectrometry method known (low amole)*
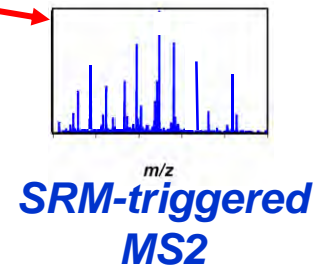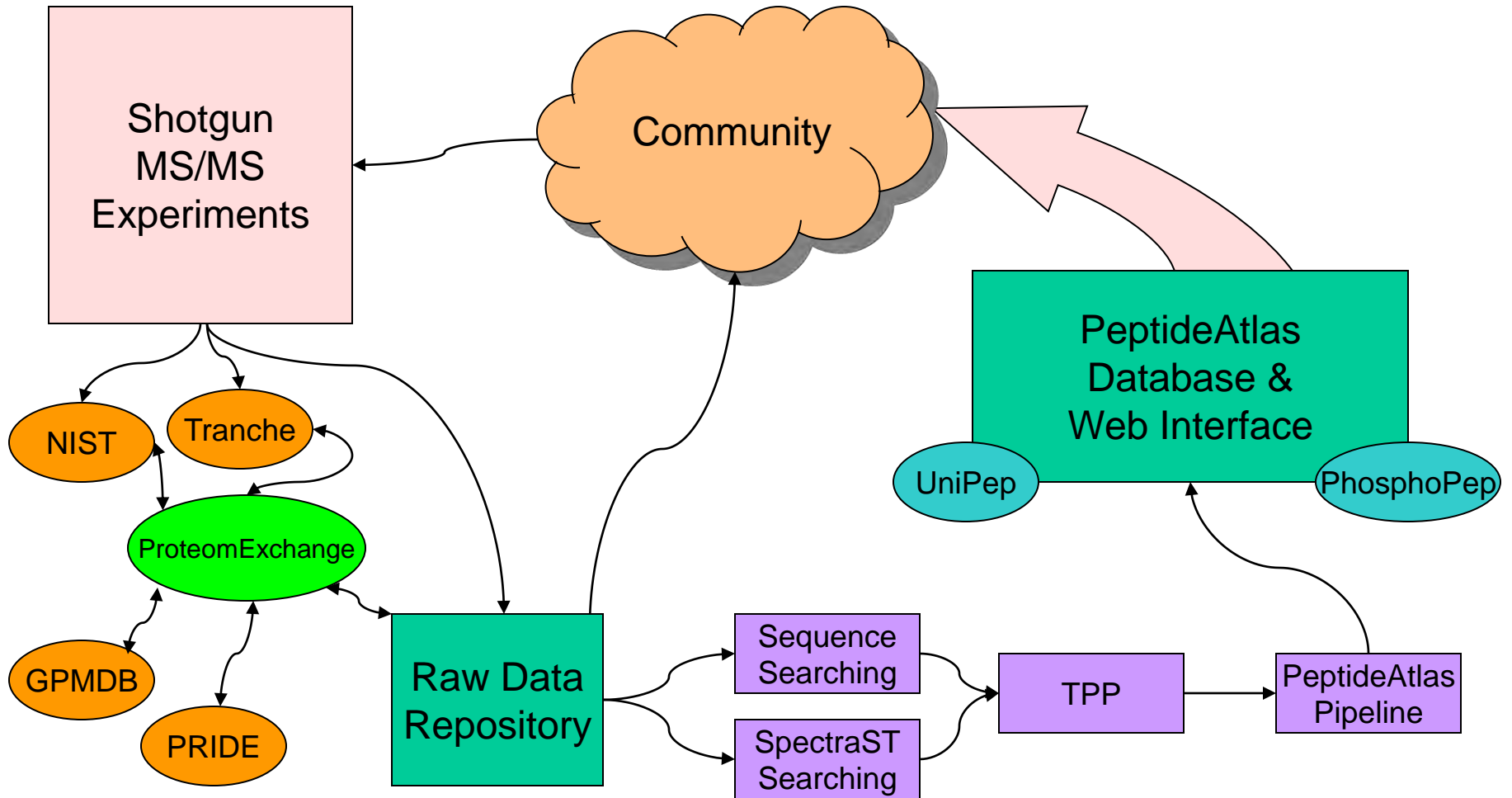
....you need to know what to look for!

(the mass spectrometrist's ELISA)

PeptideAtlas Workflow

www.peptideatlas.org, TPP: Trans Proteomic Pipeline

What is a proteotypic peptide?

Protein

Peptide region **never** observed in multiple experiments

Peptide region **consistently** observed in multiple experiments[7]

PeptideAtlas protein view page

Cytoscape view of proteins & peptides
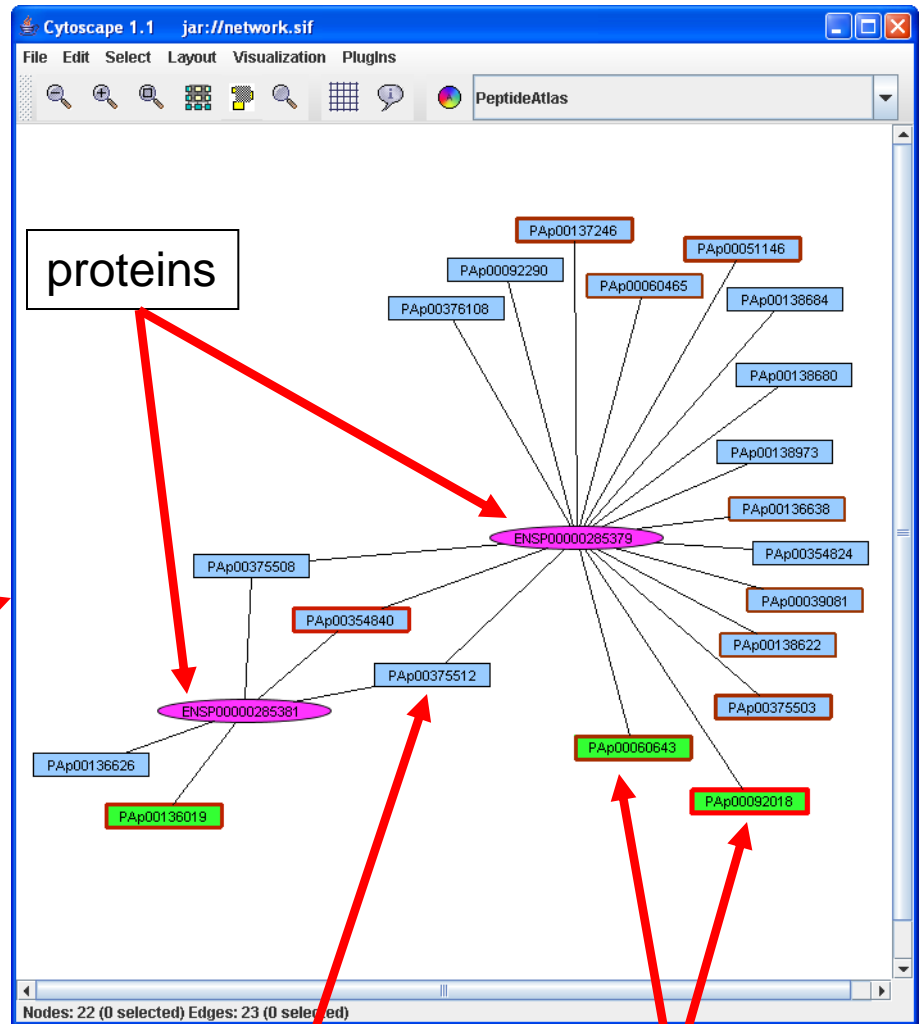
proteins

ambiguously mapped peptide

proteotypic peptides
$N_{prot} = 1$ $N_{obs} > 1$
EPS > 0.3

PeptideAtlas Build Summary

Build	# Exps	# MS Runs	Searched Spectra	IDs P>0.9	Distinct Peptides	Distinct Proteins	Swissprot
Human All	424	54 k	49 M	5.6 M	**97 k**	**12141**	**20328**
Human Plasma	76	48 k	16 M	1.8 M	18 k	2486	???
Human Glycosylated	174	1731	7.1M	690K	14K	2771	7426
Yeast	53	2957	6.5 M	1.1 M	36 k	4336	6552
Mouse	59	3097	10 M	1.4 M	51 k	7686	16085
Drosophila	43	1769	7.5 M	498 k	72 k	9124	13,600
Drosophila PhosphoPep	4	448	0.9 M	170 k	10 k	4583	???
Halobacterium	88	497	0.5 M	76 k	12 k	1975	2426
S. pyogenes	5	64	215 k	52 k	7 k	1068	3729
Mouse Plasma	4	568	7 M	0.8 M	9.4 k	2075	????

Human SRMAtlas
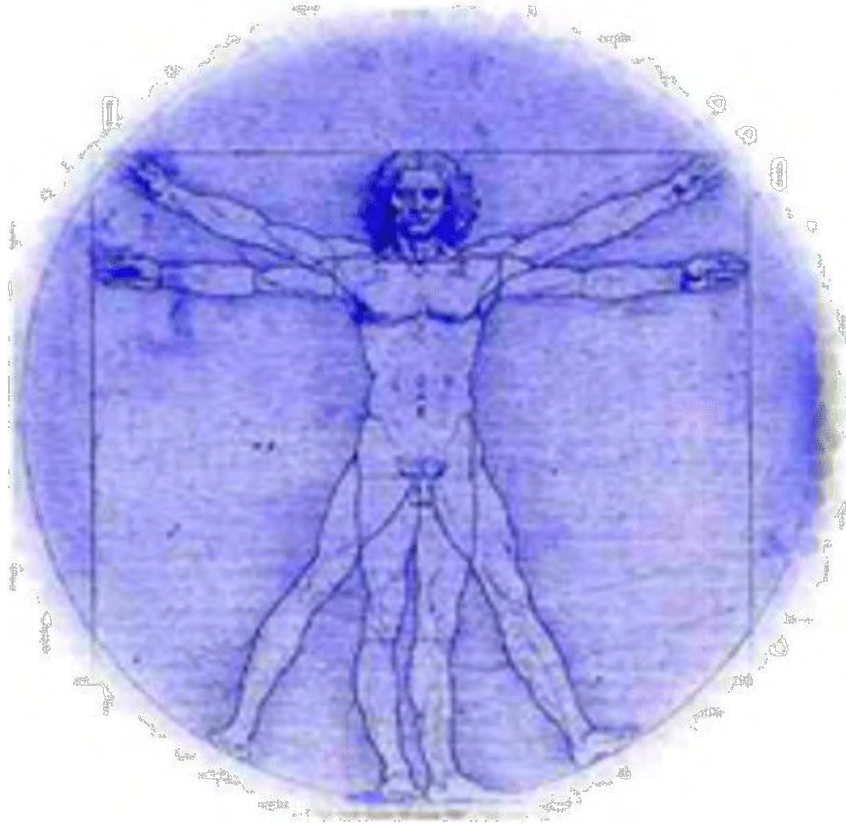
20,333 proteins (20,277 2010 version)
32,562 proteins incl. isoforms
658,684 tryptic peptides (any length)
480,284 distinct peptides (7-30aa)
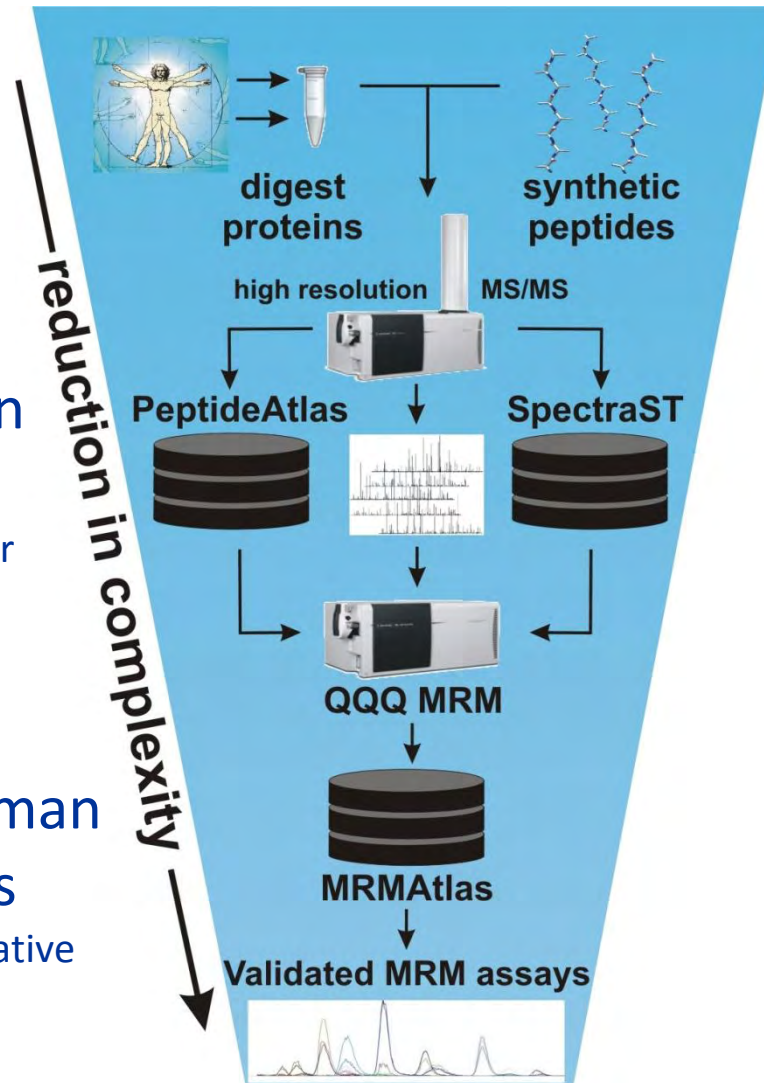

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Systems
Biology
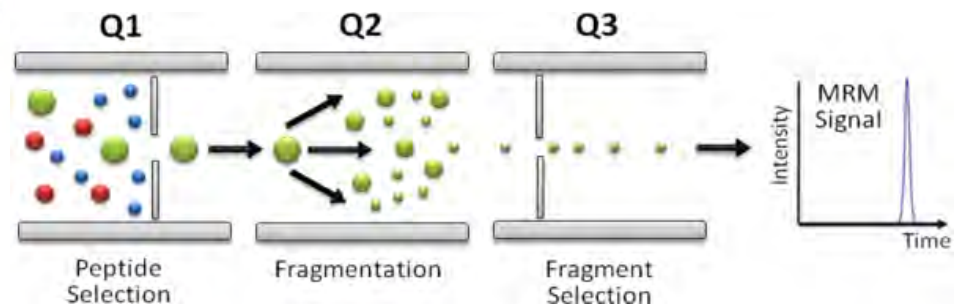
Developments at ISB - SRMAtlas

Human proteins
(from natural source or synthetic)

Develop Human PeptideAtlas
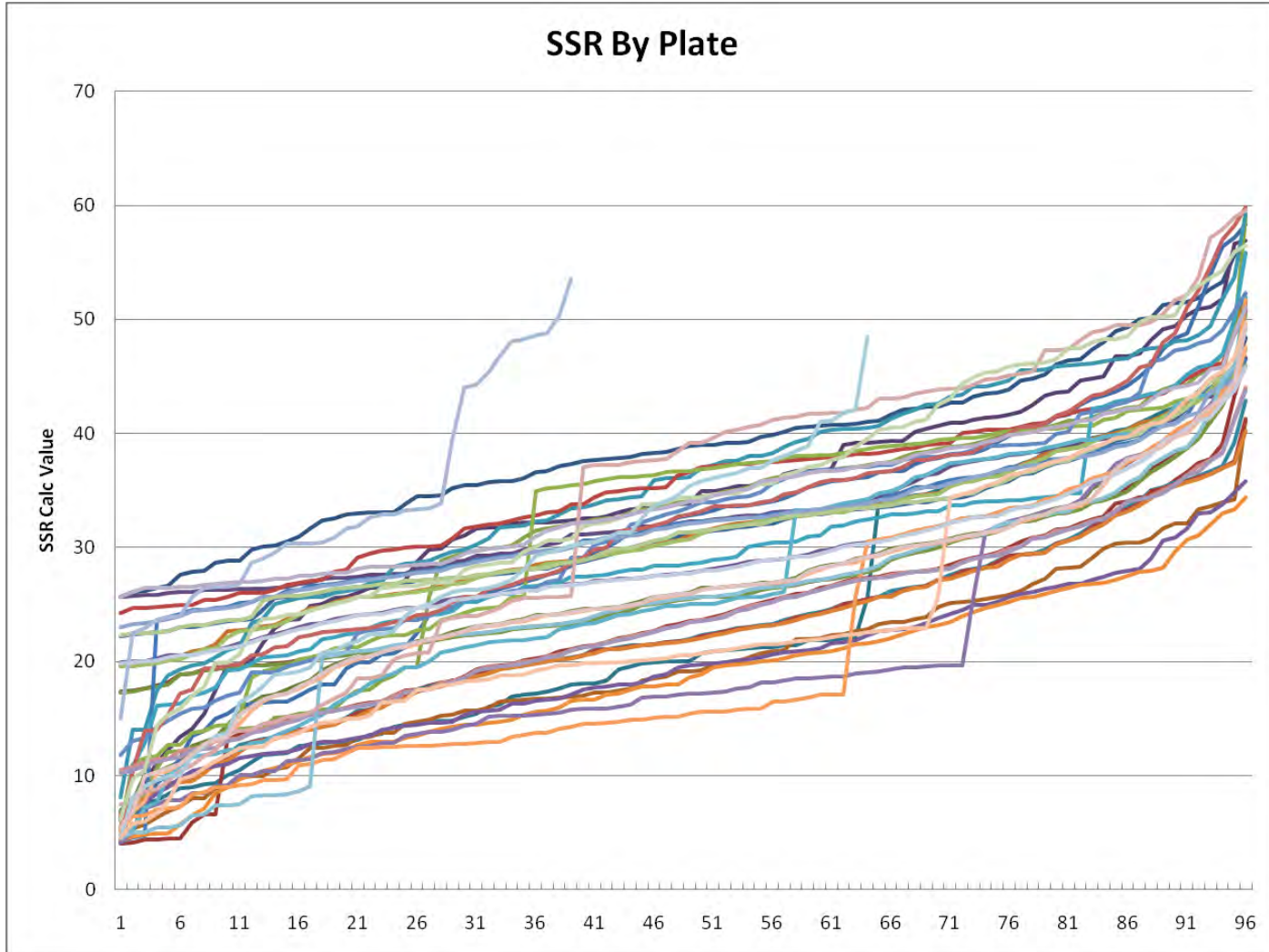(from tryptic digests or synthetic peptides)

Develop Human SRMAtlas
(verified quantitative assays)

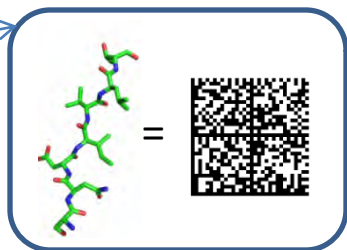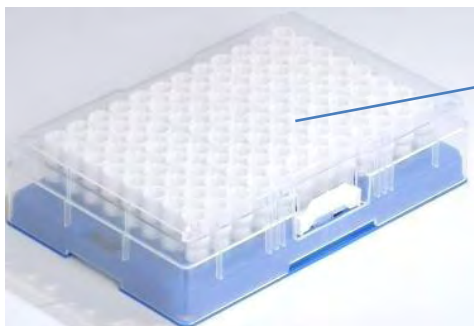Synthetic "proteotypic" peptide
(from cheap synthesis)

Develop optimized transitions from PeptideAtlas

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Biology

Peptide hydrophobicity (SSR) spread by plate

SSR By Plate

Peptide Tracking & Processing

> 150,000 peptides

ISBHJRMXX000026

= 2D-barcode tube

pools of
96 peptides

Laboratory
Information
Management
System

MRM Atlas LIMS

Create order · Search orders · Search plates · Search pools

Master Plate Detail

Plate Properties

Name:	ISBH1
Type:	MASTER
Location:	Freezer 1, Shelf 1, Rack 1, Box 1
Checked out:	NO
Last Checkout:	
Comment:	

Master Plate
ISBHTKXXX000147

Working Plate
ISBHTKXXX000147_W1

Pooled Samples
ISBHTKXXX000147_W1_P1
ISBHTKXXX000147_W1_P2
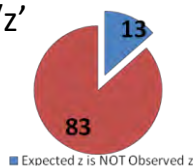ISBHTKXXX000147_W1_P3
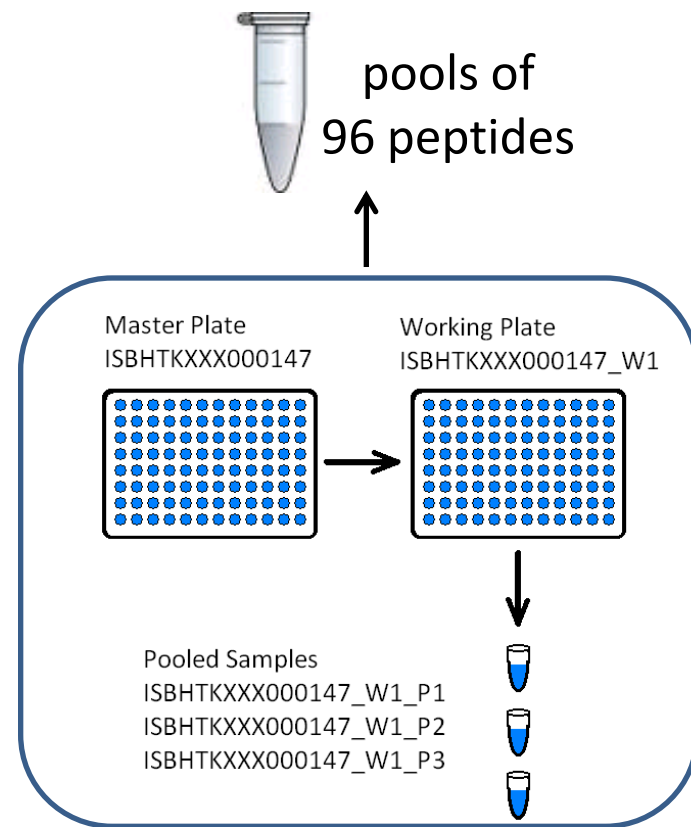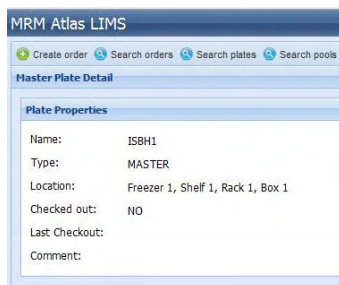
mix & dilute peptides

Data Aquisition

96 peptides

&

Inclusion List
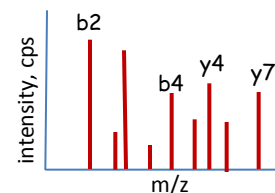
'Based on Expected m/z'
2 -> 2,3
3 -> 2,3,4
4 -> 2,3,4

Agilent AutoPreferredExcludeMSMSTable
→ exclusive
→ 5 CE steps per charge state

GQEVETSVTYYR, z=2
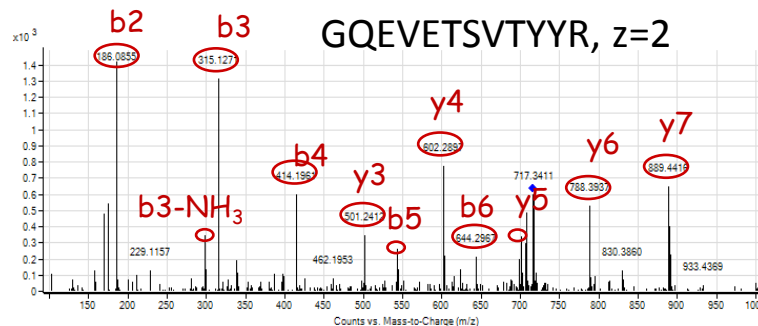
MRM Atlas LIMS

convert to mzML &
data base search

consensus spectrum

Q1	Q3	RT
691.9	659.4	25.2
691.9	730.4	25.2
691.9	900.2	25.2
691.9	303.1	25.2

transition list

Agilent
6530 QTOF

Agilent
6460 QQQ

SRM spectrum

Retention time stability from June –September 2010

14
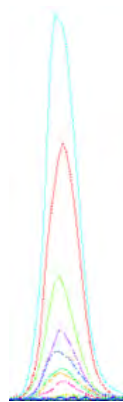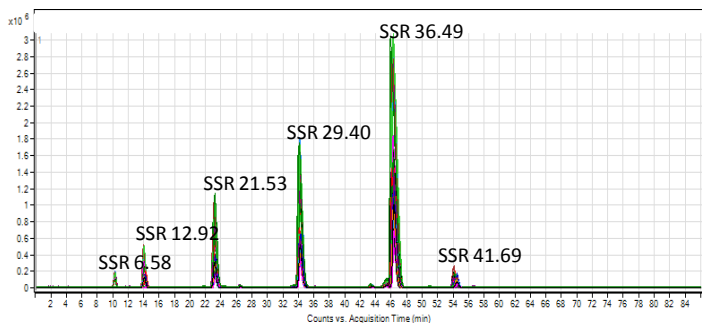
Collision Energy Optimization: QTOF

+ESI Product Ion (20.321 min) Frag=150.0V CID@29.0 (937.4493[z=2] -> **)

Original CE

937.4473
175.1157
343.1606
545.2599
856.4166
1018.4779
1260.5660

YLFPLNEIVVDFYDSLK, [M+2H]$^{2+}$

- m/z 826.4325, y142+
- m/z 899.9667, y152+
- % Precursor
- m/z 277.1547, b2

Collision Energy, V

FGYDDDLDLTPEYLFPLLK, [M+2H]$^{2+}$

- m/z1119.6449, y9
- m/z 470.3337, y4
- % Precursor

Collision Energy, V

VESVDLLAFPAYLLGIDSGR, [M+2H]$^{2+}$

- m/z1161.6262, y11
- m/z1308.6947, y12
- % Precursor

Collision Energy, V

TLLSFAQGDVITLLIPEEK, [M+2H]$^{2+}$

- m/z 1326.7515, y12
- m/z 502.2508, y4
- % Precursor

Collision Energy, V

+ESI Product Ion (20.387 min) Frag=150.0V CID@37.5 (937.4494[z=2] -> **)

New CE

343.1596
72.0805
228.1326
545.2671
458.2367
761.3419
937.4551
856.4211
1018.4783
1260.5835
1332.6221
1418.6545
1646.7503

Applied new CE values to a mixture of 120 peptides resulting in a 94% recovery rate.

QTOF Collision Energy brackets

- ➢ SRMAtlas Agilent QTOF data acquired with 5 discrete CE values*

- ➢ 2 above and 2 below 'calculated optimal' value based on z

- ➢ Initial spectral libraries, inclusion lists built using all above cutoff

+1, +2 z ions:
Base CE = (2.93*mz)/100 + 6.72 V
Step size 5.0 V

+3 z ions:
Base CE = (3.6*mz)/100 - 4.8 V
Step size 3.5 V

+4 z and above:
Base CE = (3.6*mz)/100 - 4.8 V
Step size 2.5 V

System reproducibility

Instrument to Instrument Chip reproducibility

Two Agilent 6420 triple quadrupole with Nano LC-Chip interface (QQQ-1 & QQQ-2) using the same chip on separate days. Chromatograms are overlaid for 5 injections of each instrument
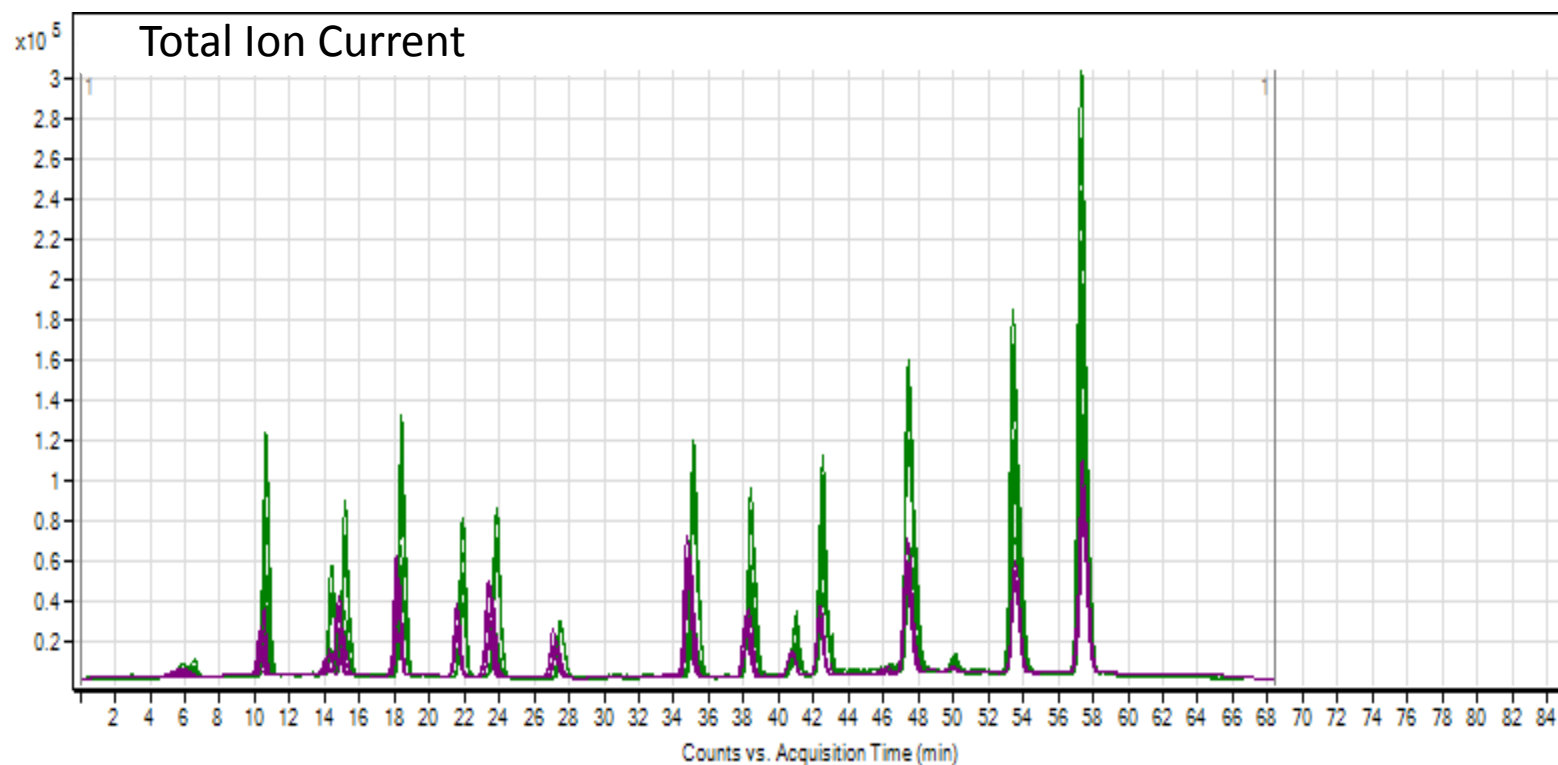
System reproducibility
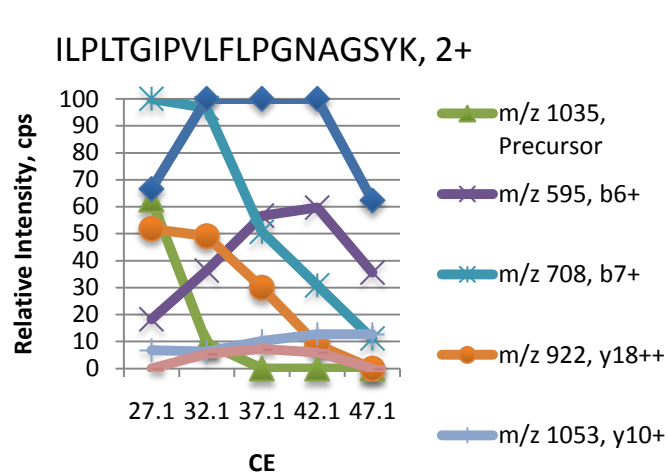
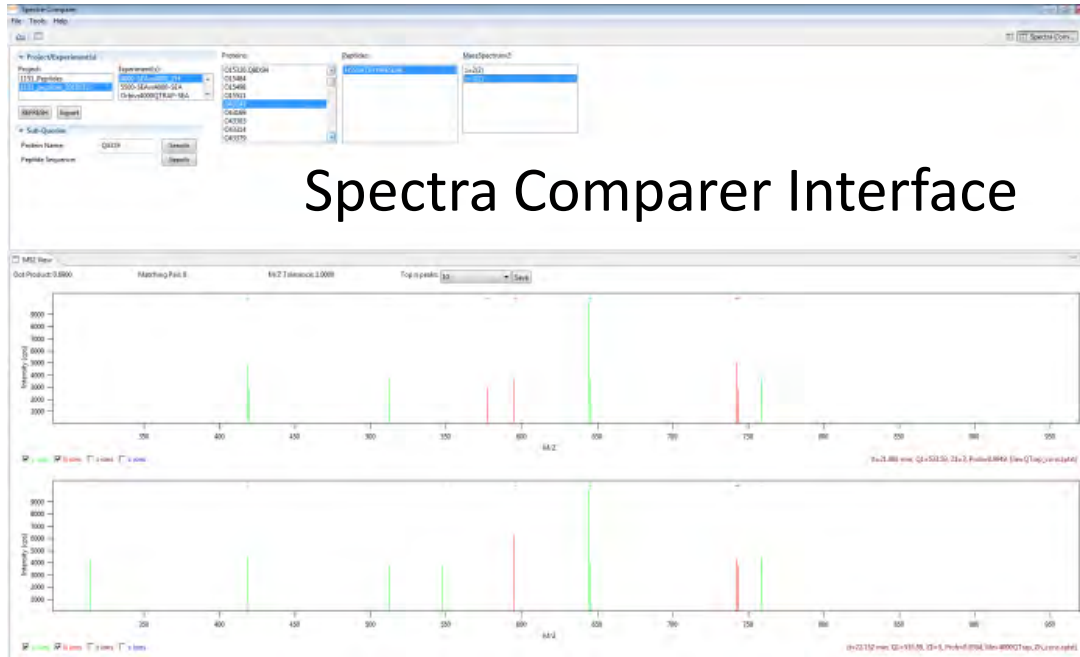## Separate Instrument Chip reproducibility
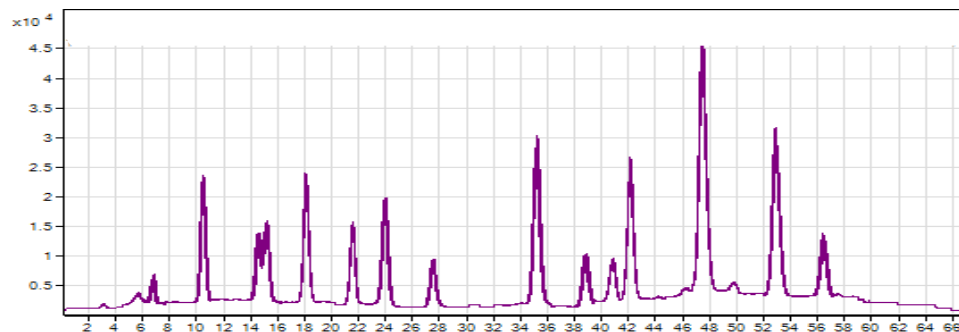
QQQ-1, 10 injections
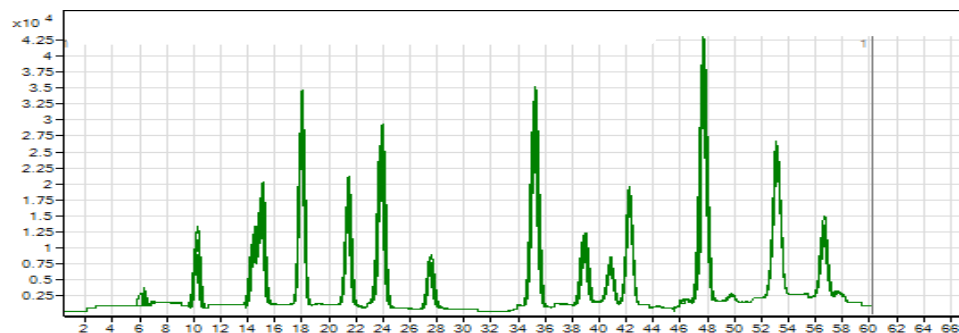
QQQ-2, 10 injections
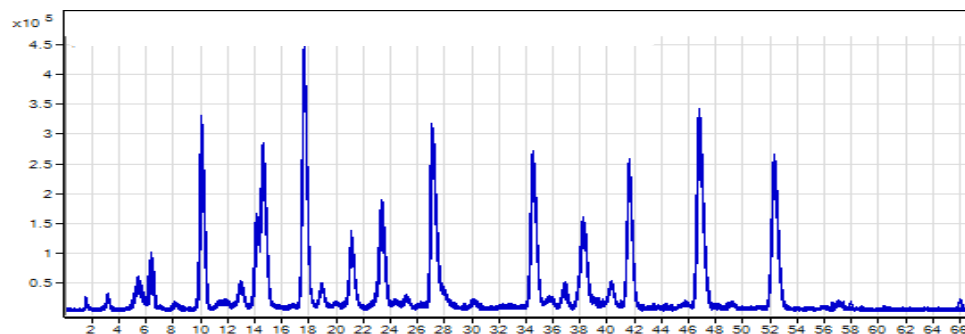
QTOF, 10 injections

Injection of ISB-Atlas RT standard, 3 separate vials, 3 separate chips, 3 separate instruments

Transition ion transportability
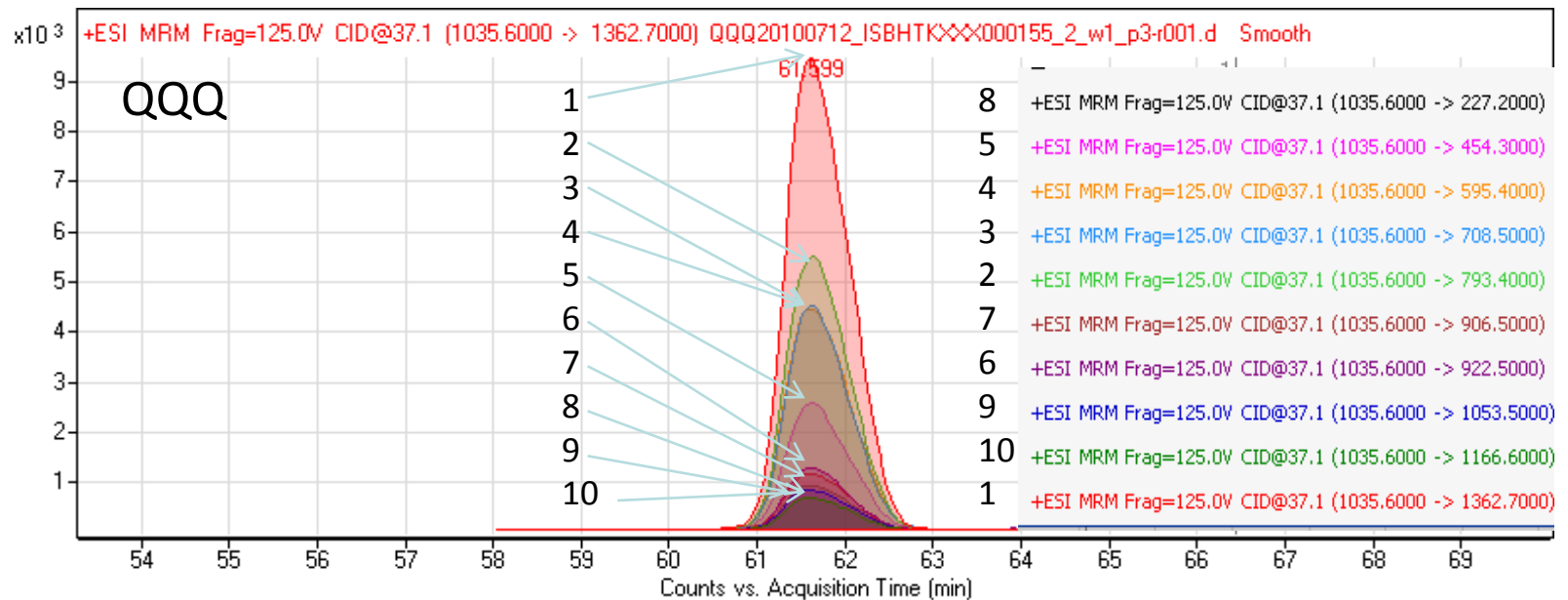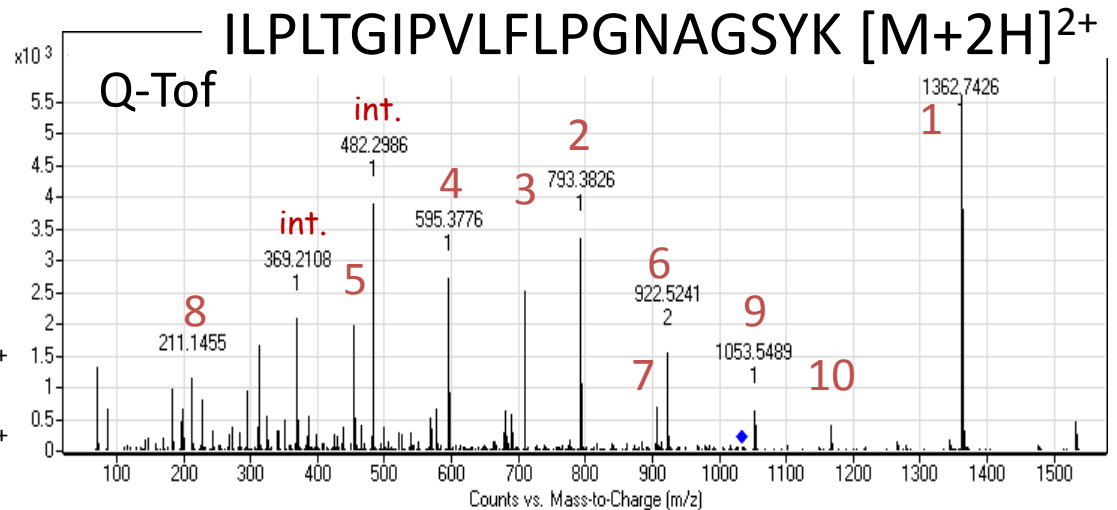
Spectra Comparer Software

Spectra Comparer Interface

SRM spectrum

consensus spectrum

- butterfly view
- value vs percent intensity
- create sptxt from SRM
  →compare SRM & MS2

PeptideAtlas
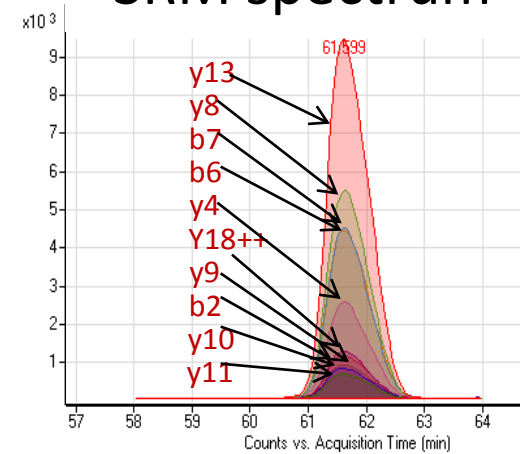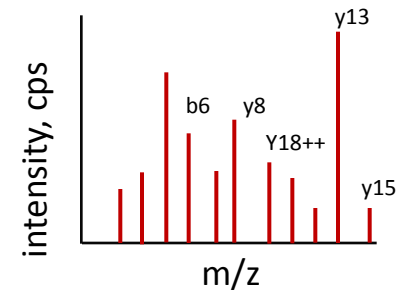
Spectra Comparer - Comparison of Abundance

Dot Prod
0.28

Matching
pair: 9

GQEVETSVTYYR, *z*=2
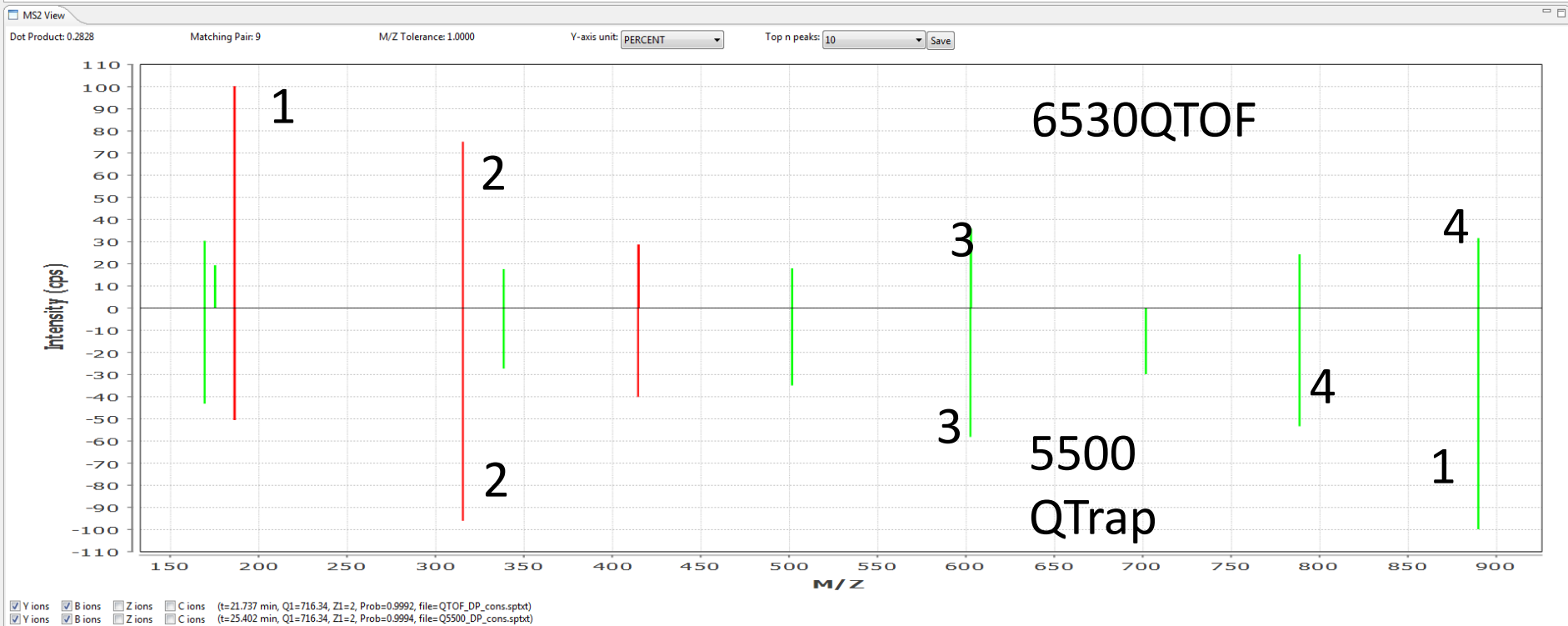
Green: y-ions

Red: b-ions   *m/z* 100-1000
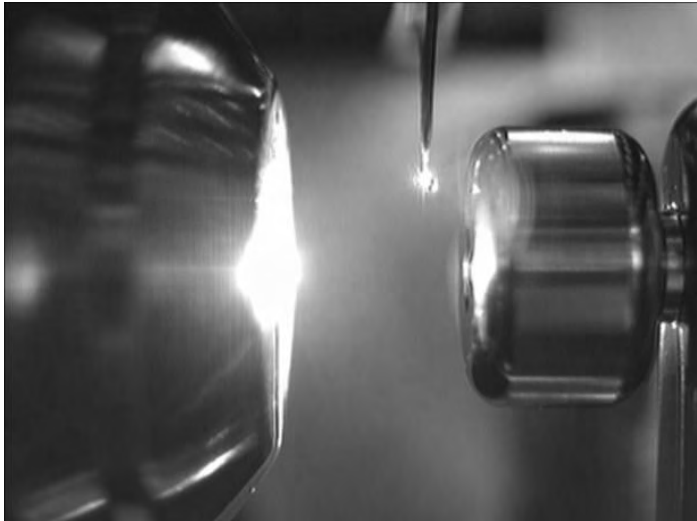
SRMAtlas interface

www.srmatlas.org

Suggested transitions,
Collision Energy plots,
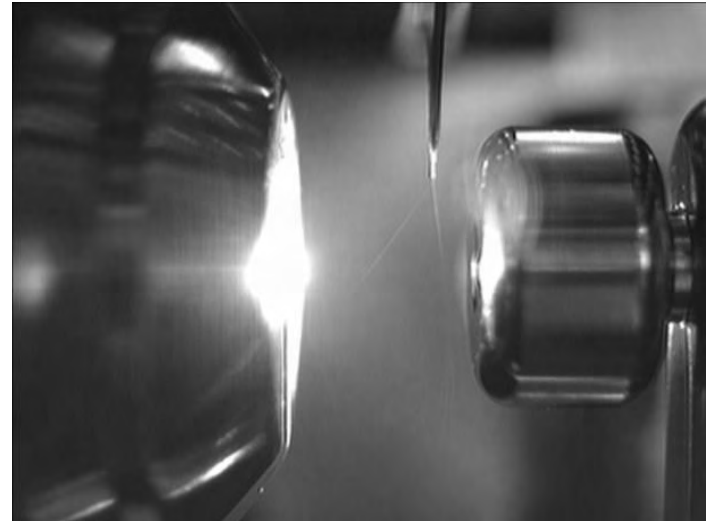*Calculated hydrophobicity*
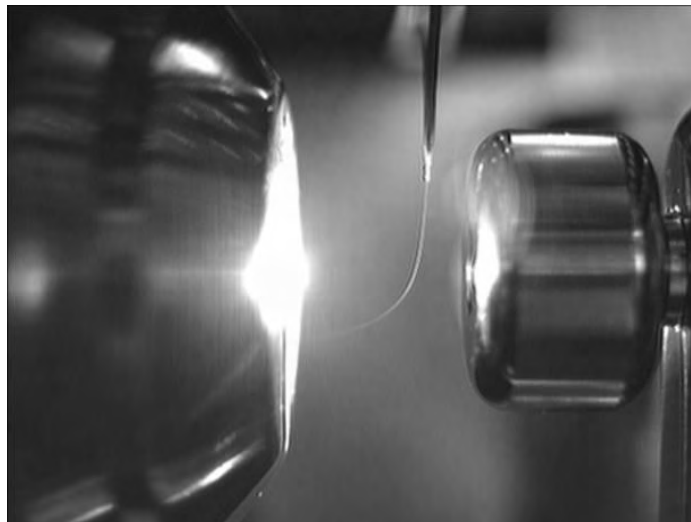*Fragment relative intensities*

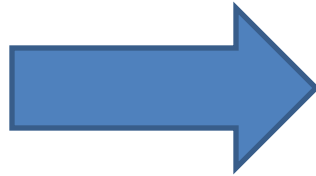**Chip Spray degradation**

Dripping spray at high voltage

Split and spitting spray

Good spray

Chip Snuggie

Care! Chip should not be bumped or snagged with tip exposed

Materials for cleaning:
Kimwipes, Gloves

clip

Polyimide Chip body

Step 1.   With gloved hands, gently press down the clip and with your other hand hold the polyimide while slowly and carefully pushing it forward to expose the tip.
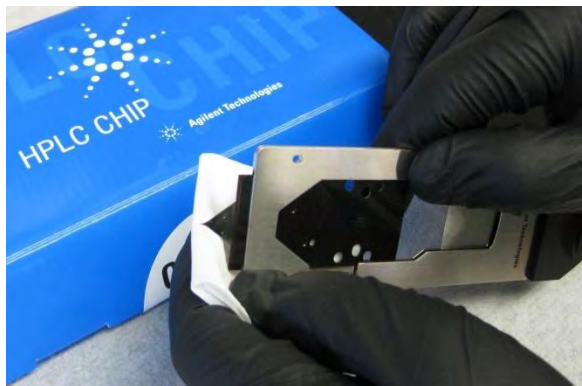
Step 2.   Gently sweep the left side of the tip across a folded Kimwipe twice. Repeat Step 2 for the right side of the tip.

Step 3. Gently sweep the top surface of the tip across a folded Kimwipe twice. Repeat Step 3 for the bottom surface of the tip.
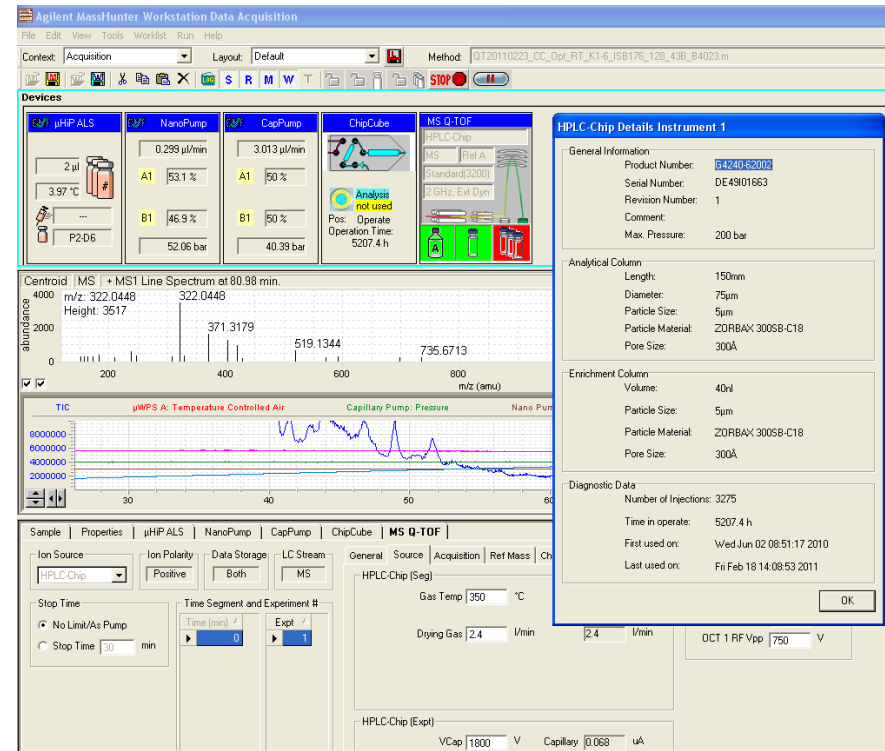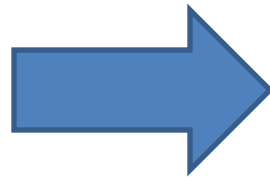

Step 4. Carefully press the clip and with your other hand gently pull the polyimide back into the chip holder until the polyimide clicks back into place.

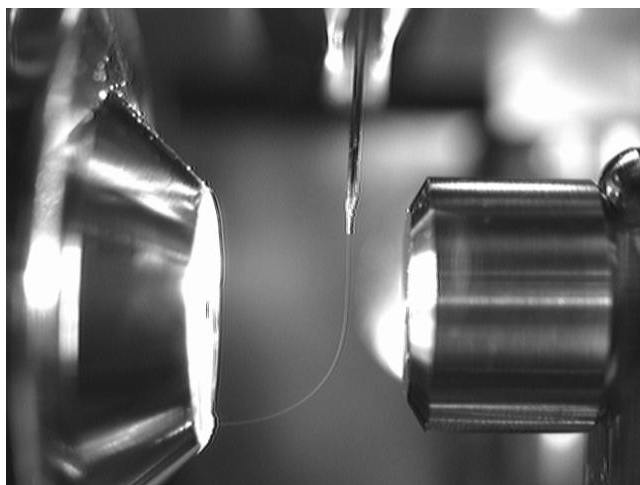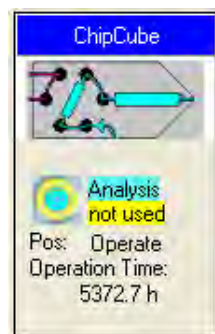**Chip is now ready for use. Lowering the Vcap voltage back to ~1750 may be necessary for achieving stable spray.**
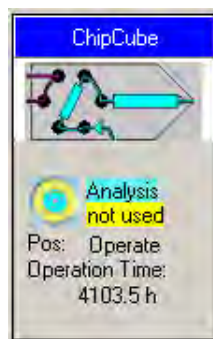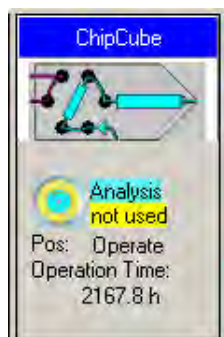
Chip Snuggie

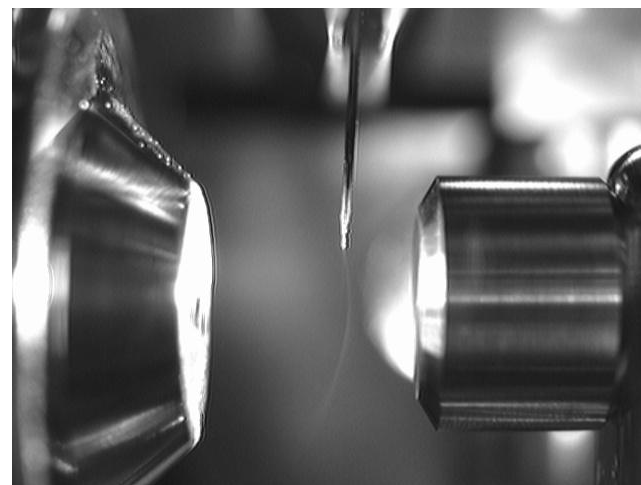After tip cleaning, you will experience many hours of enjoyment………

Chip: 3275 injections, 5207 hours
Nanopump at 0.3 uL/min at 52.0 bar
Cappump at 3.0 uL/min at 40.0 bar

Extended lifetime of the LC-Chip

Chip Snuggie

June 2010

March 2011

Acknowledgements

www.srmatlas.org

www.systemsbiology.org