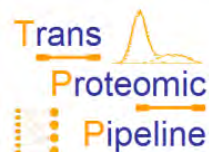


Robert Moritz

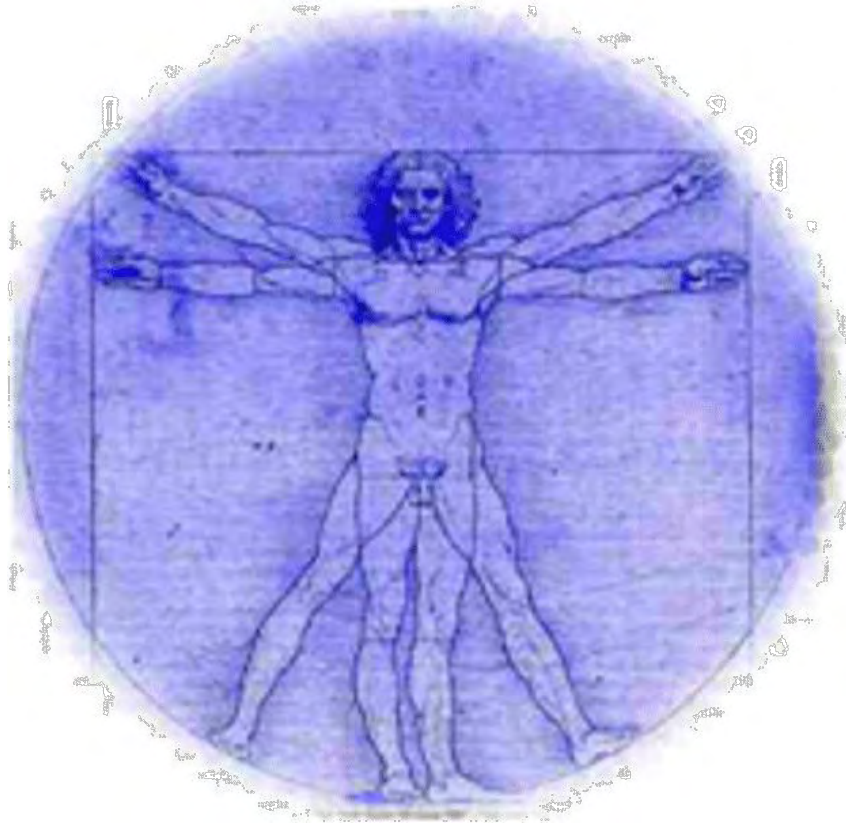
Developing a Complete and Reproducible Human SRMAtlas



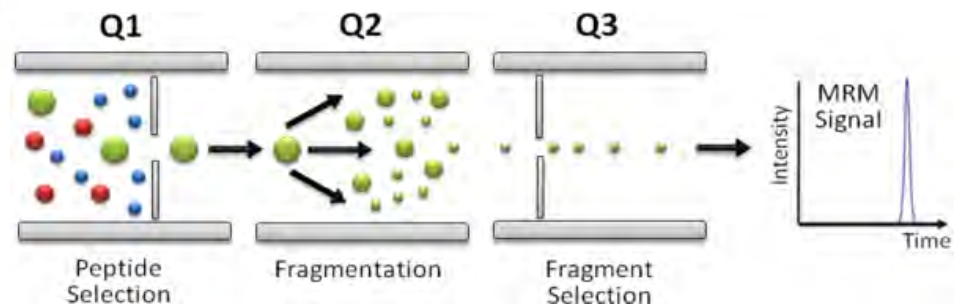
Outline

- 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

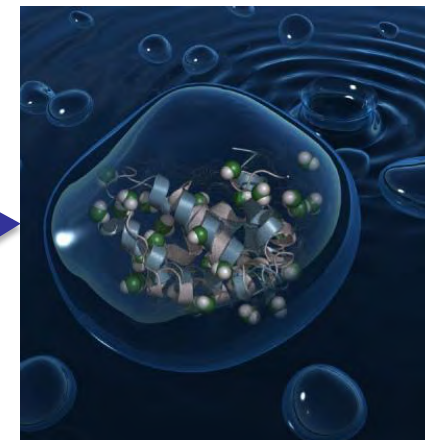
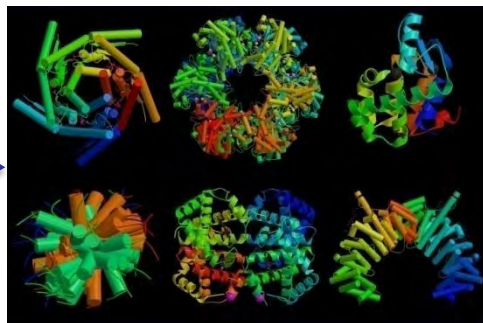
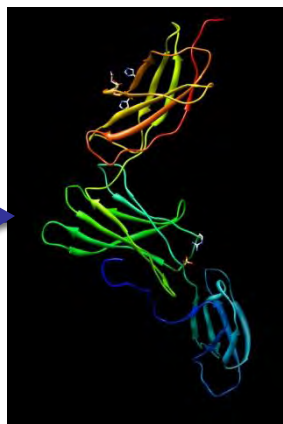
Periodic Table of the Elements

1																	2	
H																	He	
3	4											5	6	7	8	9	10	
Li	Be											B	C	N	O	F	Ne	
11	12											13	14	15	16	17	18	
Na	Mg											Al	Si	P	S	Cl	Ar	
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr	
37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe	
55	56	57	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn	
87	88	89	104	105	106	107	108	109	110									
Fr	Ra	Ac	Unq	Unp	Unh	Uns	Uno	Une	Uun									
58	59	60	61	62	63	64	65	66	67	68	69	70						
Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu					
90	91	92	93	94	95	96	97	98	99	100	101	102	103					
Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr					

Legend:

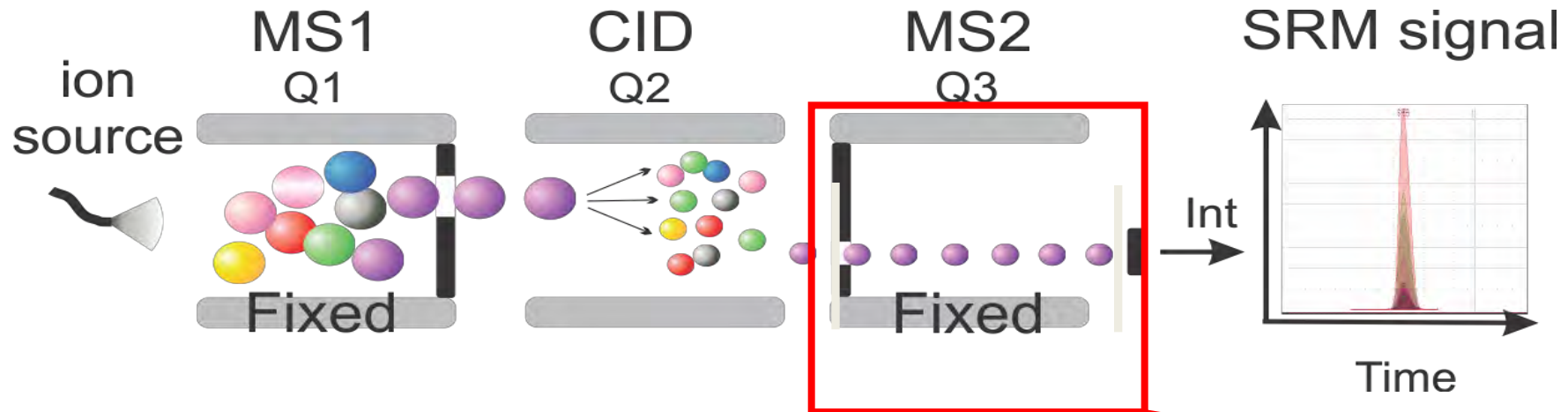
- hydrogen (green)
- alkali metals (yellow)
- alkali earth metals (light blue)
- transition metals (orange)
- poor metals (blue)
- nonmetals (white)
- noble gases (red)
- rare earth metals (grey)

Surveying to create an Atlas



Navigating the Atlas

Selected Reaction Monitoring (SRM or MRM)

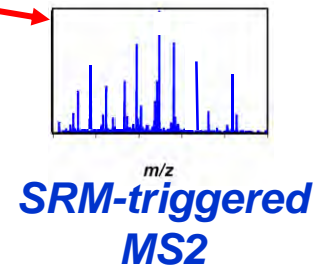


- two levels of mass selection: *high specificity*
- not scanning (Q1/Q3 static), high duty cycle: *high sensitivity*
- the most sensitive mass spectrometry method known (low amole)

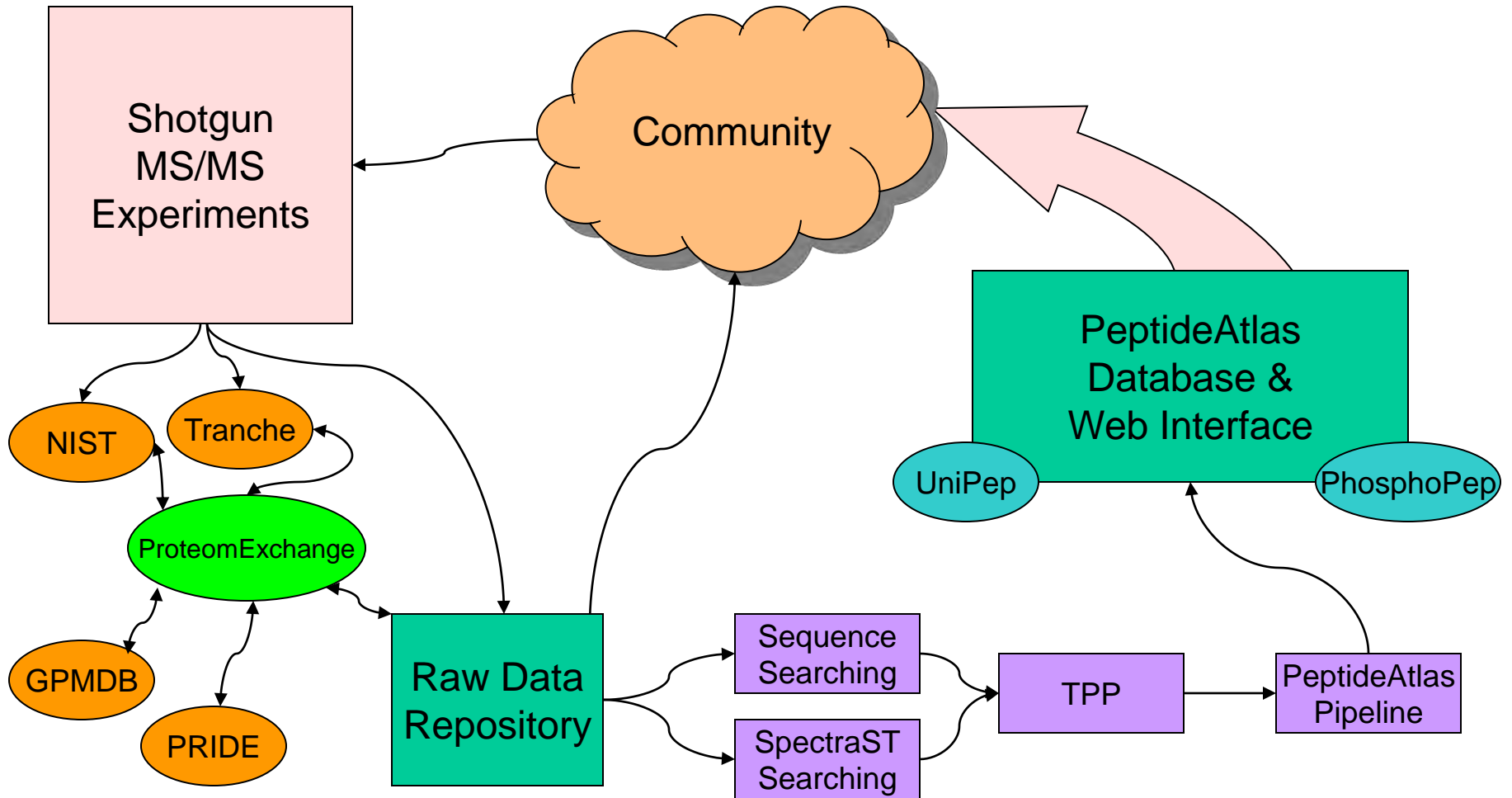
....you need to know what to look for!

(the mass spectrometrists' ELISA)

*MS/MS profile
by segmented
or trap mode*



PeptideAtlas Workflow



What is a proteotypic peptide?

Protein



Summary
Sequence
1total
Grad
LCQstdpepmix
Mix1
Mix2
Mix3
MixA
Step1
Step2

```

KCFQIENPLECQDKGEEELQKVIQESQALAKRSCGLEQ
KCFQIENPLECQDKGEEELQKVIQESQALAKRSCGLEQ
KCFQIENPLECQDKGEEELQKVIQESQALAKRSCGLEQ
KCFQIENPLECQDKGEEELQKVIQESQALAKRSCGLEQ
KCFQIENPLECQDKGEEELQKVIQESQALAKRSCGLEQ
KCFQIENPLECQDKGEEELQKVIQESQALAKRSCGLEQ
KCFQIENPLECQDKGEEELQKVIQESQALAKRSCGLEQ

```

Summary
Sequence
1total
Grad
LCQstdpepmix
Mix1
Mix2
Mix3
MixA
Step1
Step2

```

KLLACGEGAAII
KLLACGEGAAII
KLLACGEGAAII
KLLACGEGAAII
KLLACGEGAAII
KLLACGEGAAII
KLLACGEGAAII

```

Summary
Sequence
1total
Grad
LCQstdpepmix
Mix1
Mix2
Mix3
MixA
Step1
Step2

```

RRPCFSSLVVDETYVPPAFSDDKFIHKDLCIA
RRPCFSSLVVDETYVPPAFSDDKFIHKDLCIA
RRPCFSSLVVDETYVPPAFSDDKFIHKDLCIA
RRPCFSSLVVDETYVPPAFSDDKFIHKDLCIA
RRPCFSSLVVDETYVPPAFSDDKFIHKDLCIA
RRPCFSSLVVDETYVPPAFSDDKFIHKDLCIA
RRPCFSSLVVDETYVPPAFSDDKFIHKDLCIA

```

Peptide region **never** observed in multiple experiments

Peptide region **consistently** observed in multiple experiments₇

PeptideAtlas protein view page

PeptideAtlas Build: Human Plasma, PeptideAtlas 2006-08
 Protein Name: ENSP00000285381
 Gene Name: ENSG00000164879
 Description: pep.known-ccds chromosome:NCBI36:8:86537710:86548526.1 gene:ENSG00000164879 transcript:ENST00000285381 CDS:56238.1
 Entrez Gene Symbol: CA3
 Entrez GeneID: 781
 Full Name: CA3: Carbonic anhydrase 3
 IPI: IPI00216983
 RefSeq: REVIEWED/NP_005172
 UniGene: Hs.82129
 UniProt: P07451
 UniProt Symbol: CAH3_HUMAN
 Total peptides: 14

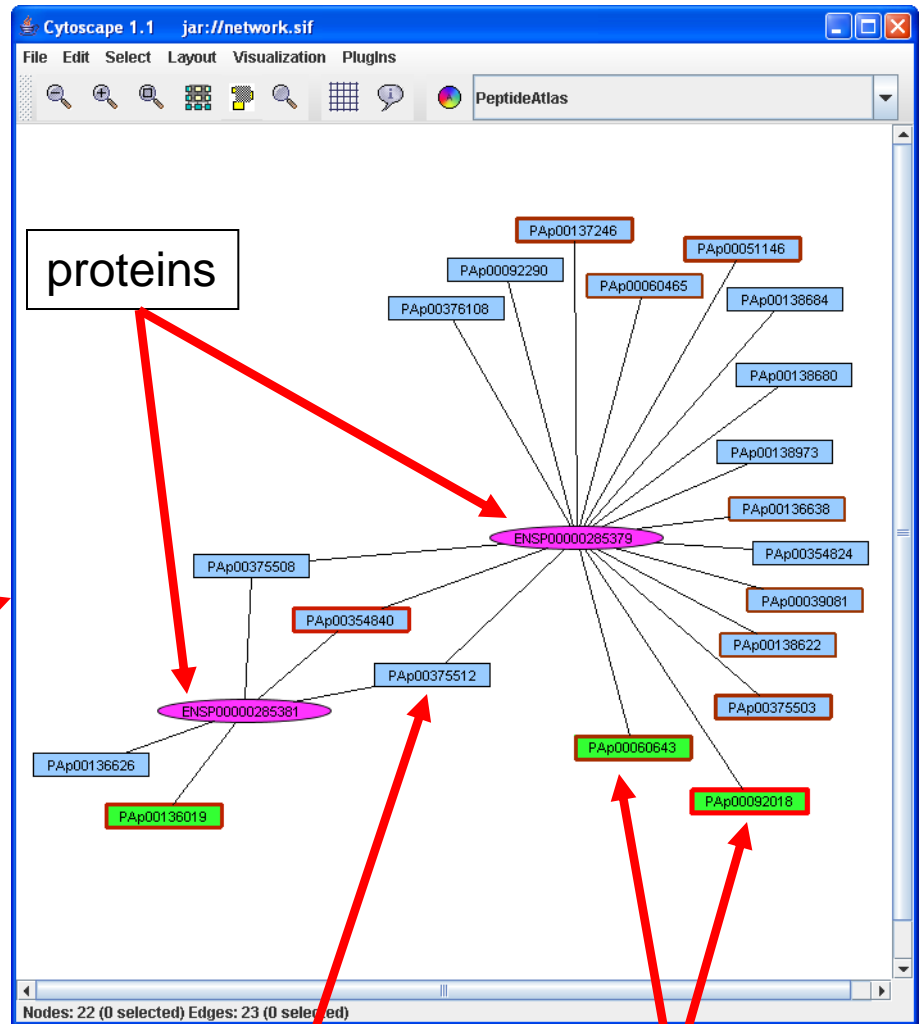
Sequence: MAKEWYASH NCPDHWELP INAKGRNQP VRLHTKEDR DPSELQWVSV YDGGSAKTL INQKTCRVF DDTYRSLD GGLPQPTPL PQLPLHWCS DQGSERTVD GPKYAEHLR VMRPPKTYTF KRALRQNDI APTGKLEK HIRGEPFL DALDKIKTKG KRAPFKPQK SCLPFAQYQI VTYGSPITP PCRCIVLWEL LKRPHTVSD QEAQLRLLS SARNEPVPVL VSNRPPQPI NNRVVAQFK

Protein Coverage = 15.7%

Peptide Accession	Peptide Sequence	Best Prob	N Obs	Empirical Proteotypic Score	SSRCalc Relative Hydrophob	N Protein Mappings	N Genome Locations	Sample IDs
PAp00136019	WFDDTYDR	0.998	5	0.50	20.73	1	1	1 199
PAp00136626	DIRHDPRLQFVWSVYDGGSAK	0.998	1	0.50	29.62	1	1	1 199
PAp00354840	YAAELHLVHV	1.000	6	0.50	32.59	2	1	2 289
PAp00375508	YAAELHLVH	0.958	1	0.50	23.41	1	1	2 289
PAp00375512	KYAAELHLVHV	0.931	1	0.50	34.37	1	1	2 289

Observed in Samples: 109: NCI1 sera Human Serum Survey

Cytoscape view of proteins & peptides



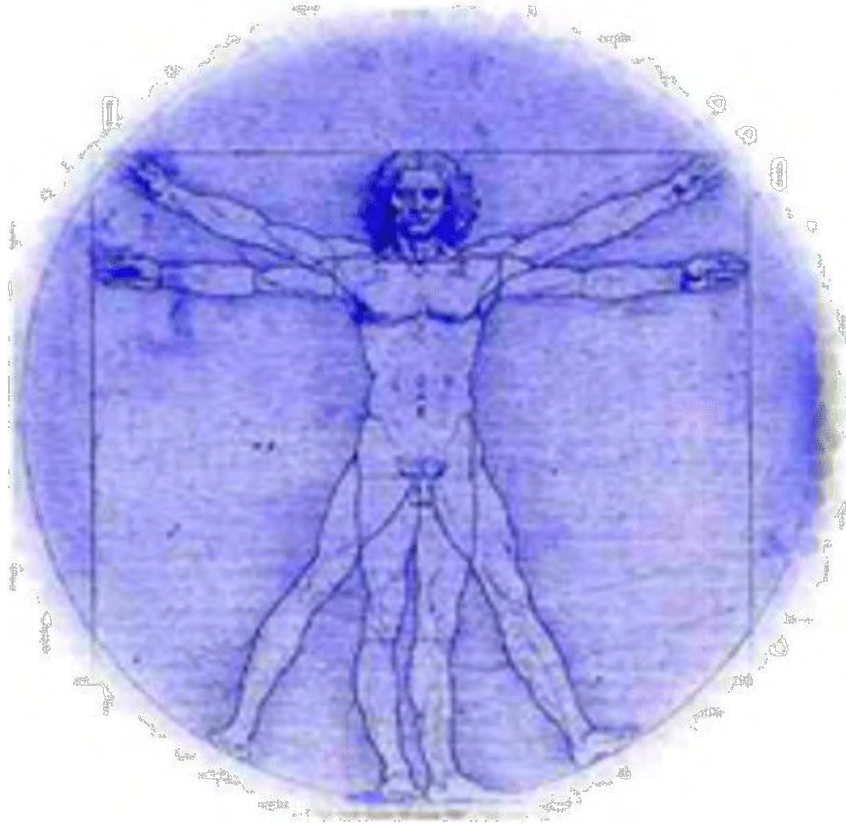
ambiguously mapped peptide

proteotypic peptides
 $N_{\text{prot}} = 1$ $N_{\text{obs}} > 8$
 $\text{EPS} > 0.3$

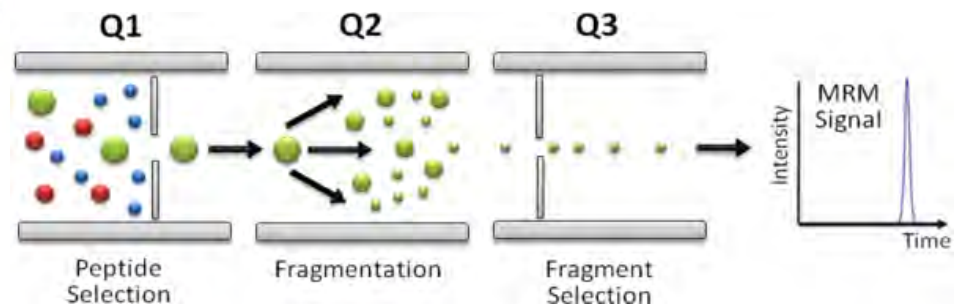
PeptideAtlas Build Summary

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)

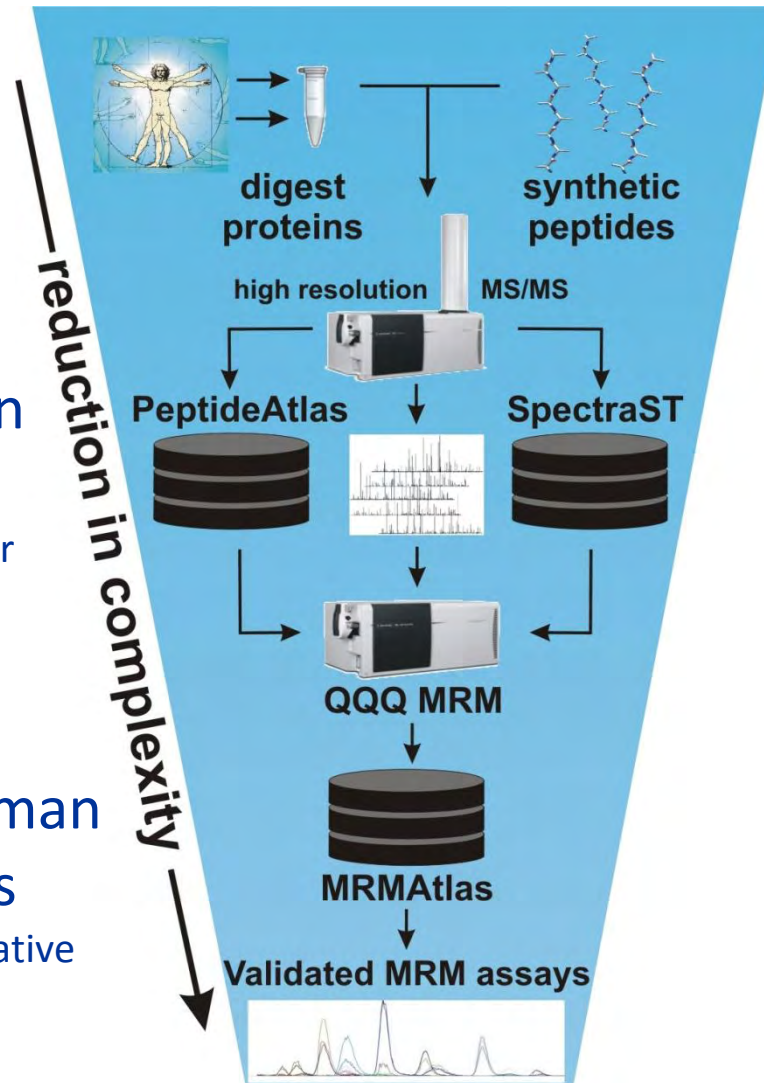


Developments at ISB - SRMAtlas

Human proteins
(from natural source or synthetic)

Develop Human
PeptideAtlas
(from tryptic digests or synthetic peptides)

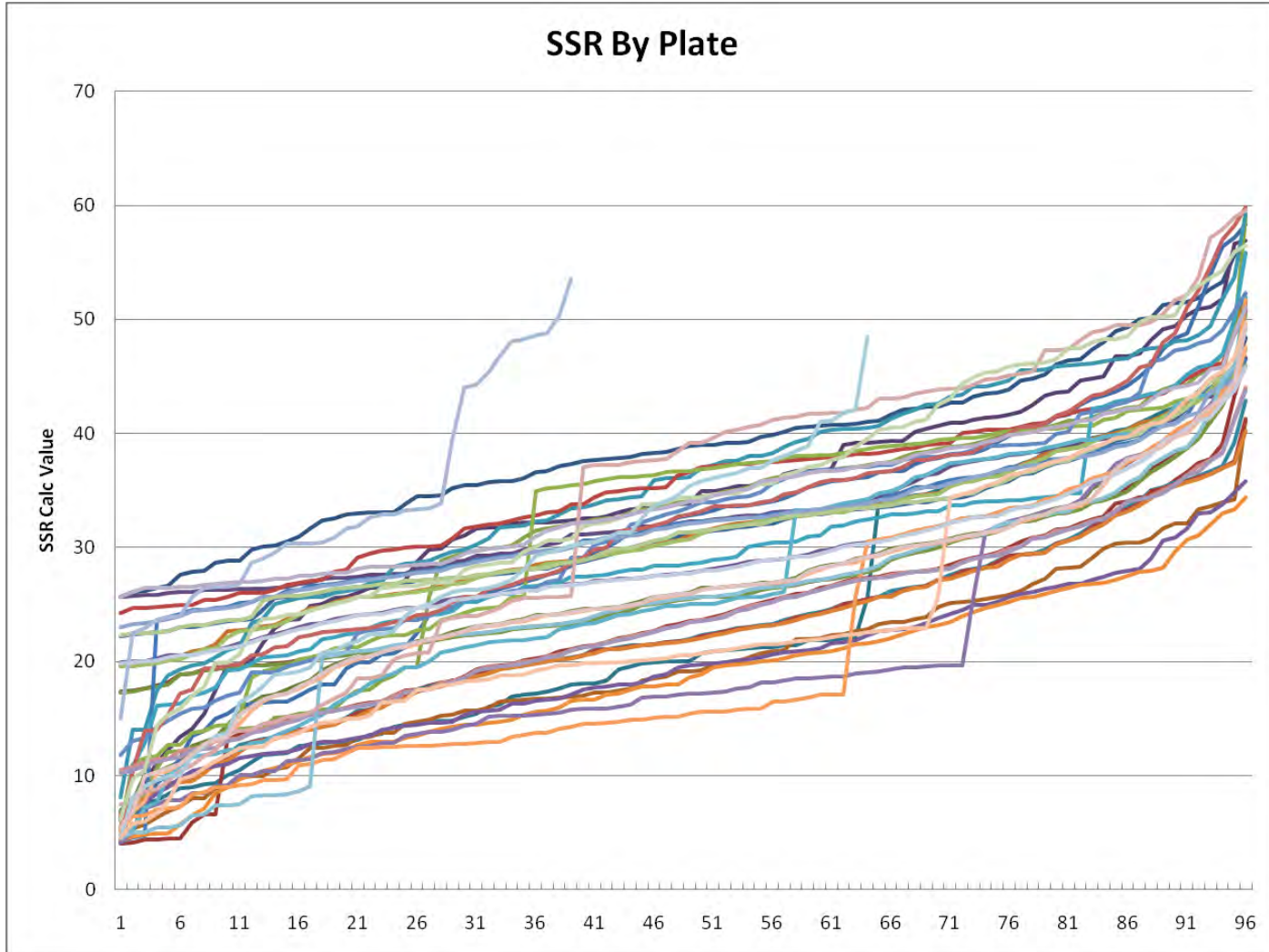
Develop Human
SRMAtlas
(verified quantitative assays)



Synthetic
“proteotypic”
peptide
(from cheap synthesis)

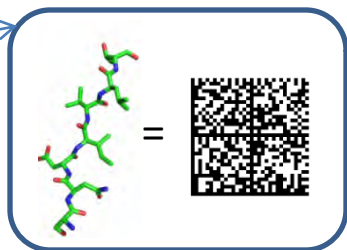
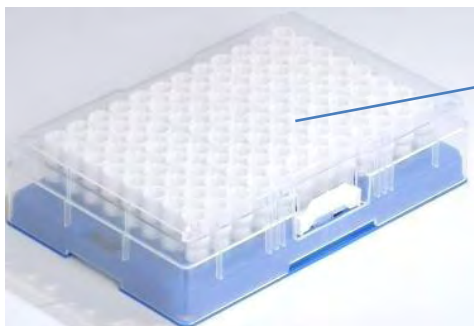
Develop
optimized
transitions from
PeptideAtlas

Peptide hydrophobicity (SSR) spread by plate



Peptide Tracking & Processing

> 150,000 peptides



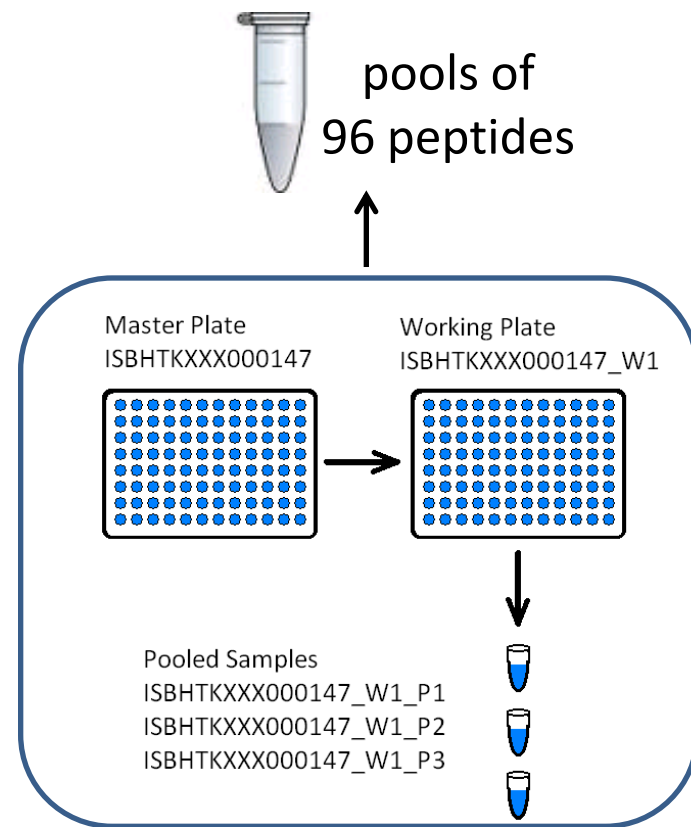
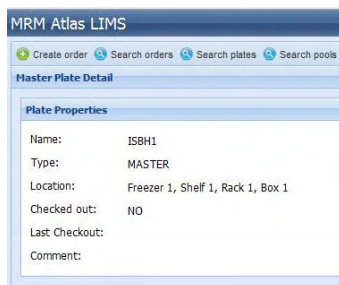
2D-barcode tube



ISBHJRMXX000026



Laboratory
Information
Management
System



mix & dilute peptides

Data Acquisition



96 peptides

&

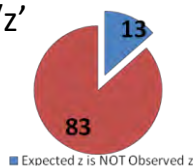
Inclusion List

'Based on Expected m/z'

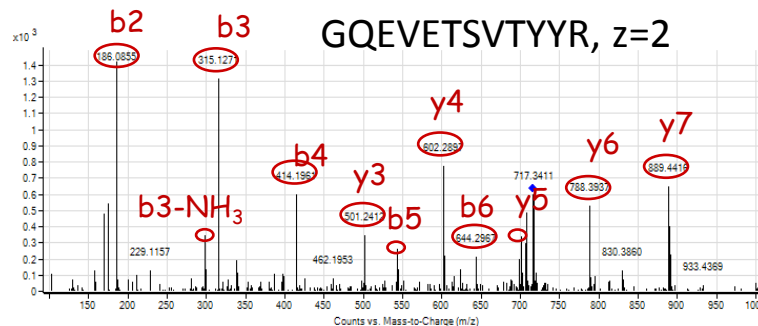
2 -> 2,3

3 -> 2,3,4

4 -> 2,3,4

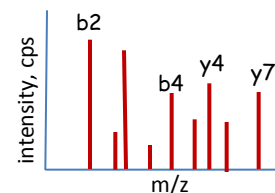


Agilent
6530 QTOF



MRM Atlas LIMS

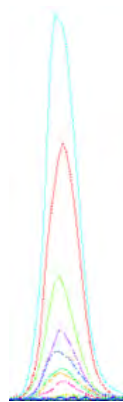
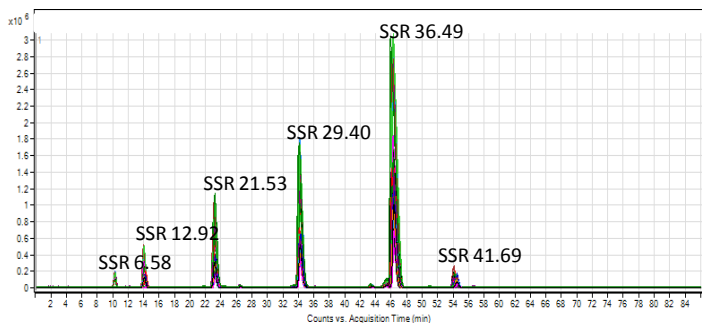
convert to mzML &
data base search



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

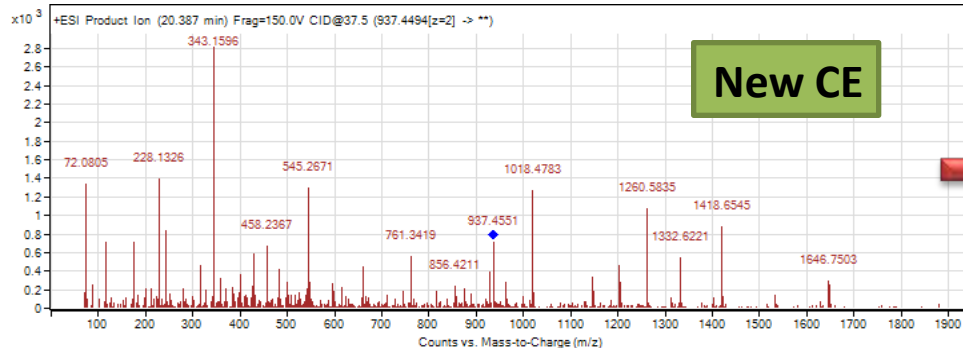
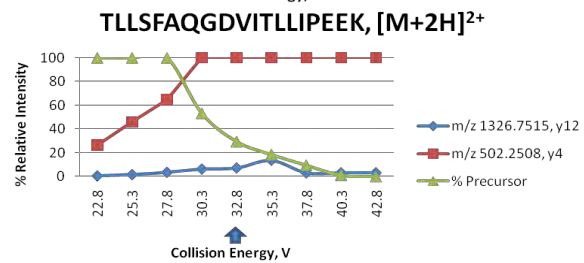
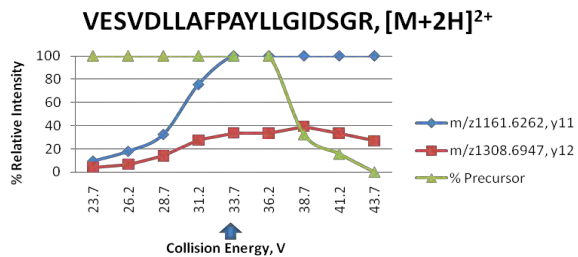
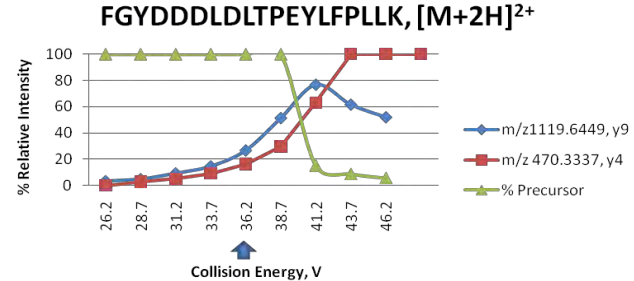
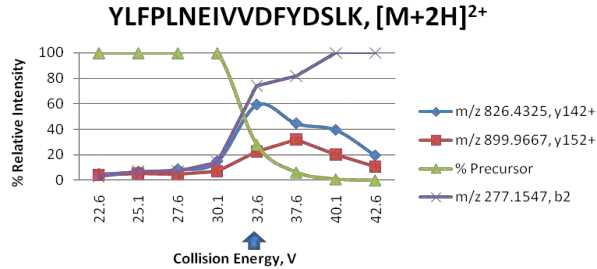
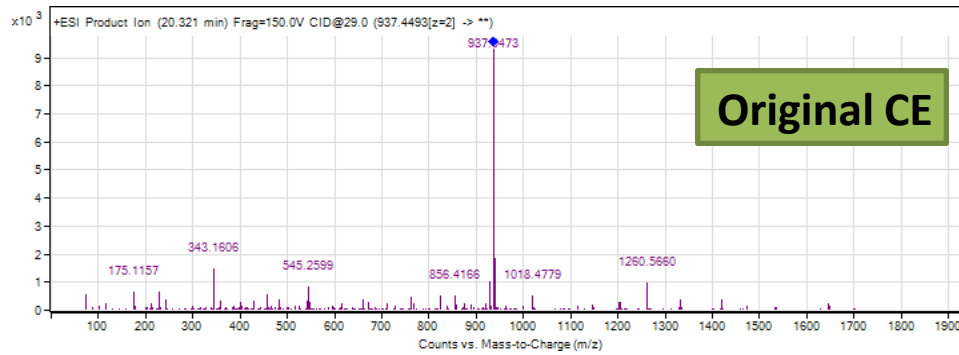


SRM spectrum



Agilent
6460 QQQ

Collision Energy Optimization: QTOF



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:

$$\text{Base CE} = (2.93 * \text{mz}) / 100 + 6.72 \text{ V}$$

Step size 5.0 V

+3 z ions:

$$\text{Base CE} = (3.6 * \text{mz}) / 100 - 4.8 \text{ V}$$

Step size 3.5 V

+4 z and above:

$$\text{Base CE} = (3.6 * \text{mz}) / 100 - 4.8 \text{ V}$$

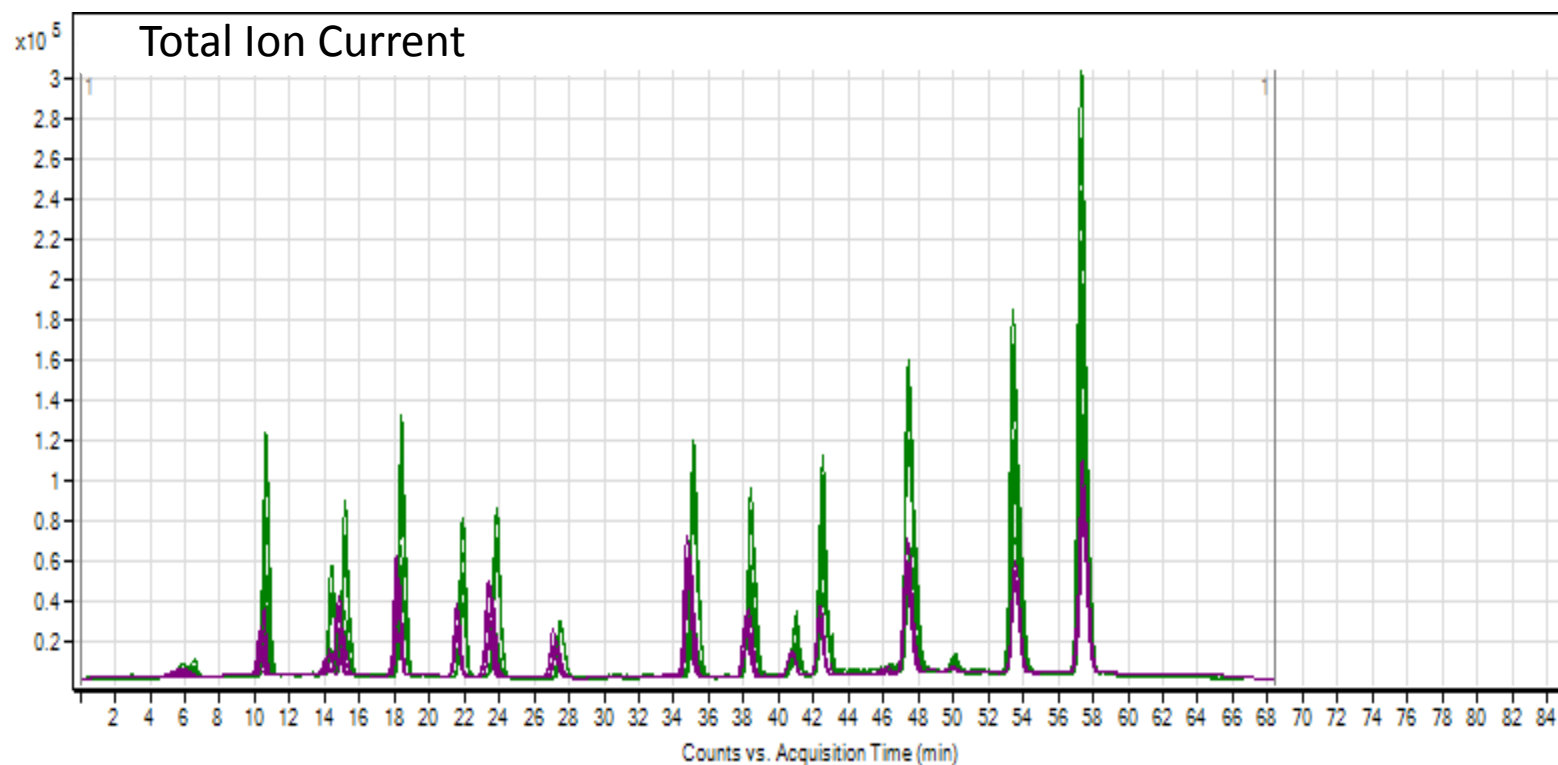
Step size 2.5 V

System reproducibility

Instrument to Instrument Chip reproducibility

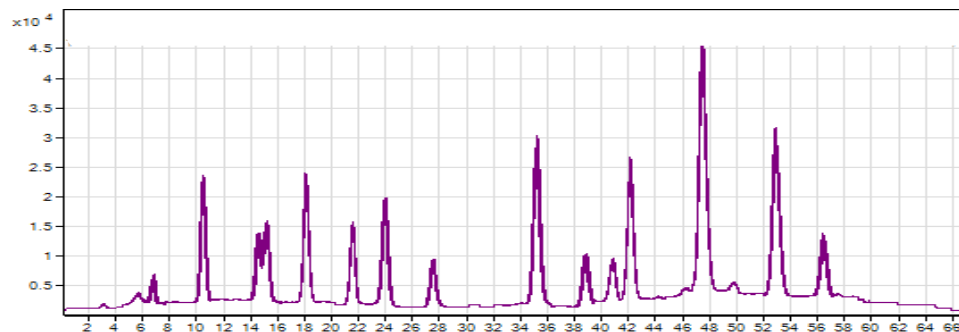
Two Agilent 6420 triple quadrupole with Nano LC-Chip interface (QQQ-1 & QQQ-2) using the same chip on separate days.

Chromatograms are overlaid for 5 injections of each instrument

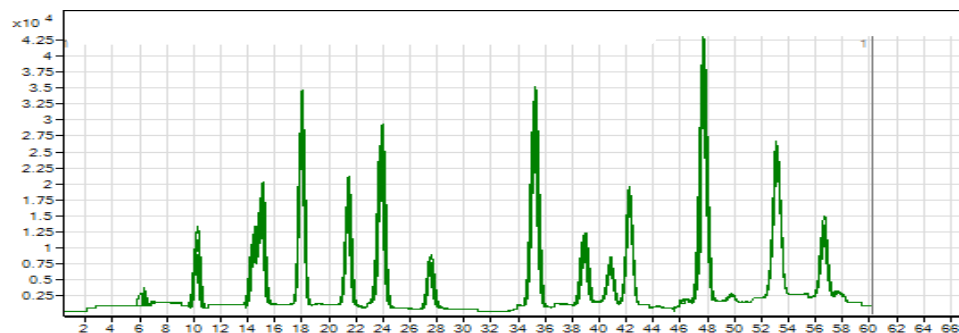


System reproducibility

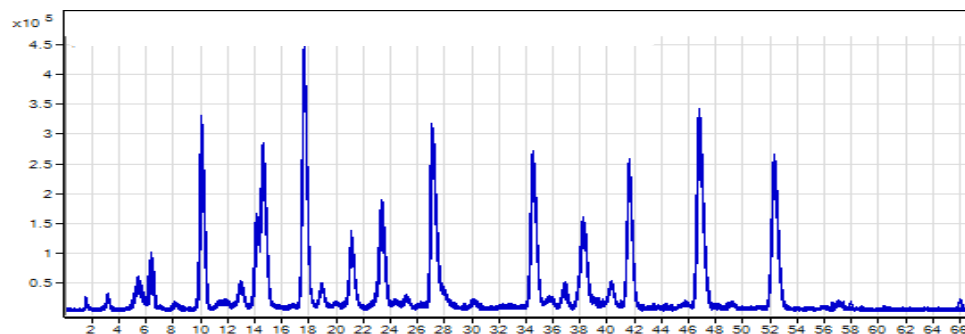
Separate Instrument Chip reproducibility



QQQ-1, 10 injections



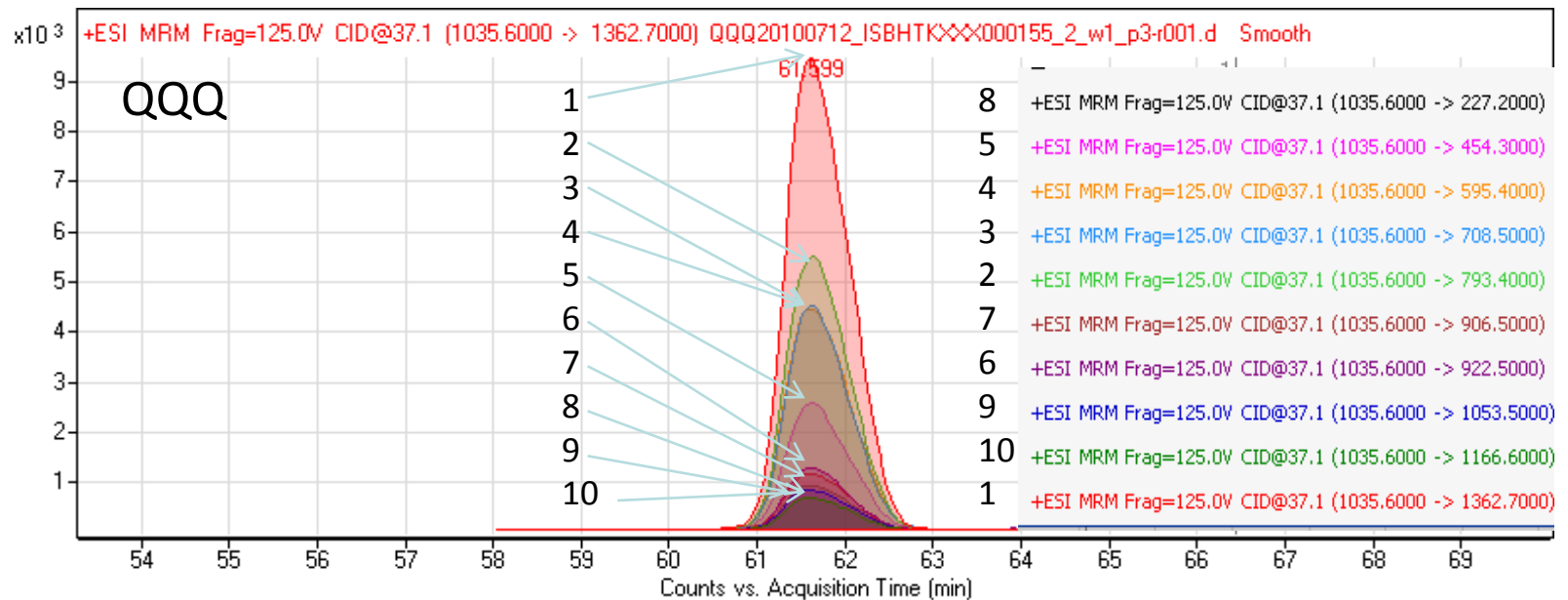
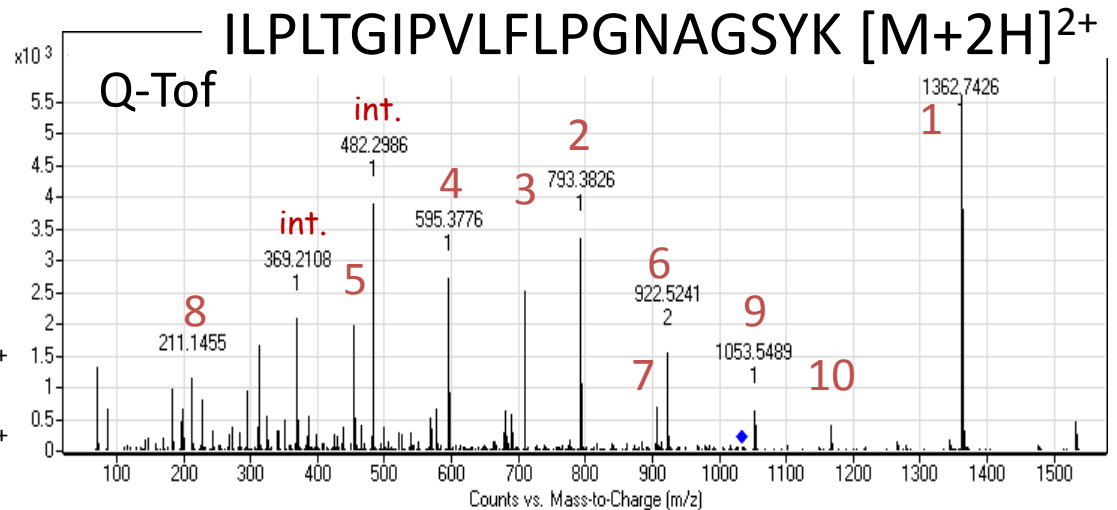
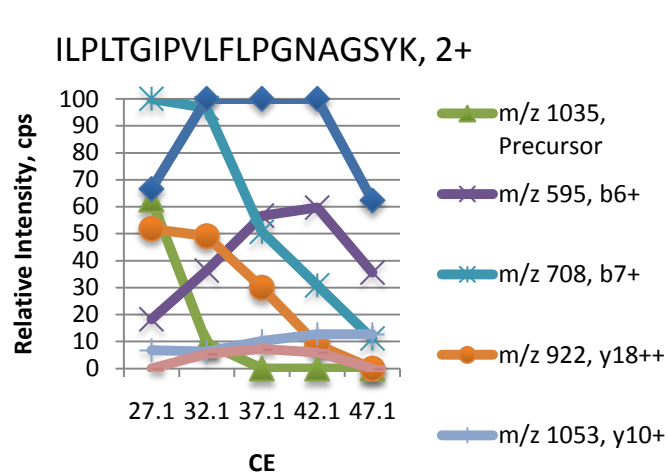
QQQ-2, 10 injections



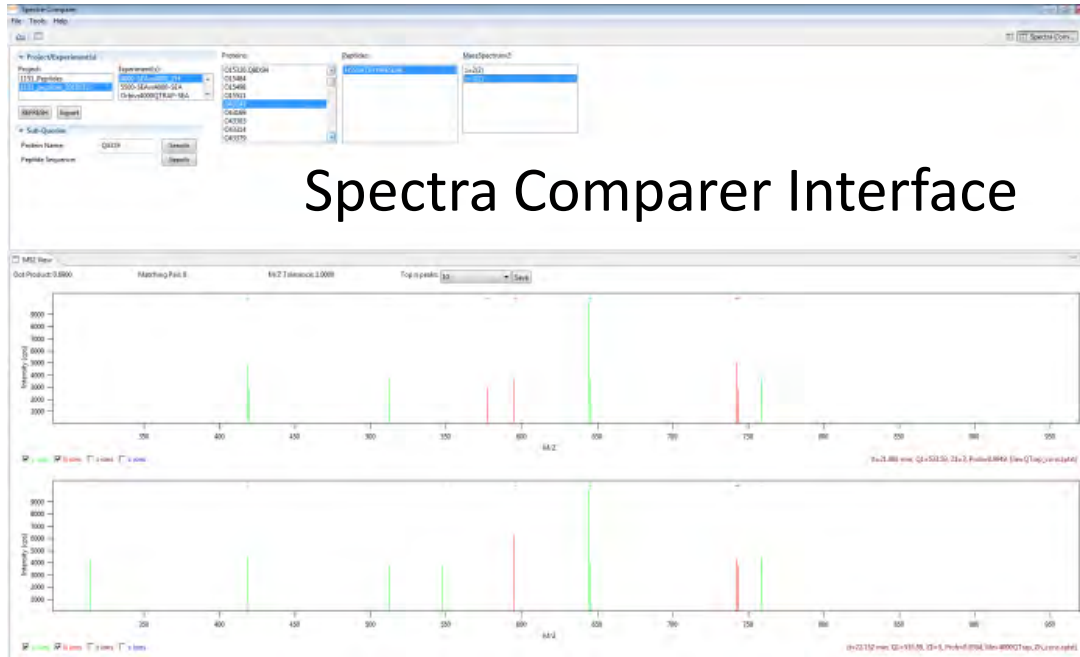
QTOF, 10 injections

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

Transition ion transportability



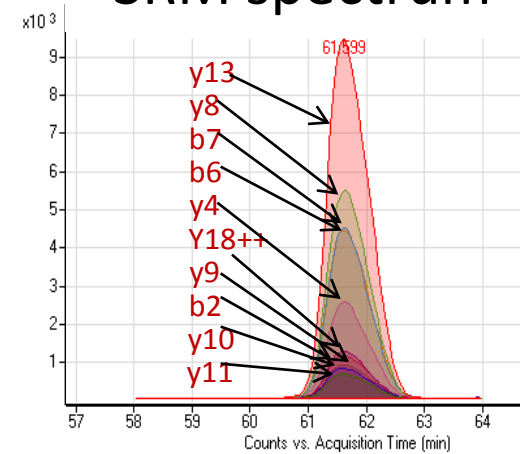
Spectra Comparer Software



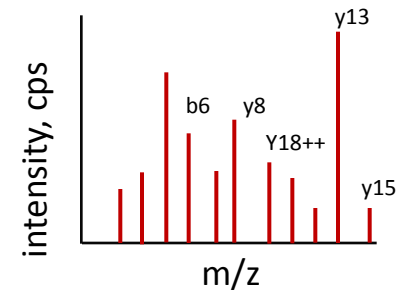
Spectra Comparer Interface

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

SRM spectrum



consensus spectrum

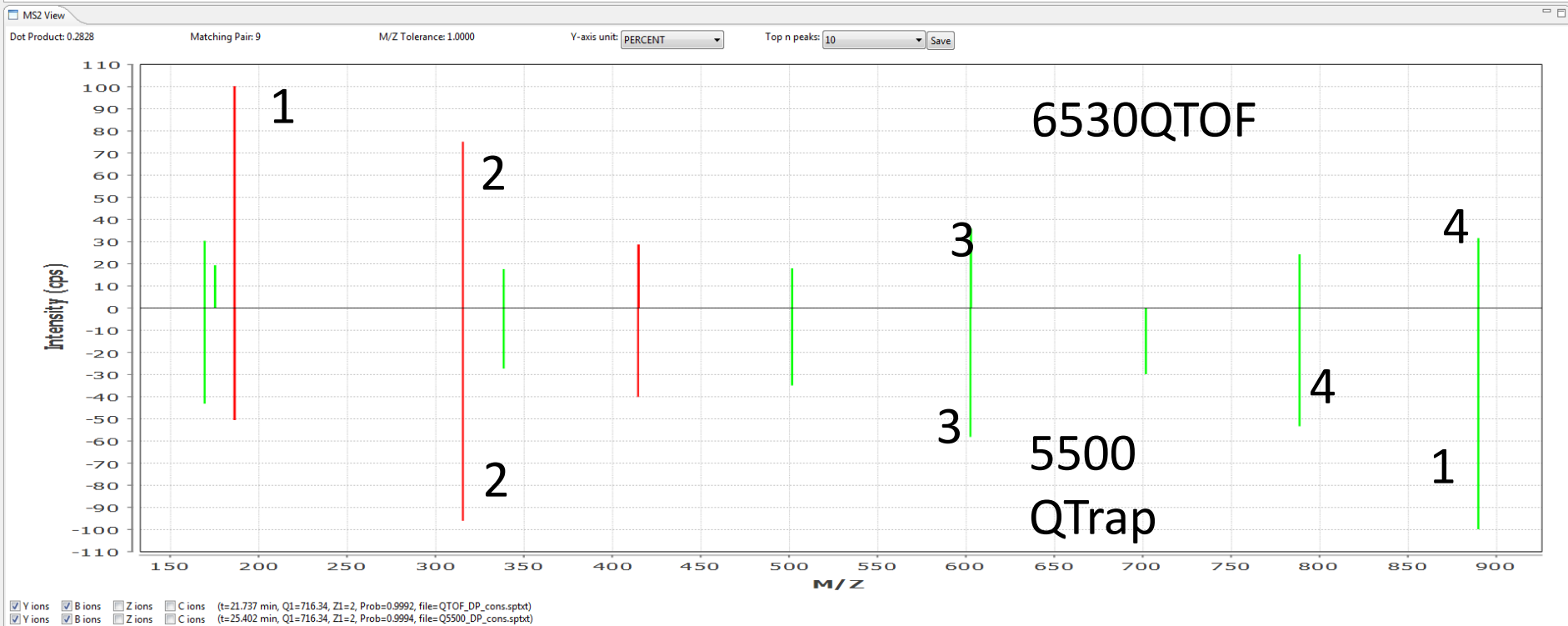


Spectra Comparer - Comparison of Abundance

Dot Prod
0.28

Matching
pair: 9

GQEVETSVTYR, z=2



Green: y-ions

Red: b-ions

m/z 100-1000

SRMAtlas interface

ISB Home

MRM ATLAS HOME

BACKGROUND
 Project Home
 Data Contributors
 Publications
 External Links
 Contacts
 SRM/MRM Assays
 SRM/MRM Glossary

DATA ACCESS
 Search Peptides
 Identified Proteins
 Pathway Search
 MRM Transitions
 Spectral Search
 Login

Current Organism:

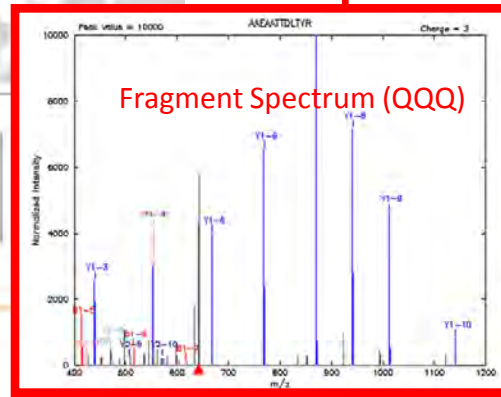
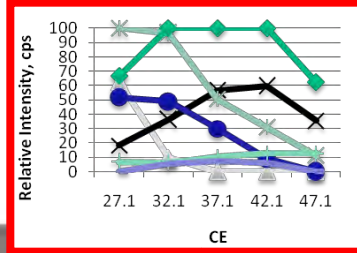
Search Select Build Browse Peptides Peptide Protein Browse Proteins

Summarize Peptide

Note: masses are mono-isotopic; results are from query to PeptideAtlas followed by peptide sequence

Download as TSV

Protein	Sequence	Chg	q1_mz	q3_mz	Intensity	Ion	CE	SSRCalc	n_obs	Annot	Spectrum
YOR230W	AAEAATDLYR	2	641.82	869.44	10000.00	y7	33.74	18.83	5	Best	
YOR230W	AAEAATDLYR	2	641.82	940.47	7154.20	y8	33.74	18.83	5	Best	
YOR230W	AAEAATDLYR	2	641.82	768.39	6543.40	y6	33.74	18.83	5	Best	
YOR230W	AAEAATDLYR	2	641.82	1011.51	4643.40	y9	33.74	18.83	5	Best	
YOR230W	AAEAATDLYR	2	641.82	667.34	3979.60	y5	33.74	18.83	5	Best	
YOR230W	AAEAATDLYR	2	641.82	552.31	3015.20	y4	33.74	18.83	5	Best	
YOR230W	AAEAATDLYR	2	641.82	439.23	2561.80	y3	33.74	18.83	5	Best	

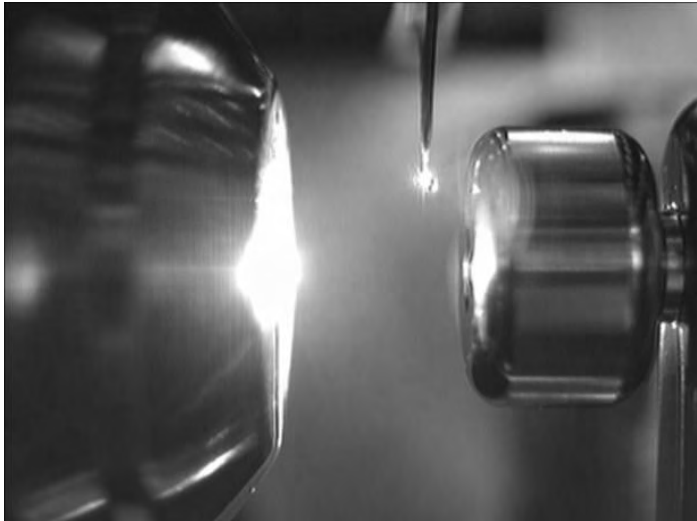


*Suggested transitions,
 Collision Energy plots,
 Calculated hydrophobicity
 Fragment relative intensities*

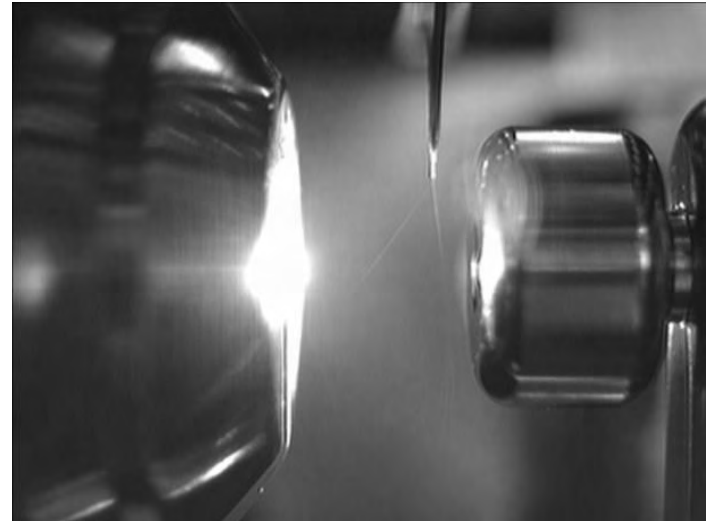


www.srmatlas.org

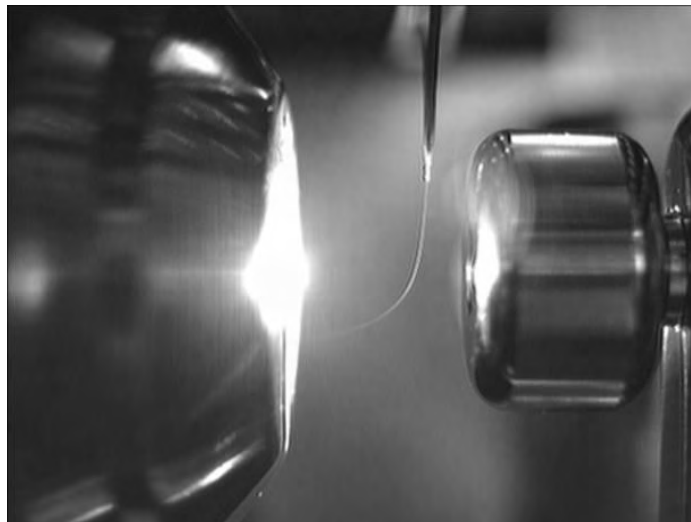
Chip Spray degradation



Dripping spray
at high voltage



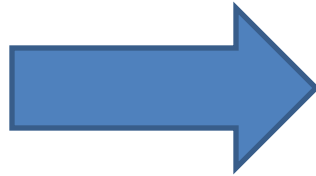
Split and
spitting spray



Good spray



Chip Snuggie

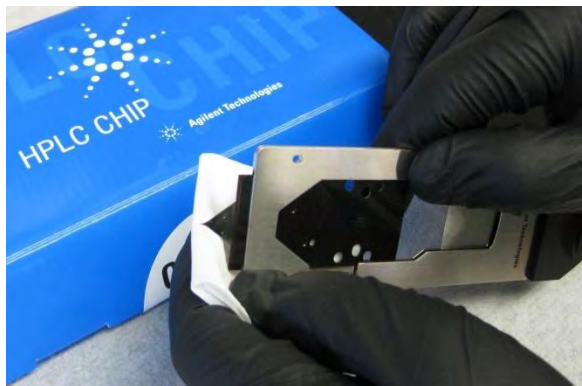


Care! Chip should not be bumped or snagged with tip exposed

**Materials for cleaning:
Kimwipes, Gloves**



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.

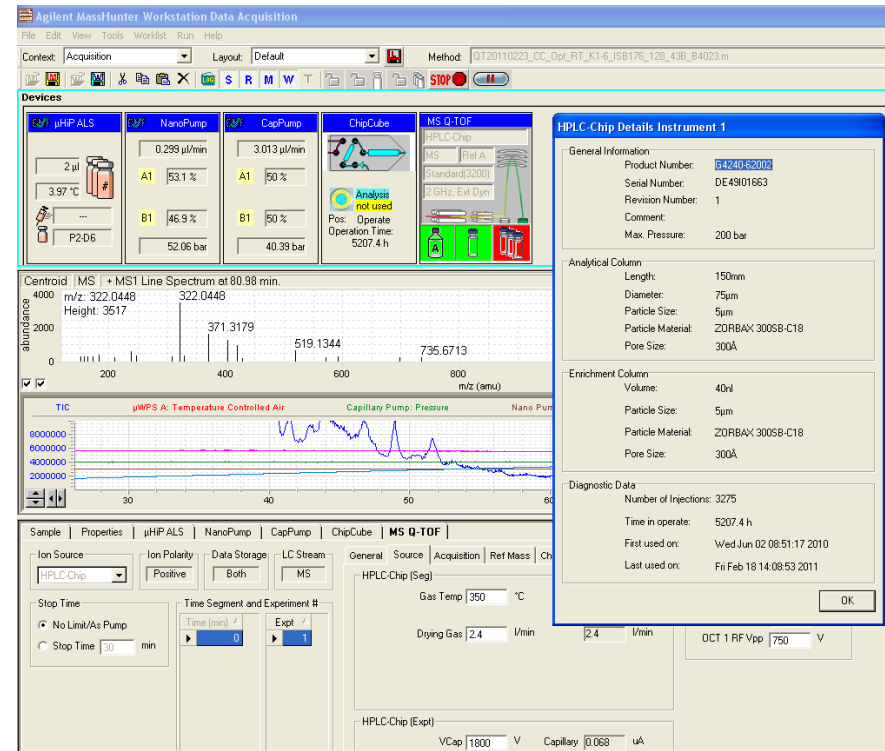
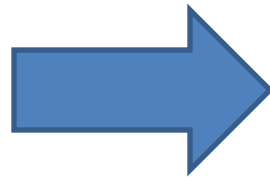


Step 4. Carefully press the clip and with your other hand gently pull the polyimide back into the chip holder until the polyimide clicks back into place.

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



Chip Snuggie

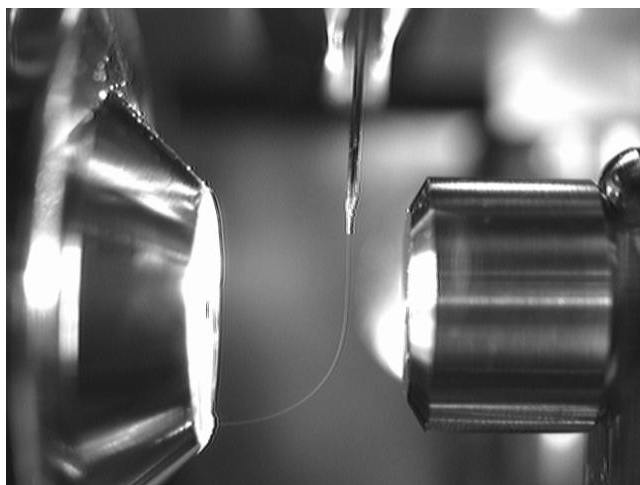
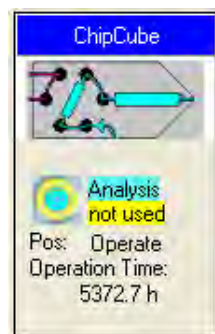
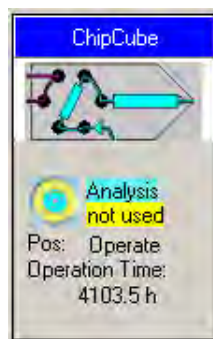
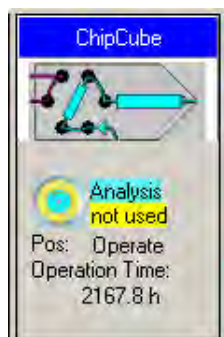


After tip cleaning, you will experience many hours of enjoyment.....

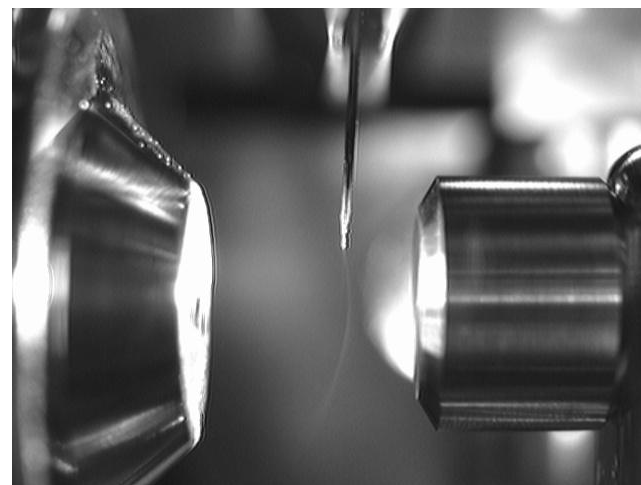
Chip: 3275 injections, 5207 hours
 Nanopump at 0.3 uL/min at 52.0 bar
 Cappump at 3.0 uL/min at 40.0 bar

Extended lifetime of the LC-Chip

Chip Snuggie



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Revolutionizing science. Enhancing life.

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