



Rapid Assay Development and Refinement for Targeted Protein Quantitation Using an Intelligent SRM (iSRM) Workflow

September 2, 2010

Reiko Kiyonami, Ph. D.

Senior Strategic Marketing Specialist – Proteomics

Thermo Fisher Scientific

What is Targeted Quantitative Proteomics?

Discovery

Fishing for differentially expressed proteins

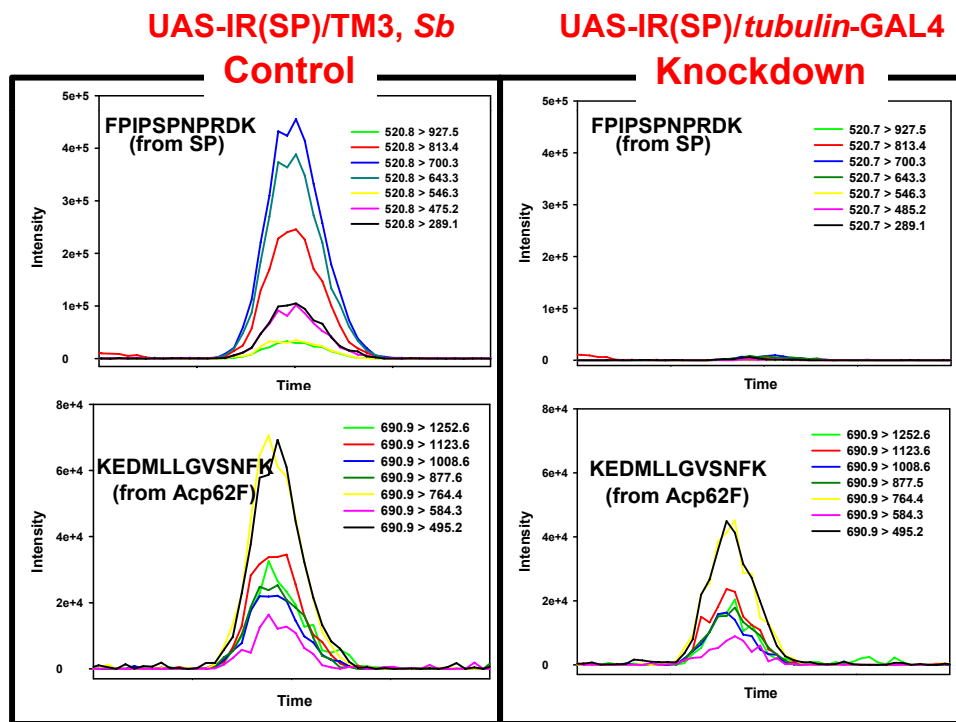
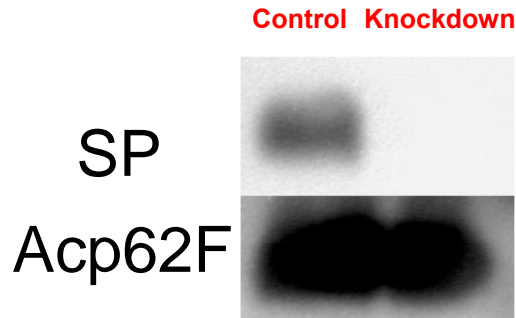


Targeted

Measuring proteins in known pathways



Why Targeted Quantitative Proteomics?



Data provided by M. J. MacCoss from Uni. of Washington

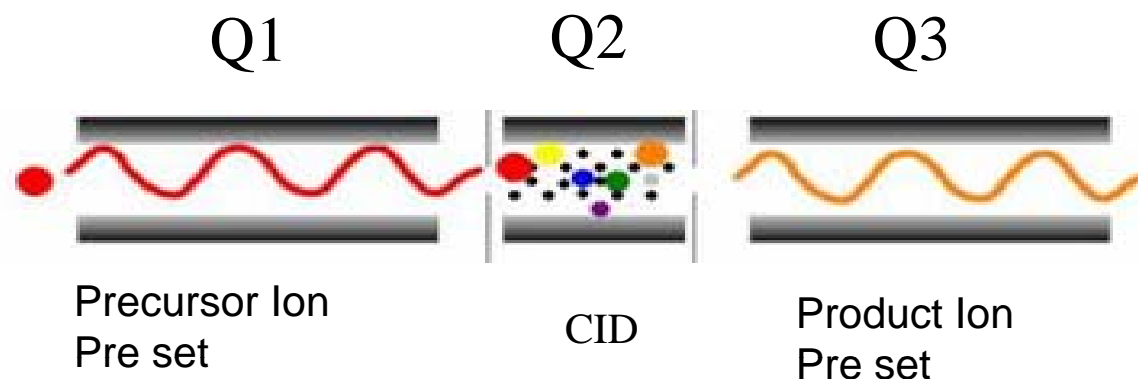
➤ Need to verify potential biomarkers which have been found in the discovery experiments to narrow down the long biomarker candidate list to a few of real promising ones for validation stage.

➤ Need to acquire consistent quantitative data for large protein sets to facilitate modeling in systems biology studies.

➤ The traditional immunoassay method is too expensive to implement on such a large scale.

➤ Alternatively, a multiple SRM assay on a triple quadrupole mass spectrometer can be used for targeting hundreds of biomarker candidates from hundreds of complex samples in a high throughput way.

SRM (Selected Reaction Monitoring) assay



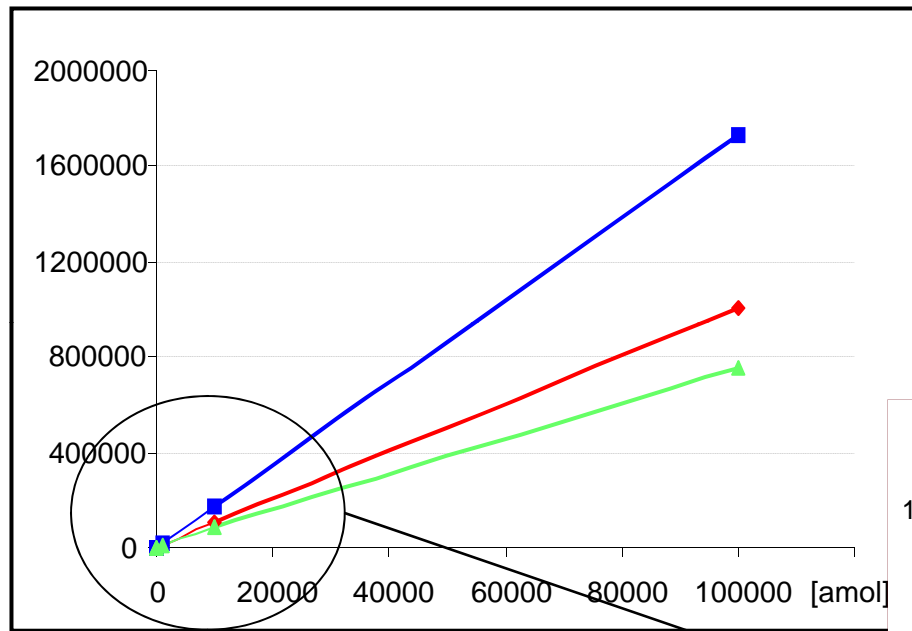
1. A “proteotypic” peptide is selected as being quantitative surrogate of a targeted protein.
 2. Q1 is set to transmit only the precursor ion of the selected peptide.
 3. Q2 is used to induce fragmentation of the precursor ion.
 4. Q3 is set to transmit a specific product ion,
- Each precursor to product ion set is called an SRM transition.

Why SRM Analysis for Targeted Protein Quantitation?

- **Highest specificity/sensitivity** – necessary for low abundant peptides in complex matrices like plasma
- **Largest linear dynamic range** for quantitation
- Well-established as the **quantitative method of choice** for small molecule applications

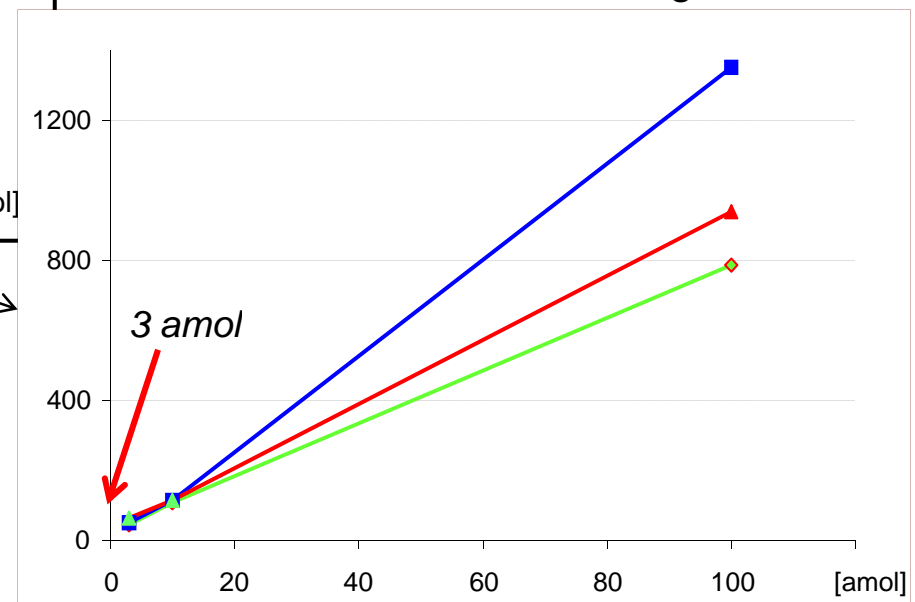
TSQ Vantage allows Low Level Quantitation in Background Matrix

Linear response over wide dynamic range



*Stable isotope labeled peptides
spiked into whole yeast lysate*

LOD in low attomole range



◆	GILFVGSGVSGGEEGAR	801.4	1072.5
■	LTILEELR	498.8	782.5
▲	LVEDPQVIAPFLGK	767.4	569.4

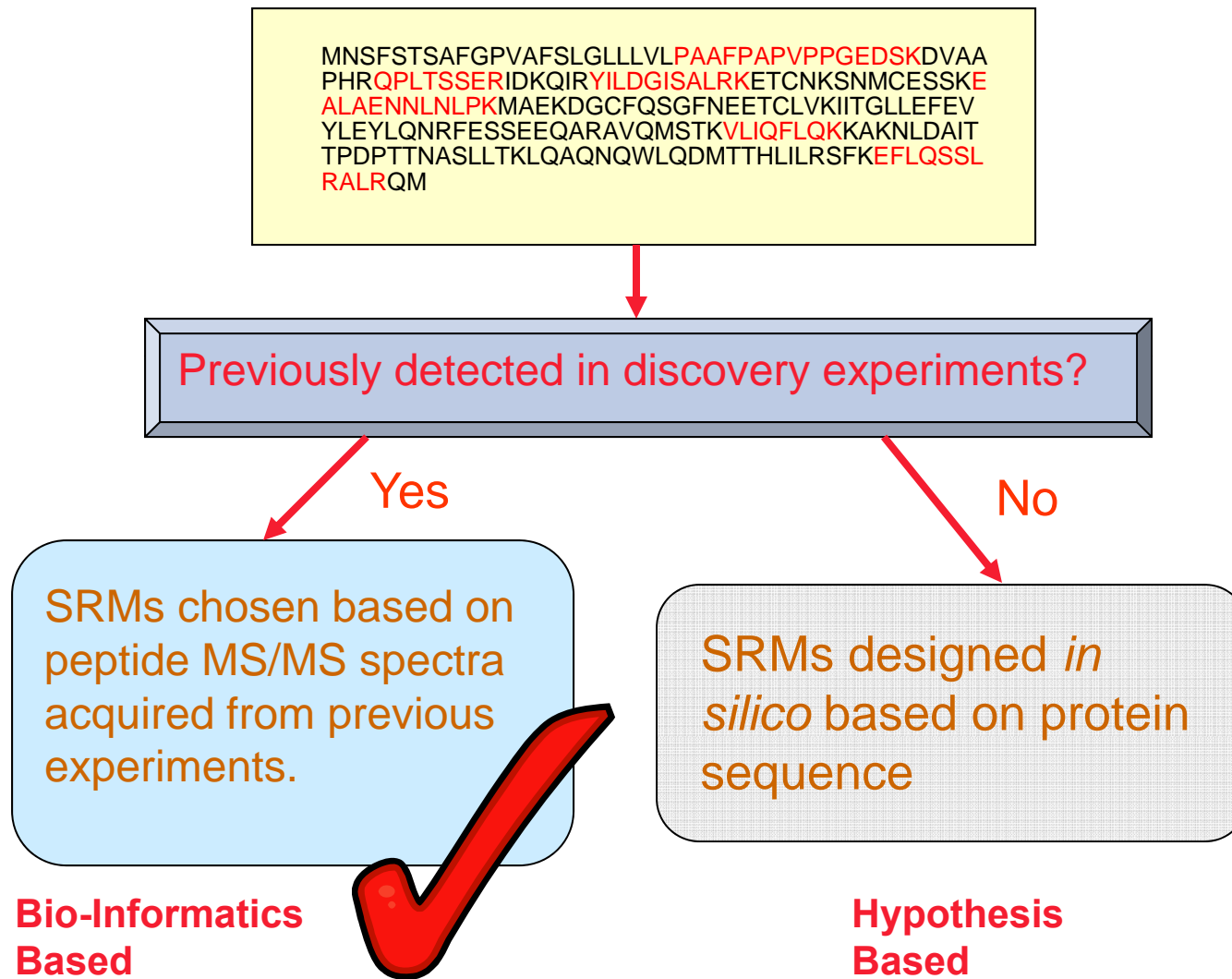
Peptide Selection and SRM Assay Design

The success and confidence of an SRM experiment directly depends on the selected proteotypic peptides and the specific transitions.

- Peptides have to be unique to the targeted protein
- Peptides should not contain Cys, Met or other commonly modified residues
- Peptides should yield excellent mass spectral signal
- Selected product ions used for SRM analysis should have higher mass over charge values for increased selectivity

From Protein...to SRM

– How can you select your peptides/transitions?



Can we use CID MS/MS data for selecting SRM transitions?

research articles **Journal of proteome**
research

Correlation between γ -Type Ions Observed in Ion Trap and Triple Quadrupole Mass Spectrometers

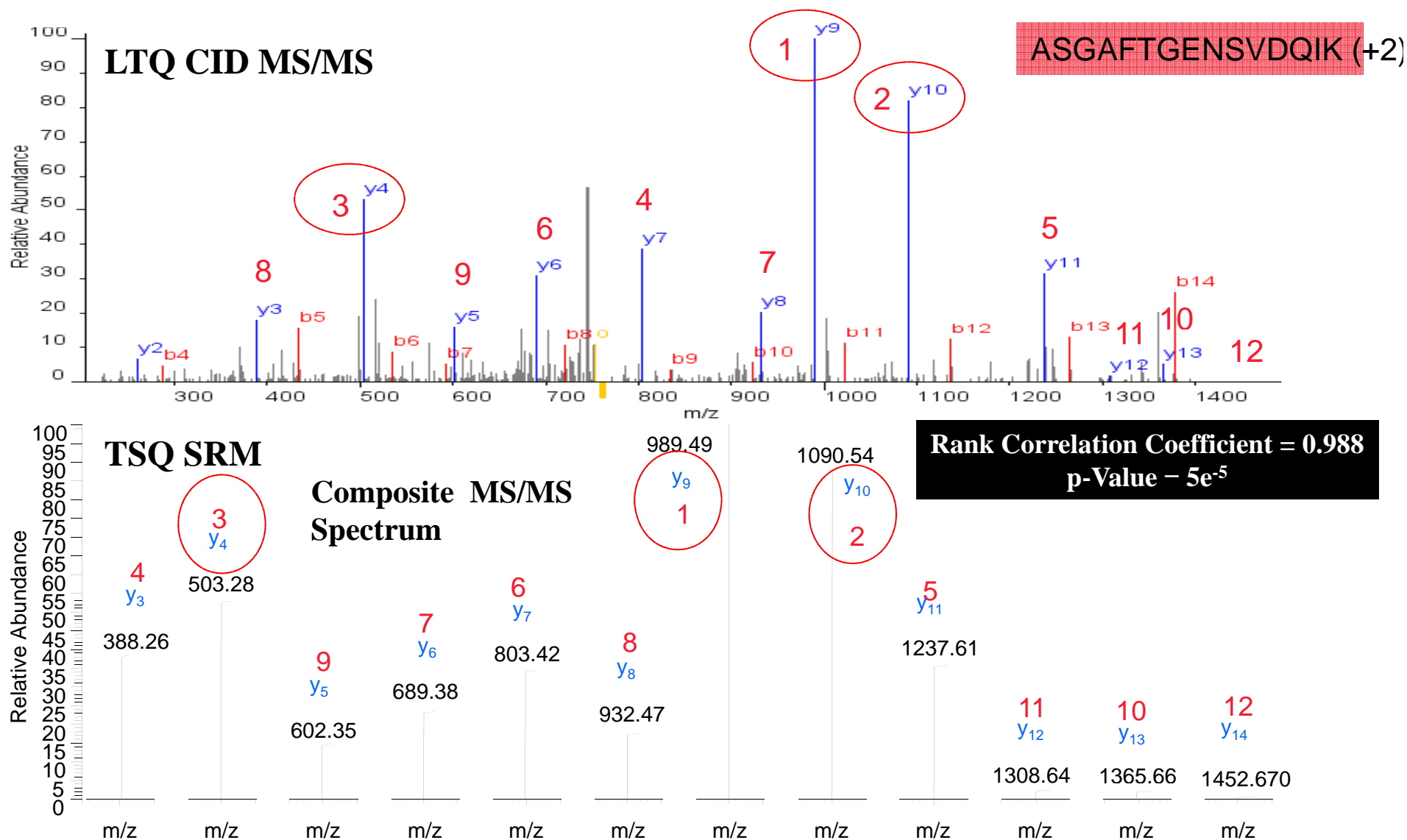
Carly A. Sherwood,[†] Ashley Eastham,[‡] Lik Wee Lee,[†] Jenni Risler,[§] Olga Vitek,^{||} and Daniel B. Martin^{*†}

Institute for Systems Biology, 1441 North 34th Street, Seattle, Washington 98103, Amgen, 1201 Amgen Court West, Seattle, Washington 98119, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue, Seattle, Washington 98112, and Purdue University, 250 North University Street, West Lafayette, Indiana 47907

Received March 31, 2009

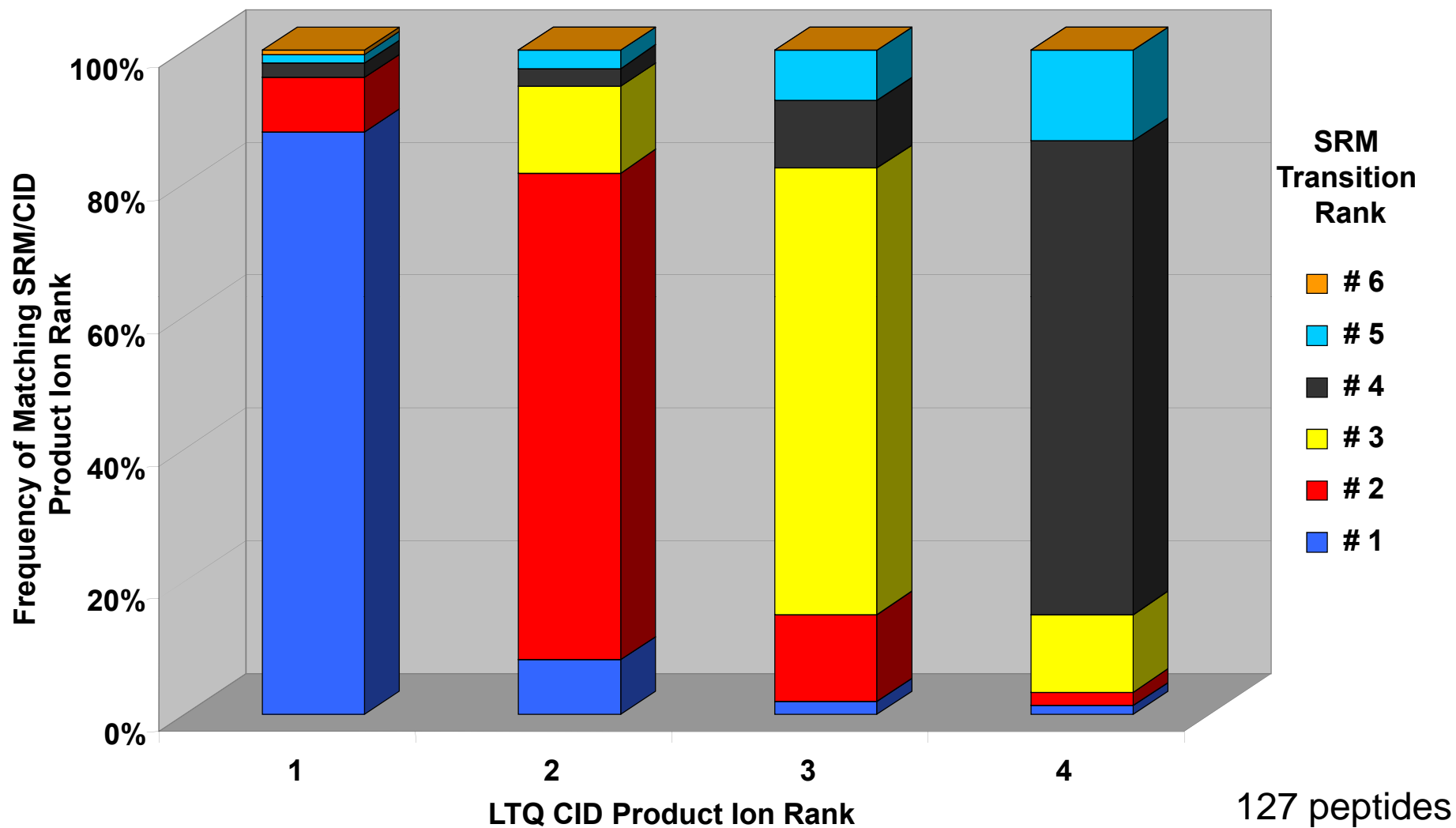
Multiple reaction monitoring mass spectrometry (MRM-MS) is a technique for high-sensitivity targeted analysis. In proteomics, MRM-MS can be used to monitor and quantify a peptide based on the production of expected fragment peaks from the selected peptide precursor ion. The choice of which fragment ions to monitor in order to achieve maximum sensitivity in MRM-MS can potentially be guided by existing MS/MS spectra. However, because the majority of discovery experiments are performed on ion trap platforms, there is concern in the field regarding the generalizability of these spectra to MRM-MS on a triple quadrupole instrument. In light of this concern, many operators perform an optimization step to determine the most intense fragments for a target peptide on a triple quadrupole mass spectrometer. We have addressed this issue by targeting, on a triple quadrupole, the top six γ -ion peaks from ion trap-derived consensus library spectra for 258 doubly charged peptides from three different sample sets and quantifying the observed elution curves. This analysis revealed a strong correlation between the γ -ion peak rank order and relative intensity across platforms. This suggests that γ -type ions obtained from ion trap-based library spectra are well-suited for generating MRM-MS assays for triple quadrupoles and that optimization is not required for each target peptide.

Comparing Product Ion Rankings – Linear Ion Trap CID vs. Triple Quadrupole SRM Transitions



MacCoss et al. *J. Proteome Research* 2008, 8(6), 2733-2739

Evaluation of Rank Overlap: LTQ CID to SRM



Automatic Peptide and Transition Selection based on MS/MS spectral library by using Pinpoint

Transition Editor
Transitions for LNSLTVGPR Batch Mode

Protein/Peptide/Precursor/Product
 Catalase
 LNSLTVGPR

Peptide sequence = LNSLTVGPR
 Molecular Wt (M) = 955.545
 Hydrophobicity Factor = 20.26

Precursor Charge State = 2
 Product Charge State = 1

b or y ion
 Water loss
 Ammonia loss
 Neutral loss

Precursor ions (Isotopes)
 478.780
 479.281
 479.783
 480.285
 480.787
 481.288
 481.790

Extra ions (separated by comma)

b series
 Precursor m/z = 478.780

<input type="checkbox"/>	b1	114.091
<input checked="" type="checkbox"/>	b2	228.134 (6.3e2)
<input type="checkbox"/>	b3	315.166 (2.7e2)
<input type="checkbox"/>	b4	428.250 (1.1e3)
<input type="checkbox"/>	b5	529.297 (2.1e3)
<input checked="" type="checkbox"/>	b6	628.366 (6.3e2)
<input type="checkbox"/>	b7	685.387 (1.8e2)
<input type="checkbox"/>	b8	782.440
<input type="checkbox"/>	b9	938.541

L
N
S
L
T
V
G
P
R

y series

<input type="checkbox"/>	y9	956.552
<input type="checkbox"/>	y8	843.468 (1.9e2)
<input checked="" type="checkbox"/>	y7	729.425 (3.6e3)
<input checked="" type="checkbox"/>	y6	642.393 (7.6e2)
<input checked="" type="checkbox"/>	y5	529.309 (2.1e3)
<input checked="" type="checkbox"/>	y4	428.261 (1.1e3)
<input checked="" type="checkbox"/>	y3	329.193 (1.5e3)
<input type="checkbox"/>	y2	272.171 (2.0e2)
<input type="checkbox"/>	y1	175.118

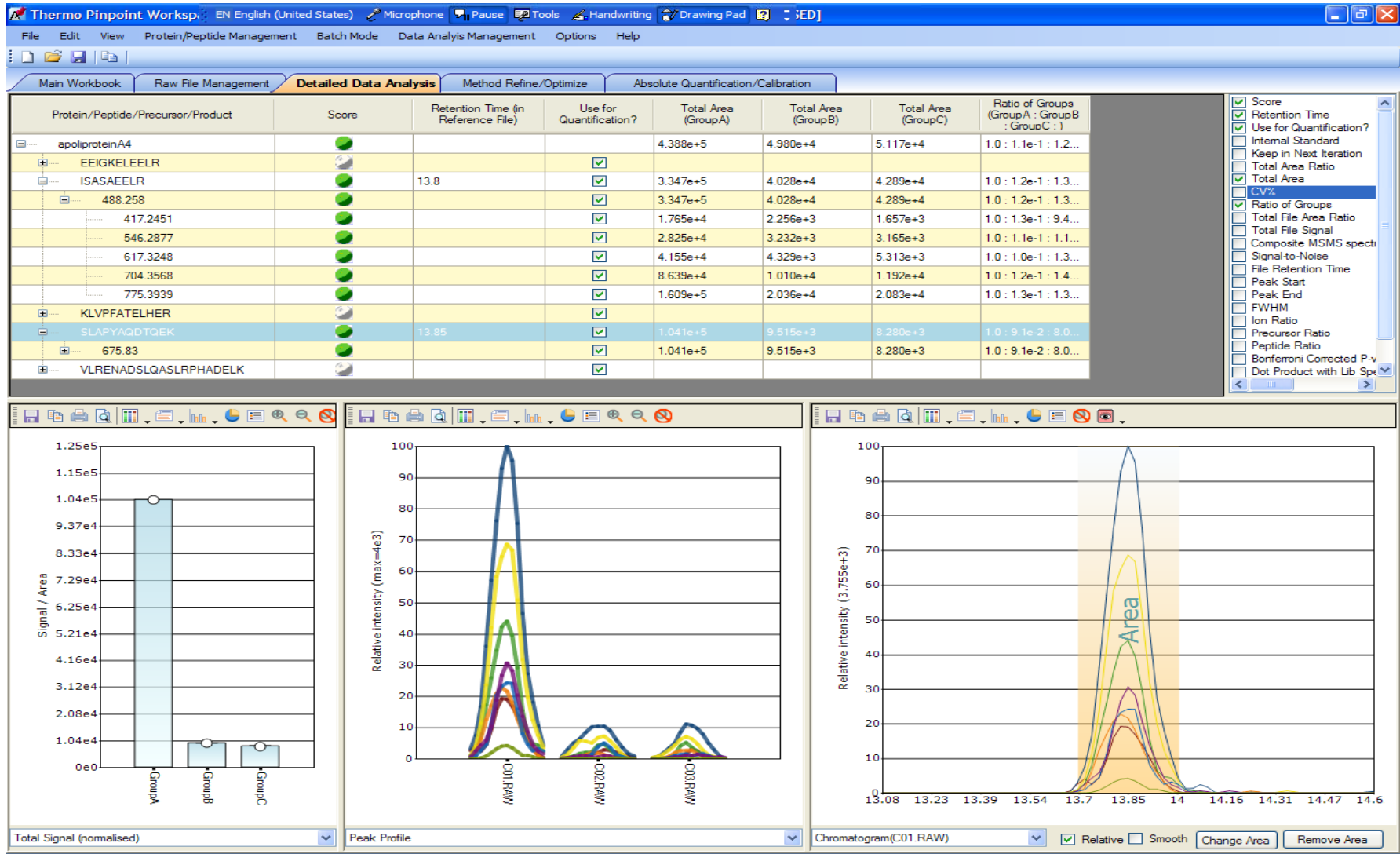
MS/MS data from previous discovery experiments

Red is the most sensitive ion by fragmentation rules
 Intensity from library spectra is given in paranthesis

Add/Edit Transitions

Automated, Easy, Selective, Robust

Automated Data Processing and Peptide Verification using Pinpoint



Biomarker Workflow



List of *candidate* markers generated

Need to *verify and validate* in expanded population



LTQ Velos



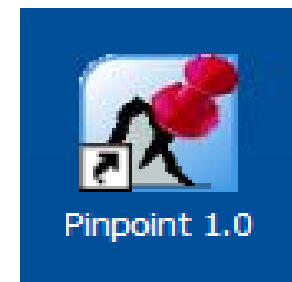
LTQ Orbitrap Velos



Unbiased



TSQ Vantage



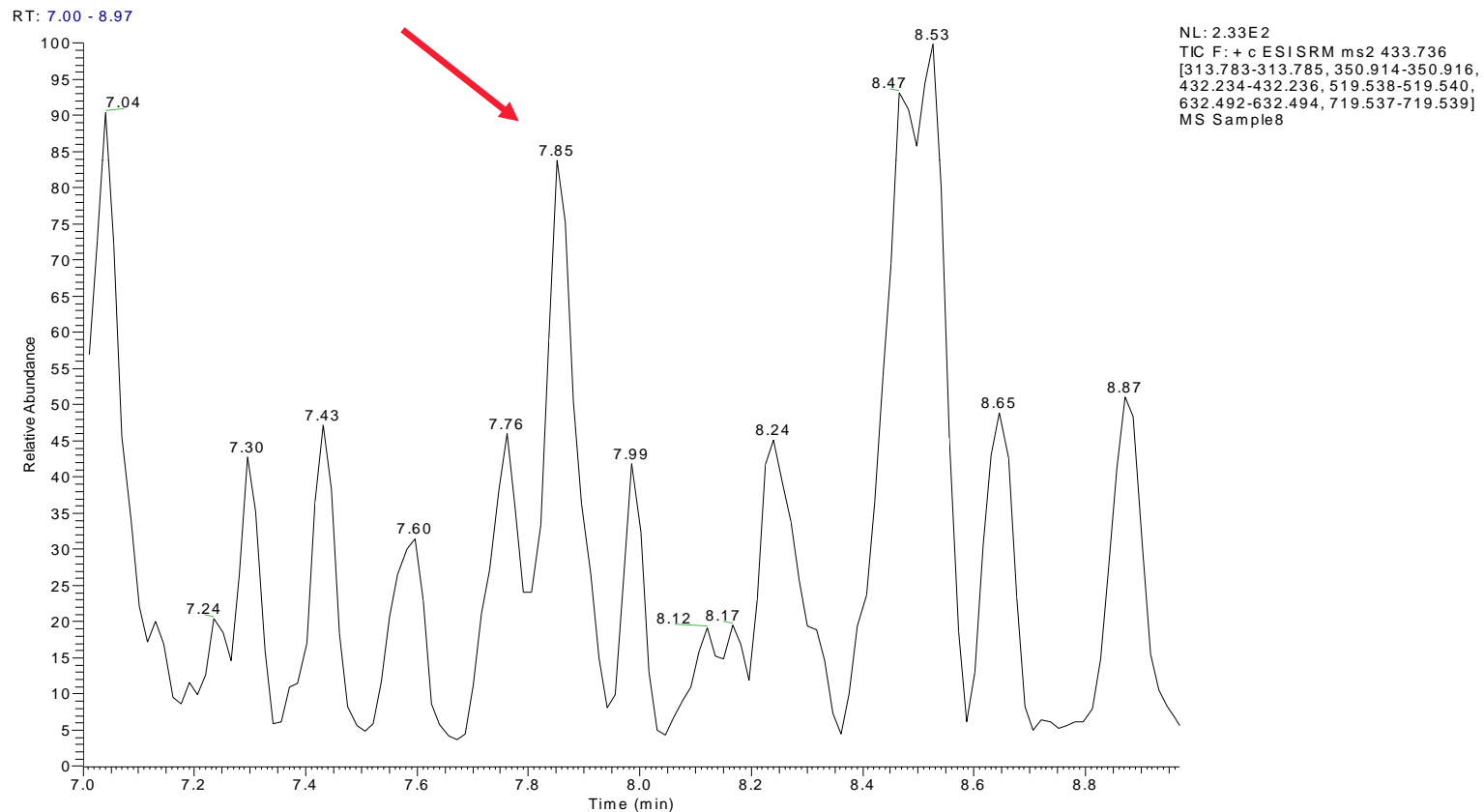
Targeted Analysis: SRM Assays

Challenge for Peptide Quantitation using SRM

How to verify if the detected peak is the right targeted peptide

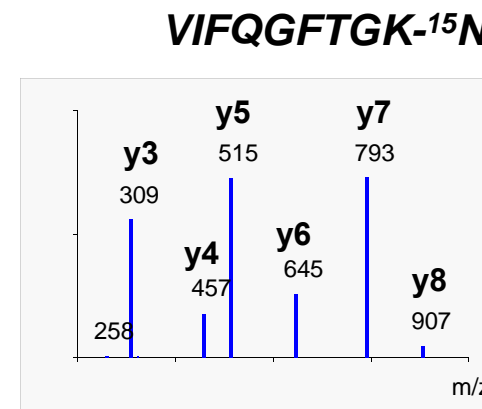
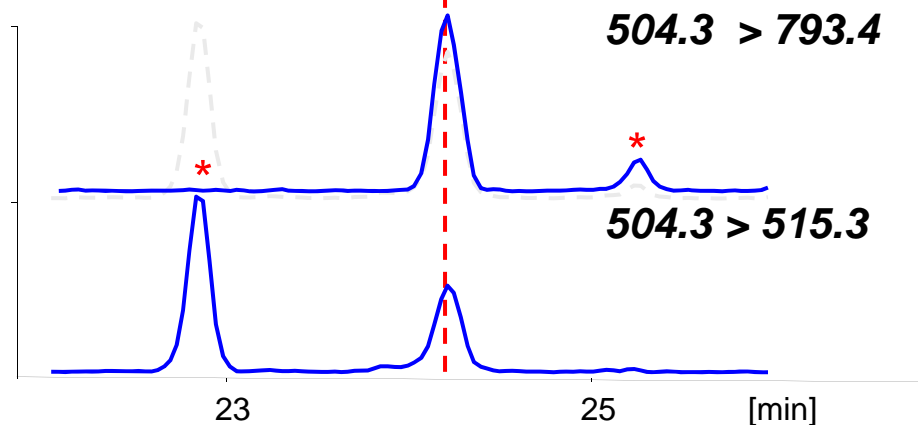
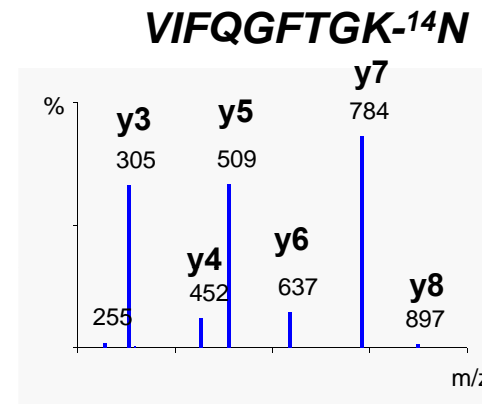
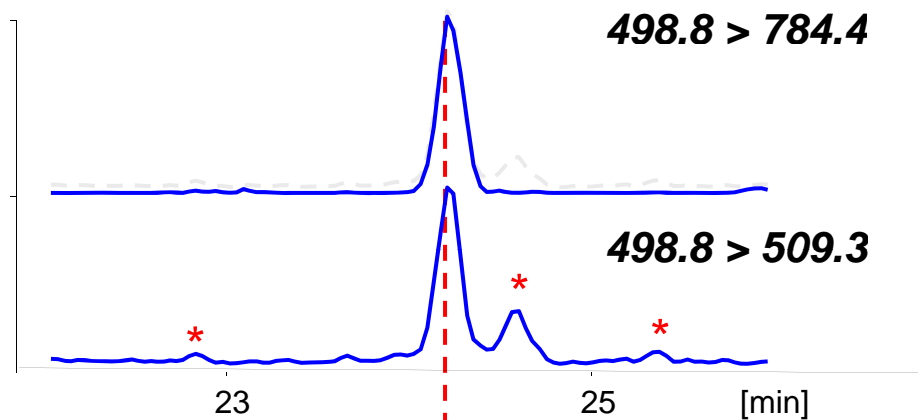
Which peak is my peptide?

Samples are very complex and background interferences require verification of the targeted peptide peak.



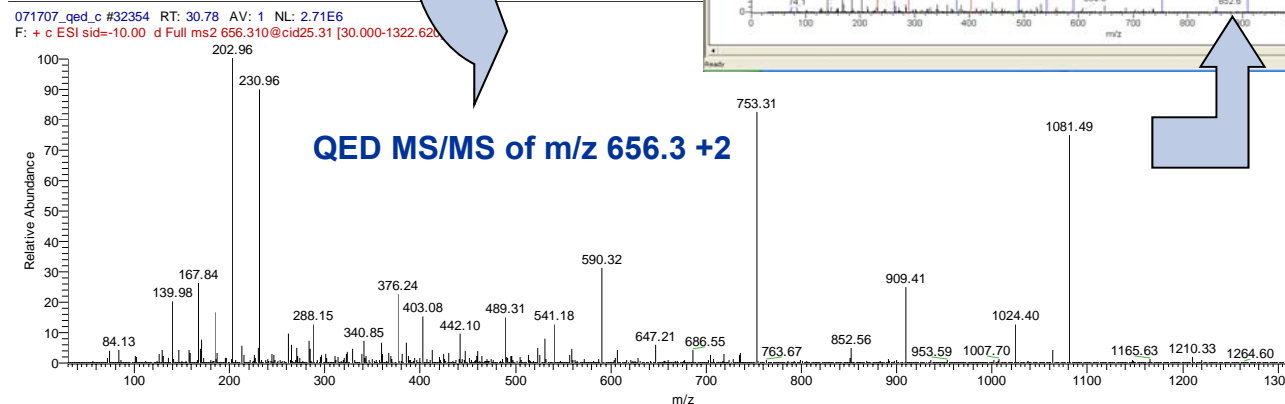
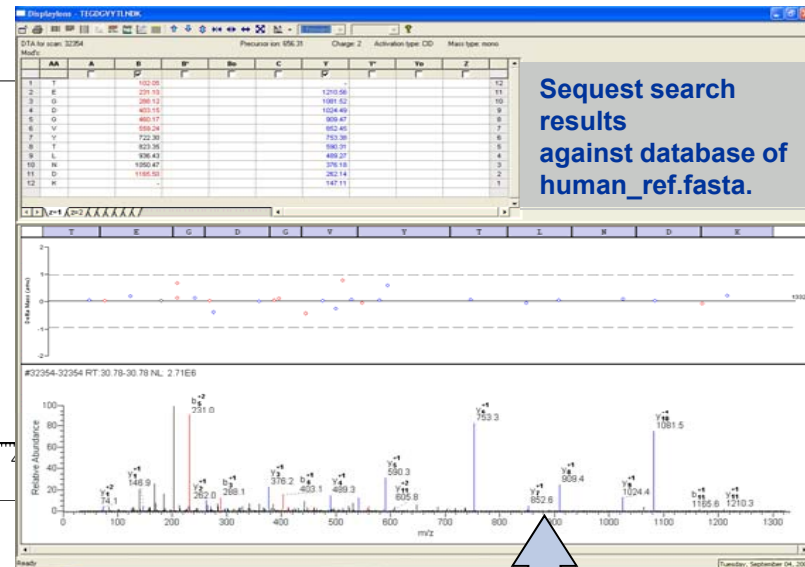
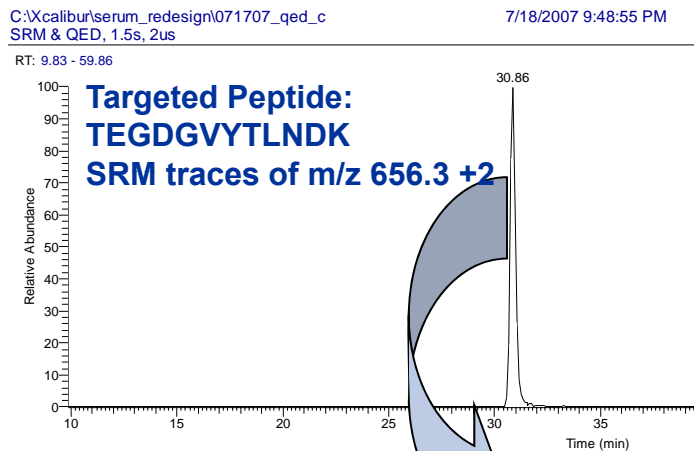
Peptide verification using isotopically labeled peptides as internal standards

- Not practical for large scale quantitative proteomics considering the cost and time to make the internal standards



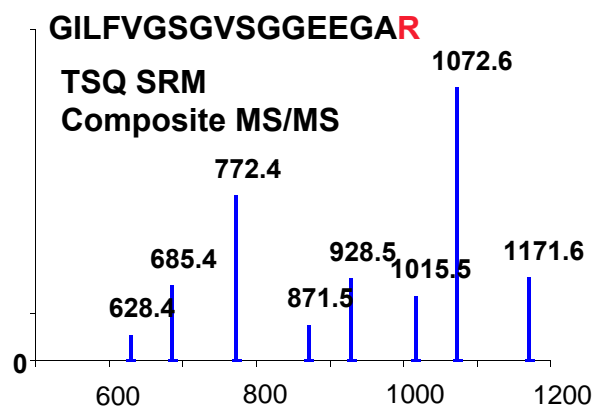
Peptide verification using SRM triggered MS/MS spectrum

- Requires a broad mass selection window
- MS/MS spectra often contain multiple components for complex biological samples
- Need longer cycle time and less sensitive

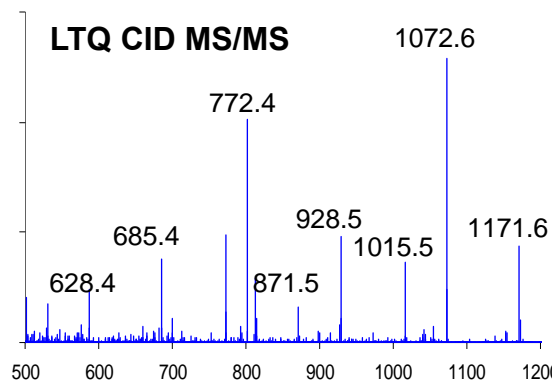
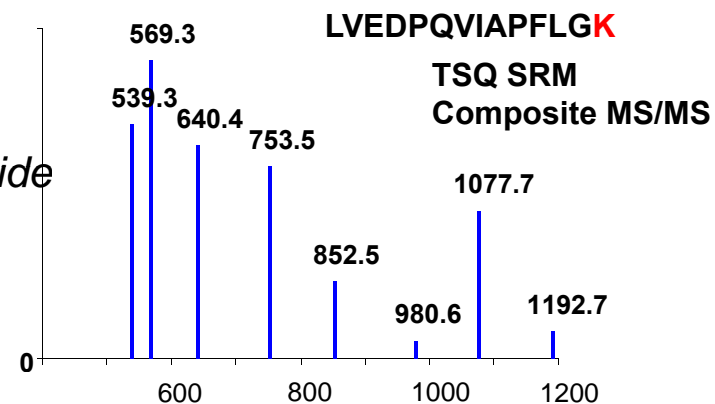


Peptide verification using composite MS/MS spectra (constituted with a large number of transitions per peptide)

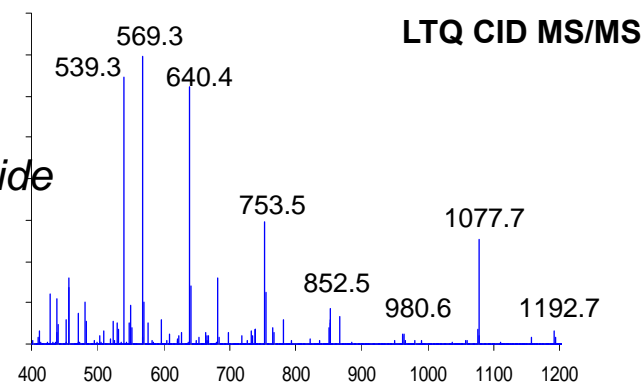
- Measurements of multiple transitions per peptide - a composite tandem mass spectrum
- Narrow mass selection window (higher selectivity; Higher sensitivity)
- Co-elution of all ions allows to eliminate false positive calls
- Limited throughput if constantly monitoring large number of transitions per peptide



Heavy peptide

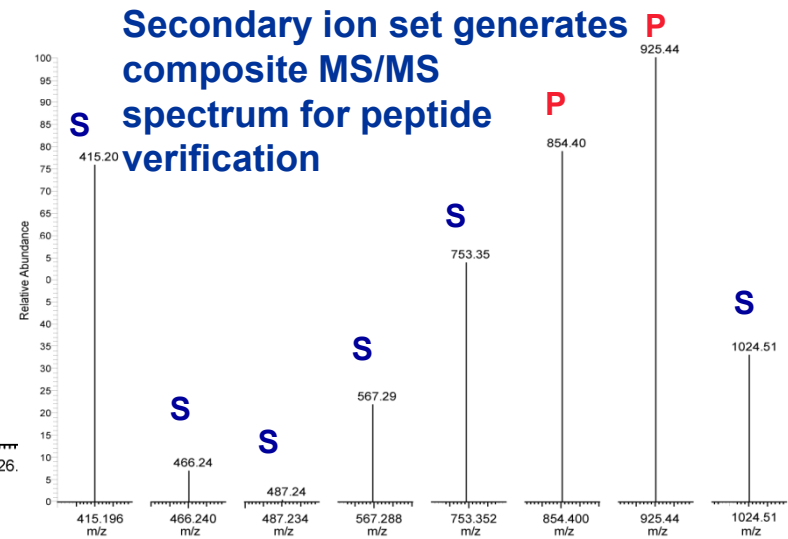
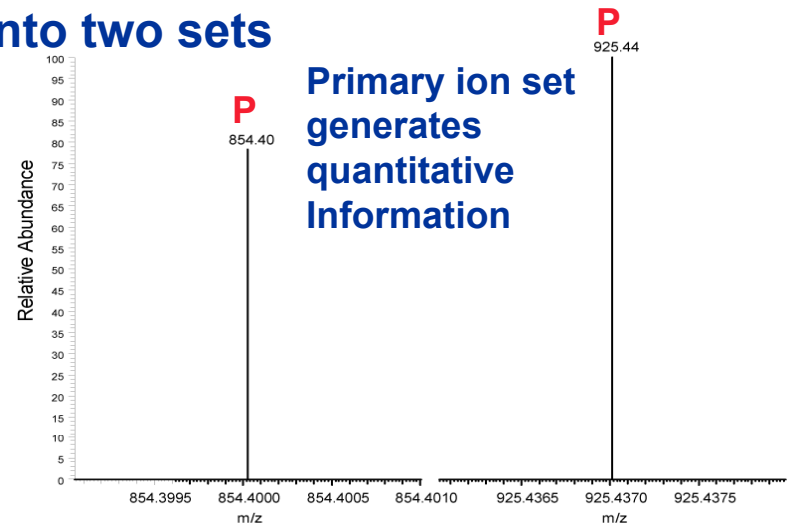
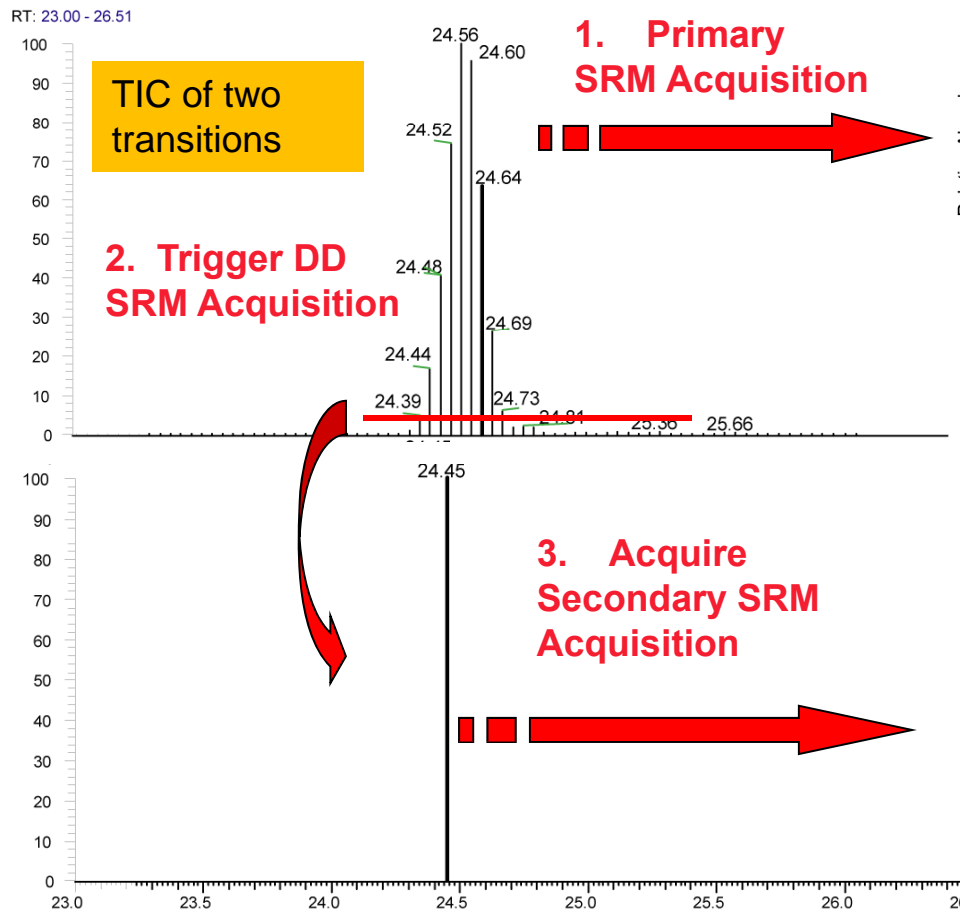


Heavy peptide



Introducing intelligent SRM (iSRM)

Large number of transitions are split into two sets

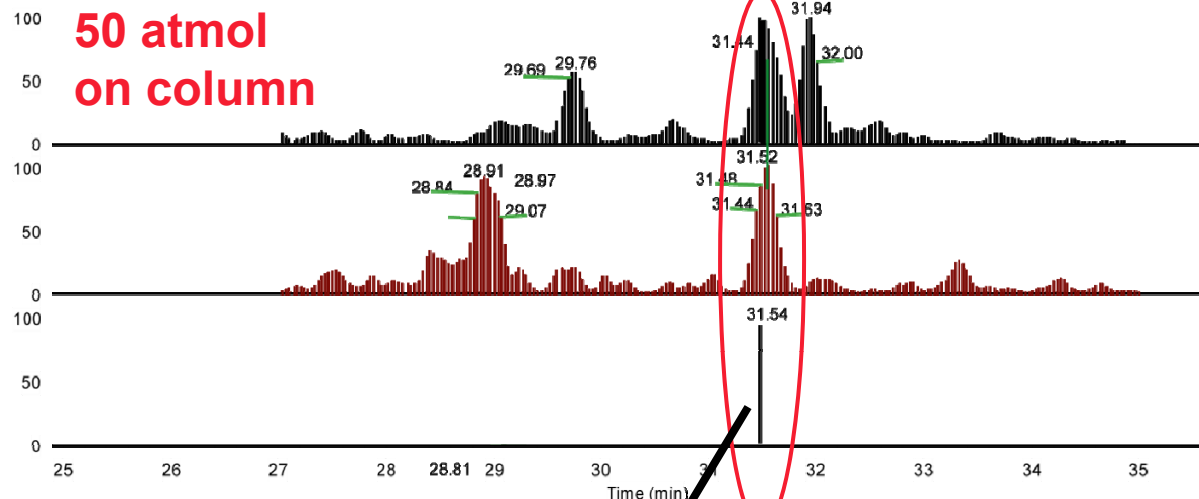


iSRM – An advantage for verifying low level targets in biological matrices

C:\Xcalibur...\spikedyeast50atmol_01
heavy pep mix spiked into 0.8ug/ul of yeast, 50ttomol/ul

1/8/2009 1:18:44 PM

RT: 24.89 - 35.92



**50 atmol
on column**

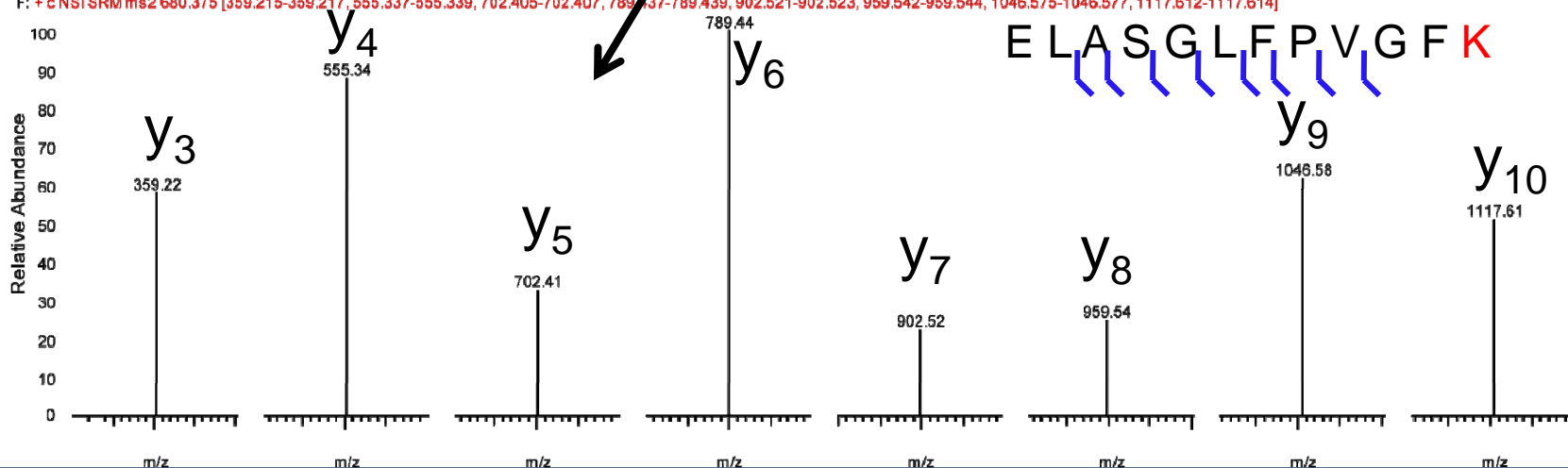
Primary SRM Transition
m/z 680.37 → 789.44
NL: 2.48E2

Primary SRM Transition
m/z 680.37 → 959.54
NL: 1.50E2

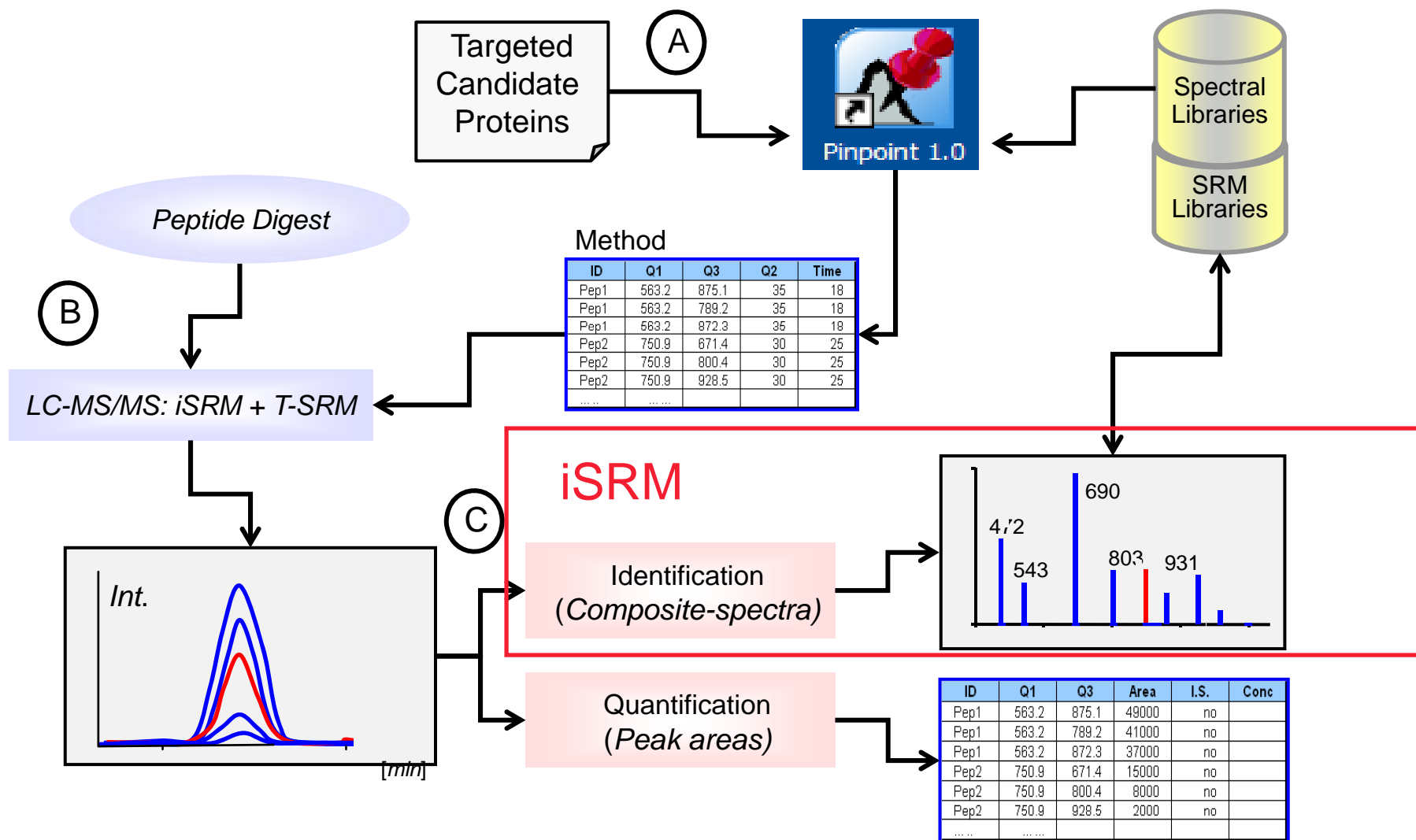
Data Dependent SRM
Primary and Secondary SRM
Transitions
NL: 1.12E3

spikedyeast50atmol_01 #3288 RT: 31.54 AV: 1 NL: 2.57E2

F: +c NSI SRM ms2 680.375 [359.215-359.217, 555.337-555.339, 702.405-702.407, 789.437-789.439, 902.521-902.523, 959.542-959.544, 1046.575-1046.577, 1117.612-1117.614]



Pinpoint software is integrated with iSRM workflow for automate assay design and data processing



What can iSRM and Pinpoint software provide you?

The combination of Pinpoint and iSRM provides the capability to simultaneously verify and quantitate up to 1000 targeted peptides in a single HPLC/MS/MS experiment.

- Rapid SRM assay design and refinement for the targeted protein quantification based on the discovery data.
- Provide high throughput screening methods to narrow down a large number of candidate biomarkers coming out of discovery to the most promising few.
- Provide high throughput screening methods for signaling pathway studies.



Rapid SRM assay development and refinement by using iSRM
- Targeting 40 known yeast proteins

Initial peptide selection based on previous proteomics discovery data

The screenshot displays the Proteome Discoverer interface. On the left, a sidebar contains icons for 'Add Protein', 'Digest', 'Add/Edit transitions', 'Add/Edit modifications', and 'Export to CSV'. Below these are statistics: '#protein targets=40/40', '#peptide targets=369/369', and '#transitions=2952'. A list of protein/peptide entries is shown in the main window, with a yellow box highlighting a subset of peptides. A red arrow points from this box to the 'Spectral Libraries' dialog box on the right. The dialog box shows a list of spectral libraries, with 'C:\Xcalibur\yeast_proteins\yeast.msf' selected. Below the list are buttons for 'Add library' and 'Remove'. Further down, there are settings for 'Library MS2 m/z accuracy (Da)' set to 1.0, a checkbox for '?Generate all library peptides', and a section for search engines (Sequest, MASCOT, Peptide Prophet) with various parameters like XCorr and Delta Cn. An 'Apply library' button is at the bottom.

1

2

Peptides detected in the discovery experiment are selected

Spectral Libraries

C:\Xcalibur\yeast_proteins\yeast.msf

Add library Remove

Library MS2 m/z accuracy (Da) 1.0

?Generate all library peptides

Sequest MASCOT Peptide Prophet

XCorr (charge=1) >= 1.50

XCorr (charge=2) >= 2.00

XCorr (charge=3) >= 2.50

Delta Cn >= 0.10

Rank <= 1

Charge = All

Apply library

Automatic transition selection based on the MS/MS spectral library

The screenshot displays the ThermoFisher software interface for protein/peptide management. The main window shows a list of peptides with their precursor and product m/z values. A dialog box titled "Select transitions in an automated fashion" is open, allowing for the selection of transitions based on various criteria. The dialog box includes options for selecting peptides and transitions, and a section for selecting transitions based on the spectral library. A yellow circle with the number "3" is placed near the "Select Peptides" section. A red circle highlights the text: "Two primary and additional six secondary transitions per peptide are selected based on the fragment ion intensity." A red arrow points from the "Number of primary ions" field in the dialog box to the "Primary" checkbox in the left sidebar.

Primary transitions

3

Two primary and additional six secondary transitions per peptide are selected based on the fragment ion intensity.

Number of primary ions: 2

Maximum m/z allowed in the instrument: 1500

Build transition list Cancel

#protein targets=40/40
#peptide targets=369/369
#transitions=2952

Primary

Export initial transitions to csv file for generating the initial iSRM method

Write CSV

Export Protein Level | Export Peptide Level | **Export Transitions Level (Instrument method)** | Export Optimization | Export Data analysis

Instrument parameters

- Precursor m/z
- Product m/z
- Collision Energy
- CE Start and Stop
- Scan Time
- Start Time
- End Time
- S Lens
- Polarity
- Trigger
- Primary/Secondary

Information for book-keeping

- Internal Std. Amount
- Peptide Sequence
- Protein Description
- Krokhin Hydrophobicity Factor
- Precursor charge state
- Product charge state
- Ion Type (e.g., y5, b2 or reporter)

873.406	374.214	33	0	50	1	300	1
873.406	489.241	33	0	50	1	300	1
873.406	560.278	33	0	50	1	300	1
873.406	760.394	33	0	50	1	300	1
873.406	844.895	33	0	50	1	300	1
873.406	946.458	33	0	50	1	300	0
873.406	1131.538	33	0	50	1	300	0
873.406	1260.581	33	0	50	1	300	1
584.789	417.22	23	0	50	1	300	1
584.789	450.202	23	0	50	1	300	0
584.789	493.736	23	0	50	1	300	1
584.789	518.268	23	0	50	1	300	1
584.789	528.247	23	0	50	1	300	1
584.789	633.295	23	0	50	1	300	1
584.789	762.337	23	0	50	1	300	0
584.789	899.396	23	0	50	1	300	1
587.309	362.203	23	0	50	1	300	0
587.309	443.753	23	0	50	1	300	1
587.309	508.274	23	0	50	1	300	1
587.309	551.79	23	0	50	1	300	1
587.309	574.283	23	0	50	1	300	0
587.309	702.378	23	0	50	1	300	1
587.309	815.462	23	0	50	1	300	1
587.309	886.499	23	0	50	1	300	1
623.817	347.228	25	0	50	1	300	1
623.817	423.737	25	0	50	1	300	1
623.817	452.248	25	0	50	1	300	0
623.817	475.323	25	0	50	1	300	1
623.817	516.769	25	0	50	1	300	0
623.817	574.283	25	0	50	1	300	1
623.817	590.35	25	0	50	1	300	1
623.817	903.489	25	0	50	1	300	1
459.256	275.171	19	0	50	1	300	1
459.256	346.208	19	0	50	1	300	1
459.256	352.19	19	0	50	1	300	1
459.256	402.714	19	0	50	1	300	1
459.256	561.299	19	0	50	1	300	0
459.256	632.336	19	0	50	1	300	1
459.256	703.373	19	0	50	1	300	0
459.256	804.421	19	0	50	1	300	1
541.765	274.187	22	0	50	1	300	1
541.765	358.682	22	0	50	1	300	1
541.765	403.229	22	0	50	1	300	1
541.765	491.241	22	0	50	1	300	1
541.765	518.256	22	0	50	1	300	1
541.765	716.357	22	0	50	1	300	0
541.765	787.394	22	0	50	1	300	1
541.765	844.415	22	0	50	1	300	0
423.738	131.081	18	0	50	1	300	1
423.738	161.155	18	0	50	1	300	1

Number of files to split into

Collision energy is calculated automatically by using formula of $0.034 \times m/z$ of peptide + 2

4

Export to CSV | Cancel

iSRM instrument method set up

40yeastproteins_final_rttest.meth - Instrument Setup

File TSQ Help

Scan Editor | Divert Valve | Tune Method | Method Summary |

Run Settings

MS Acquire Time (min): 60.00 Experiment Type: iSRM 1

Chrom Filter Peak Width (s): 10.0 Collision Gas Pressure (mTorr): 1.2 Use Tuned S-Lens Value

iSRM Settings

Q1 Peak Width (FWHM): 0.70 Cycle Time(s): 2.000 2

#	Parent	Product	SRM Collision Energy	Start Time	Stop Time	Polarity	Trigger	Reaction Category
1	544.770	744.399	22	11.23	15.23	+	300	Secondary
2	544.770	859.426	22	11.23	15.23	+	300	Secondary
3	544.770	232.140	22	11.23	15.23	+	300	Secondary
4	544.770	346.183	22	11.23	15.23	+	300	Secondary
5	544.770	475.225	22	11.23	15.23	+	300	Secondary
6	544.770	494.246	22	11.23	15.23	+	300	Secondary
7	544.770	532.247	22	11.23	15.23	+	300	Primary
8	544.770	645.331	22	11.23	15.23	+	300	Primary
9	423.738	131.081	18	11.58	15.58	+	300	Secondary
10	423.738	261.155	18	11.58	15.58	+	300	Secondary
11	423.738	392.192	18	11.58	15.58	+	300	Secondary
12	423.738	338.685	18	11.58	15.58	+	300	Secondary
13	423.738	488.282	18	11.58	15.58	+	300	Secondary
14	423.738	575.314	18	11.58	15.58	+	300	Primary
15	423.738	676.367	18	11.58	15.58	+	300	Primary

Scan Parameters

Scan Time (s): 0.100 3 Charge State: 1 Q1 Peak Width (FWHM): 0.70

Advanced Data Dependent Settings And Activation

Dynamic Exclusion

Advanced Settings...

Copy ScanEvent Paste Append List Import List 5 Export List Help Tune

Data Dependent Settings

Global Settings

Dynamic Exclusion

Repeat Count: 5 4

Repeat Duration (min): 0.32

Exclusion List Size: 50

Exclusion Duration (min): 0.32

User Defined Scan Window

First Mass: 10.000

Last Mass: 1500.000

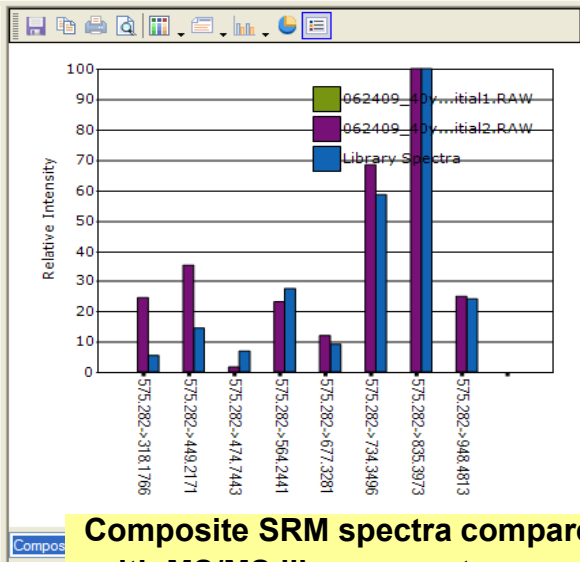
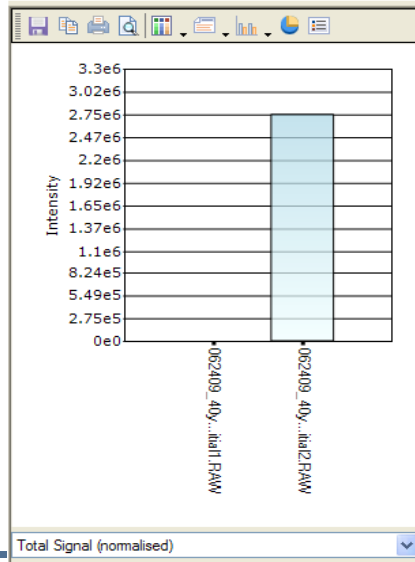
OK Cancel Help

NOT SAVED

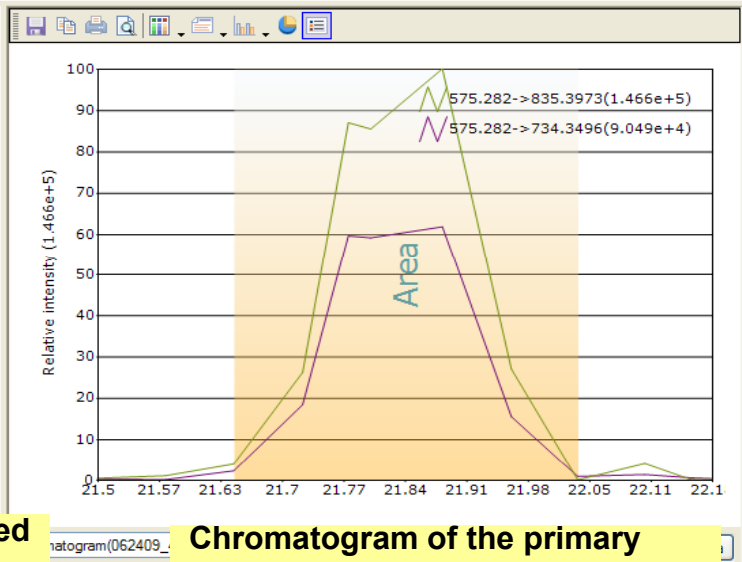
Peptide sequence verification and quantitation from the initial SRM assay

Protein/Peptide/Precursor/Product	Score	Retention Time (in reference file)	Use for quantification?	Total file Signal (062409_40y...ital1.RAW)	Bonferroni corrected p-value of Correlation with Lib Spectra	Total file Signal (062409_40y...ital2.RAW)	Bonferroni corrected p-value of Correlation with
>gil6324951ref\NP_015020.1 Gdh1p [Sac...				3.015e+5		5.891e+6	
AANLGGVAVSGLLEMAQNSQR		30.83	<input checked="" type="checkbox"/>	1.831e+5			
986.9971			<input checked="" type="checkbox"/>	1.831e+5	1.803e-2		
FIAEGSNMGSTPEAIAVFETAR		34.37	<input checked="" type="checkbox"/>			1.763e+5	
1149.557			<input checked="" type="checkbox"/>			1.763e+5	2.728e-4
GANIASFIK		26.98	<input checked="" type="checkbox"/>			2.333e+6	
460.7636			<input checked="" type="checkbox"/>			2.333e+6	4.437e-2
HIGQDTPVAGDIGVGGRR		22.46	<input checked="" type="checkbox"/>			7.936e+4	
882.4372			<input checked="" type="checkbox"/>			7.936e+4	1.736e-3
NSLTGLDMGGGK		21.8	<input checked="" type="checkbox"/>			2.746e+6	
575.282			<input checked="" type="checkbox"/>			2.746e+6	7.168e-3
SLEQIVNEYSTFSENK		36.47	<input checked="" type="checkbox"/>	1.184e-5			
STATGPSEAVWYGGPPK		25.29	<input checked="" type="checkbox"/>				
VTWENDKGEQEAQGYR			<input checked="" type="checkbox"/>				
>gil14318506ref\NP_116640.1 Hsp12p [Sa...				2.987e+6			
ASEALKPDSQK		1.06	<input checked="" type="checkbox"/>	1.698e+6			
587.3091			<input checked="" type="checkbox"/>	1.698e+6	2.480e-5		

Correlation p-value between composite SRM spectra and MS/MS library spectra



Composite SRM spectra compared with MS/MS library spectra



Chromatogram of the primary and secondary transitions. Area calculated based on primary

SRM method refinement based on the initial SRM assay data

**153 green colored peptides
With confirmed nice LC peak
shapes were selected from
the initial targeted 368 peptides
for the final iSRM method.**

Next iteration

Select peptides/transitions

- Keep Green peptides/transitions
- Keep Yellow peptides/transitions
- Keep White peptides/transitions

Scheduling window size for identified transitions (min) = +/- 2.00

Filter by analysis table column 'Keep in next iteration'

Automatically select best transitions 8

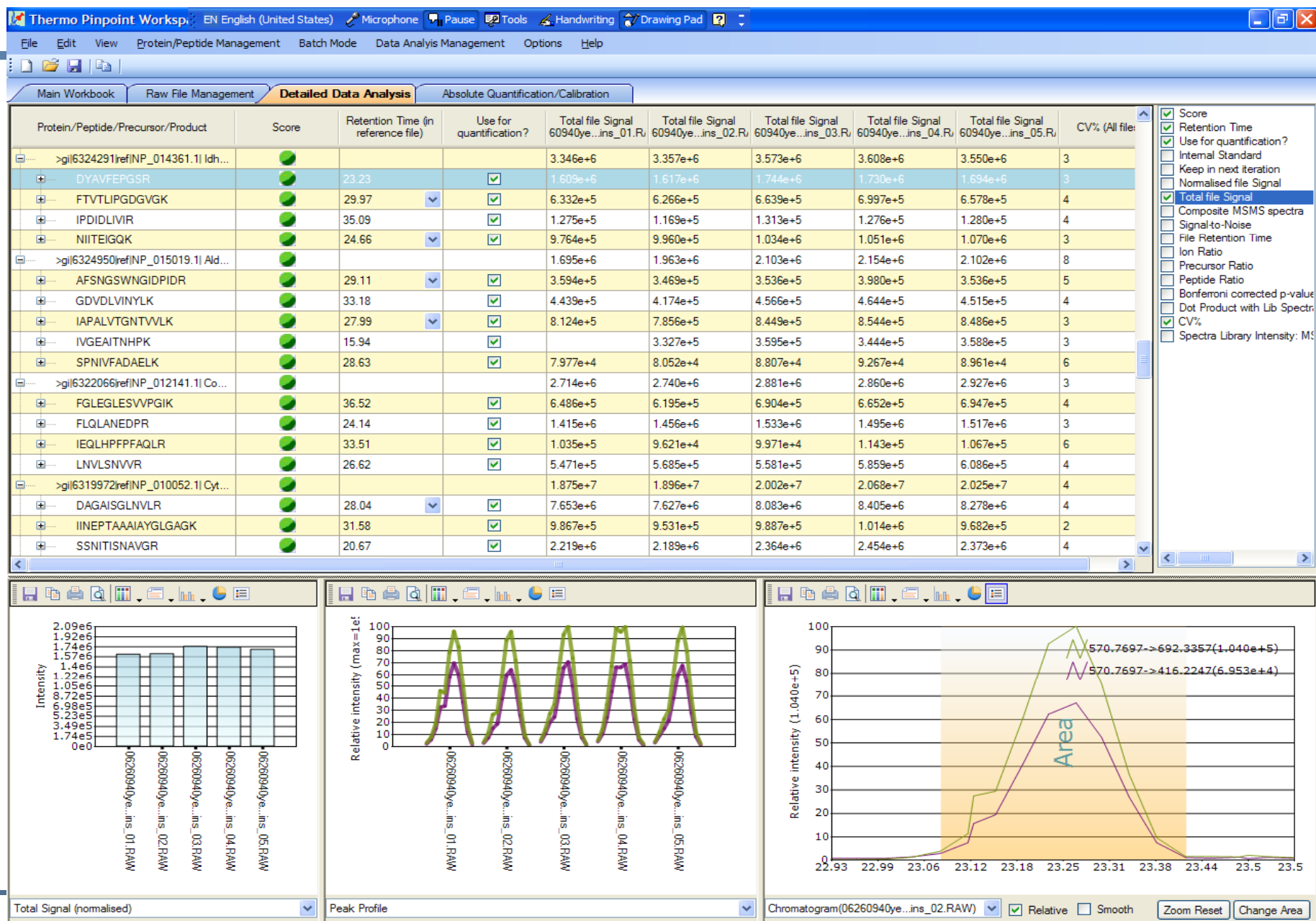
Generate next iteration Cancel

#protein targets=40/40
#peptide targets=153/368
#transitions=1224

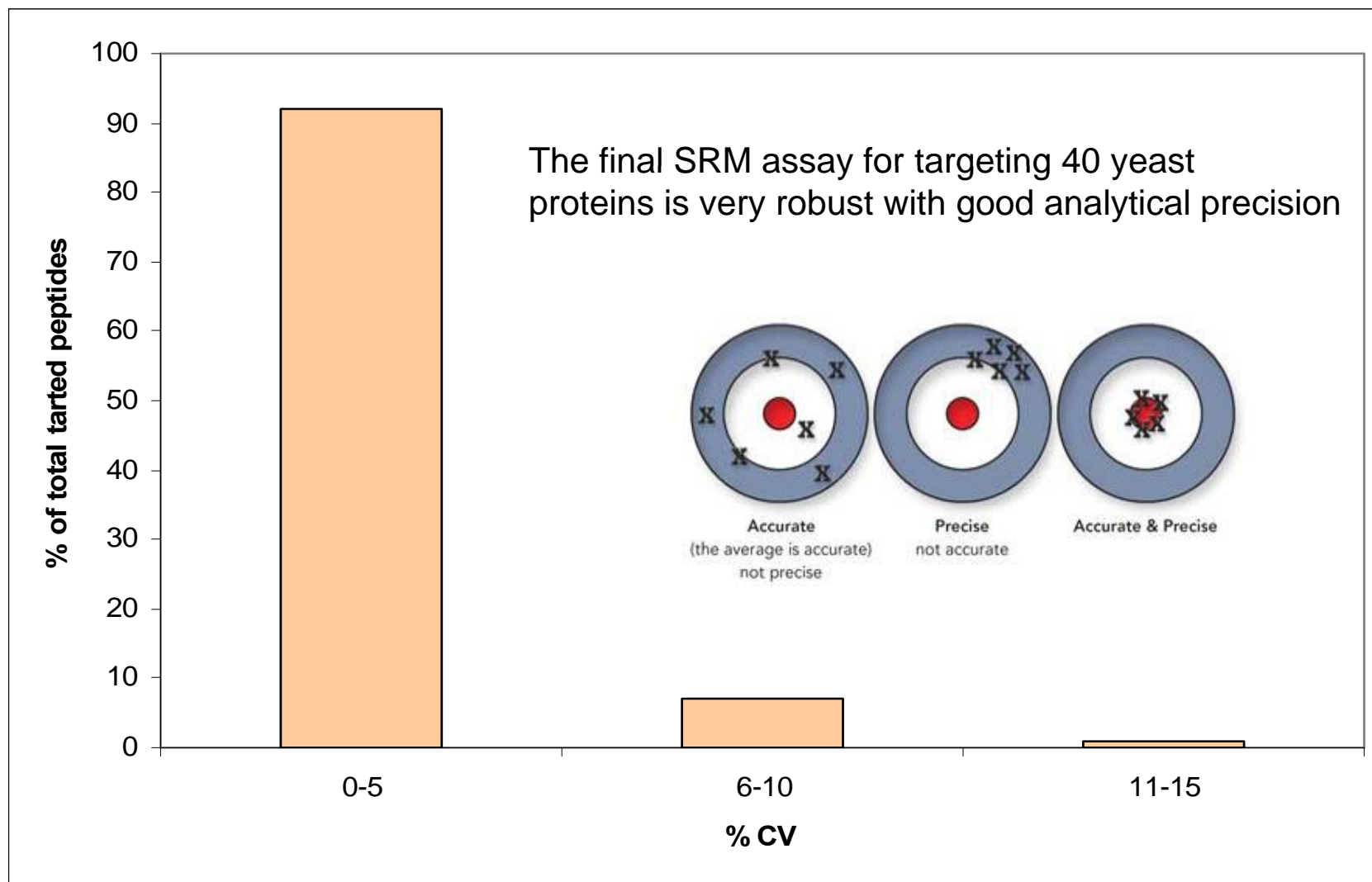
Protein/Peptide/Precursor/Product

Protein/Peptide/Precursor/Product	Retention Time	m/z
.....0..... GFGYAGSPFHR.....0.....	598.2858	700.352
.....0..... KVESLGSPSGATK.....0.....	481.2713	685.3874
.....0..... LYNDIVPK.....0.....	481.2713	244.165
.....0..... VESLGSPSGATK.....0.....	566.7958	904.4729
.....0..... VIPDFMLQGGDFTAGNGTGGK.....0.....	1041.502	935.4252

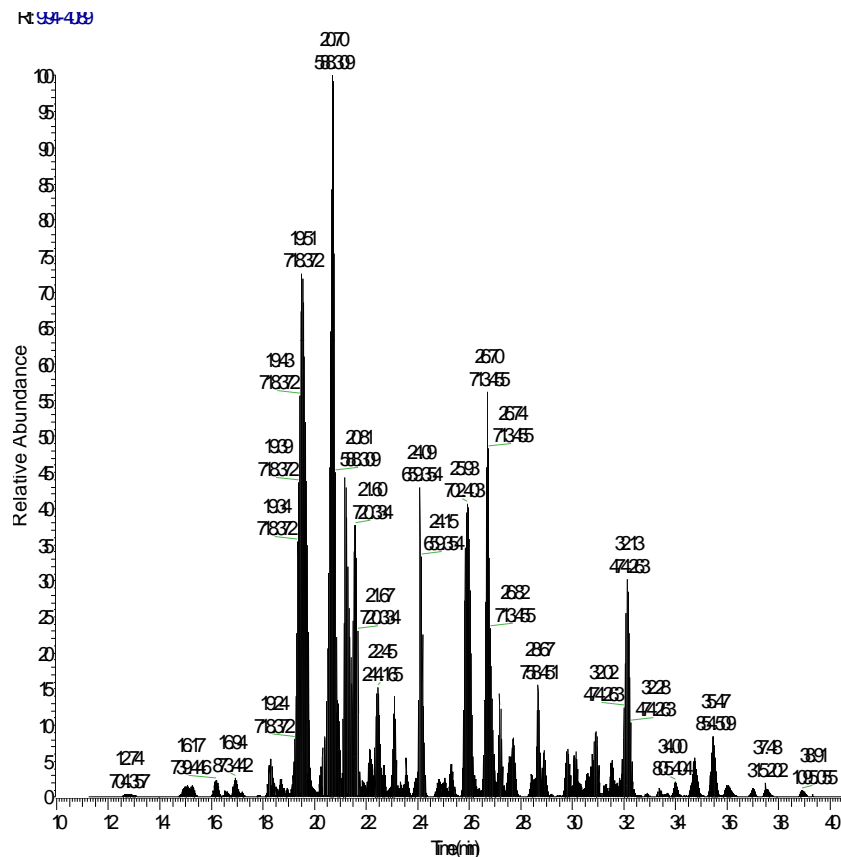
Peptide sequence verification and quantitative precision from the refined iSRM assay



CV percentage for targeting 153 verified peptides using iSRM



A robust SRM assay for quantitation of 40 targeted yeast proteins was developed in a few short hours



Step 1- Select Target Peptides:

369 peptides/2952 transitions (738 primary, 2214 secondary) were selected by Pinpoint based on the discovery data (20 min).

Step 2- Acquire data:

Data acquisition using above initial SRM assay (60 min).

Step 3 – Verify data & optimize conditions:

Data verification and generate optimized final SRM assay which kept only peptides that were confidently identified by composite MS/MS data and gave strong signal intensity. 153 peptides/1224 transitions (306 primary and 918 secondary) were retained in the final SRM assay (40 min) .

Step 4 – Validate final assay:

Validate the robustness and precision of final SRM assay by triplicate acquisition (60 min each, total 180 min).

Additional sensitivity can be obtained by optimizing collision energy in a single injection

Write CSV

Export Protein Level | Export Peptide Level | Export Transitions Level (Instrument method) | **Export Optimization** | Export Data Analysis

On column

Parameters

Collision Energy Optimization: Range = predicted CE +/- 6 eV; Step = 2 eV

For example, if the predicted CE=22eV, transitions will be created for 16 18 20 22 24 26 28 eV

Number of files to split into: 1

Export to CSV | Cancel

568.7904	341.116	16	9.8	29.8
568.7904	341.118	18	9.8	29.8
568.7904	341.12	20	9.8	29.8
568.7904	341.122	22	9.8	29.8
568.7904	341.124	24	9.8	29.8
568.7904	341.126	26	9.8	29.8
568.7904	341.128	28	9.8	29.8
568.7904	428.216	16	9.8	29.8
568.7904	428.218	18	9.8	29.8
568.7904	428.22	20	9.8	29.8
568.7904	428.222	22	9.8	29.8
568.7904	428.224	24	9.8	29.8
568.7904	428.226	26	9.8	29.8
568.7904	428.228	28	9.8	29.8
568.7904	434.216	16	9.8	29.8
568.7904	434.218	18	9.8	29.8
568.7904	434.22	20	9.8	29.8
568.7904	434.222	22	9.8	29.8
568.7904	434.224	24	9.8	29.8
568.7904	434.226	26	9.8	29.8
568.7904	434.228	28	9.8	29.8
568.7904	490.716	16	9.8	29.8
568.7904	490.718	18	9.8	29.8
568.7904	490.72	20	9.8	29.8
568.7904	490.722	22	9.8	29.8
568.7904	490.724	24	9.8	29.8
568.7904	490.726	26	9.8	29.8
568.7904	490.728	28	9.8	29.8
568.7904	557.216	16	9.8	29.8
568.7904	557.218	18	9.8	29.8
568.7904	557.22	20	9.8	29.8
568.7904	557.222	22	9.8	29.8
568.7904	557.224	24	9.8	29.8
568.7904	557.226	26	9.8	29.8
568.7904	557.228	28	9.8	29.8
568.7904	720.316	16	9.8	29.8
568.7904	720.318	18	9.8	29.8
568.7904	720.32	20	9.8	29.8
568.7904	720.322	22	9.8	29.8
568.7904	720.324	24	9.8	29.8
568.7904	720.326	26	9.8	29.8
568.7904	720.328	28	9.8	29.8
568.7904	867.316	16	9.8	29.8
568.7904	867.318	18	9.8	29.8
568.7904	867.32	20	9.8	29.8
568.7904	867.322	22	9.8	29.8
568.7904	867.324	24	9.8	29.8
568.7904	867.326	26	9.8	29.8
568.7904	867.328	28	9.8	29.8

Retention time shift correction on-the-fly provides robust quantitative results across different columns and instruments

C:\Xcalibur...heavypep_25fmolmatrix05 9/1/2009 2:46:42 PM
heavypep_25fmolmatrix05
heavy peptides,25fmol in matrix, 400nl

heavypep_25fmolmatrix05
TSQ Vantage

Experiment Type: iSRM
Chrom Filter Peak Width (s): 10.0
Collision Gas Pressure (mTorr): 1.2
Use Tuned S-Lens Value: Yes
Q1 Peak Width (FWHM): 0.70
Display Time Range for SRM table: Yes
Cycle Time (s): 2.000
DCV (V): Not used

SRM Table:

Parent	Product	CE	Start	Stop	Pol	Trigger	React Cat	Name
609.310	347.228	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
609.310	524.257	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
609.310	532.308	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
609.310	660.367	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
609.310	717.388	24	13.65	21.65	+	1.000e+03	Primary	(empty)
609.310	846.431	24	13.65	21.65	+	1.000e+03	Primary	(empty)
609.310	960.474	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
609.310	1047.506	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
613.317	355.243	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
613.317	528.264	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
613.317	540.323	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
613.317	668.381	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
613.317	725.403	24	13.65	21.65	+	1.000e+03	Reference	(empty)
613.317	854.445	24	13.65	21.65	+	1.000e+03	Reference	(empty)
613.317	968.488	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
613.317	1055.520	24	13.65	21.65	+	1.000e+03	Secondary	(empty)
521.793	451.266	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
521.793	588.325	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
521.793	659.362	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
521.793	730.399	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
521.793	801.436	22	20.33	24.33	+	1.000e+03	Primary	(empty)
521.793	914.520	22	20.33	24.33	+	1.000e+03	Primary	(empty)
521.793	985.557	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
526.797	461.274	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
526.797	598.333	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
526.797	669.370	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
526.797	740.407	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
526.797	811.444	22	20.33	24.33	+	1.000e+03	Reference	(empty)
526.797	924.528	22	20.33	24.33	+	1.000e+03	Reference	(empty)
526.797	995.565	22	20.33	24.33	+	1.000e+03	Secondary	(empty)
769.888	376.219	30	24.75	28.75	+	1.000e+03	Secondary	(empty)

The TSQ series of triple quadrupole instruments allows user to assign single or multiple peaks as RT reference compounds. With the changes of chromatographic conditions, the retention time shifts from these reference compounds are captured and a linear regression curve will be generated on-the-fly that provides an offset and slope correction to the tSRM windows of the compounds eluted afterwards. This approach enables the use of narrow timed-SRM window without missing peaks due to retention time shifts, caused by column/mobile phase replacement, switching instrument, column clog or changing gradient conditions.

Targeted peak was detected by correcting the RT shift on the fly

The time shift was created by increasing the flow rate from 300 nl/min to 400 nl/min

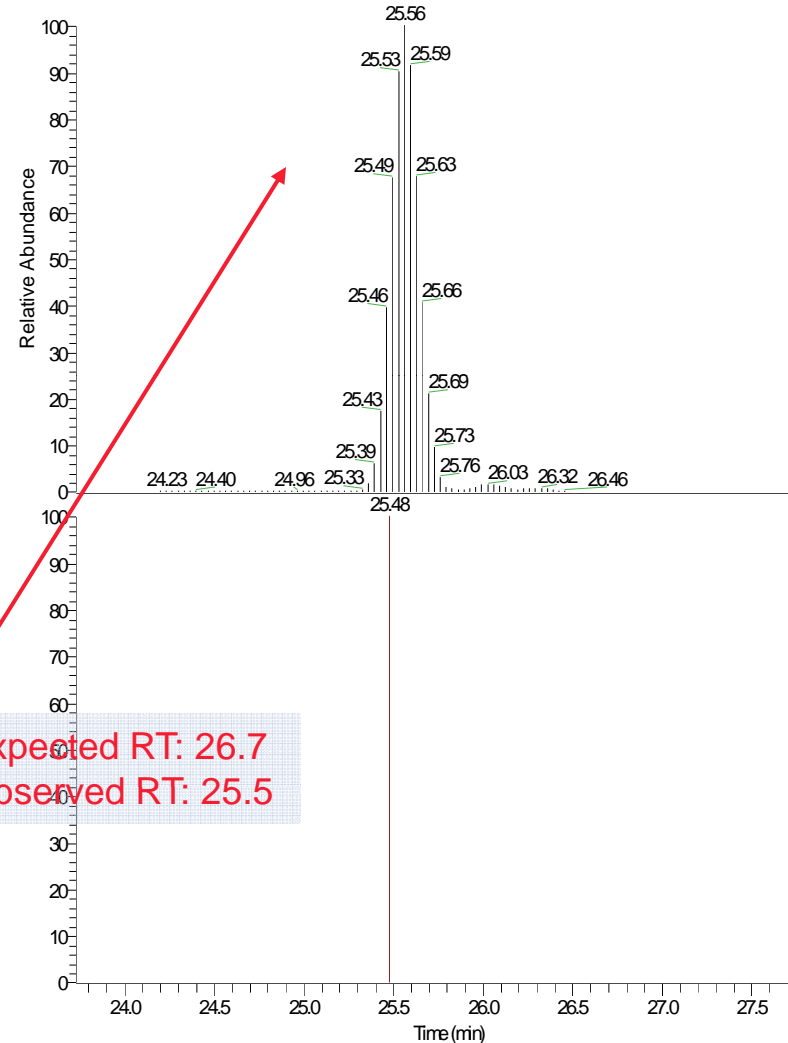
heavypep_25fmolmatrix_2minwindow
 heavy peptides,25fmol in matrix, 400nl

9/1/2009 4:14:00 PM

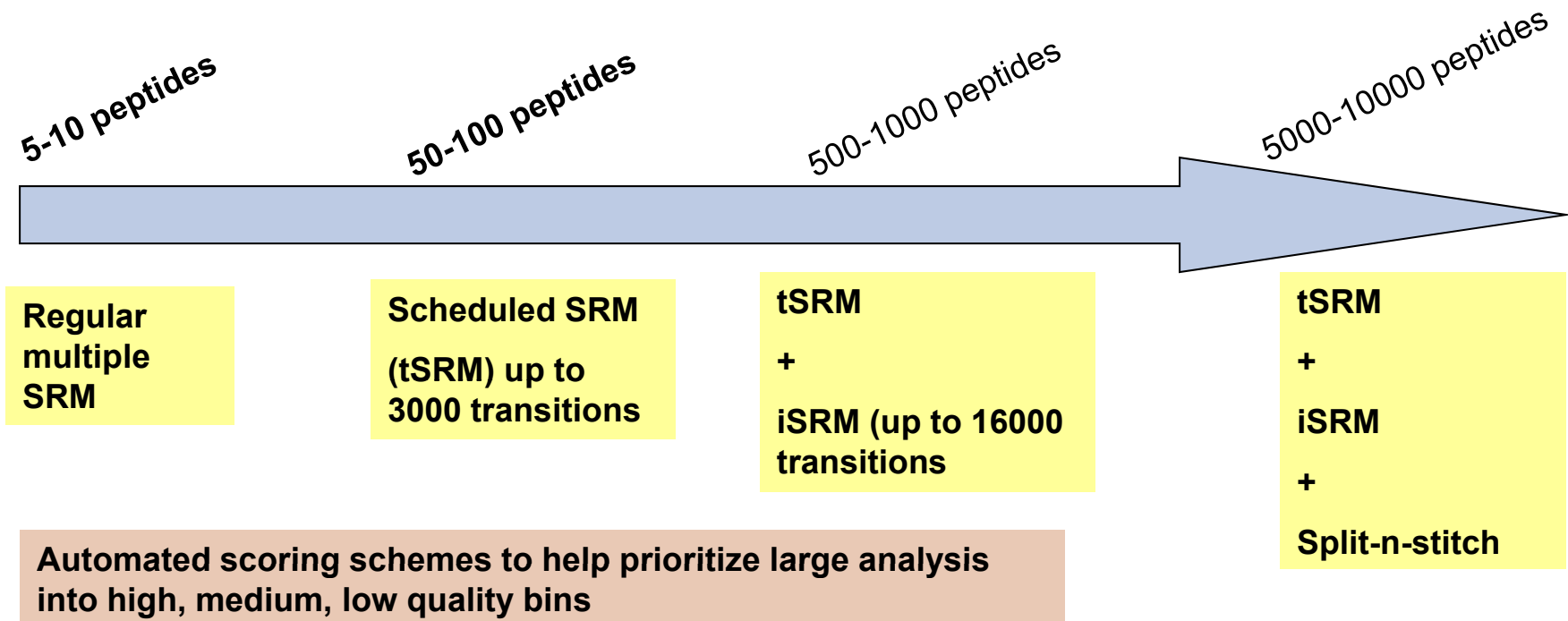
heavypep_25fmolmatrix_2minwindow
 TSQ Vantage

613.317	854.445	24	11.65	23.65	+ 1.000e+03	Reference
613.317	968.488	24	11.65	23.65	+ 1.000e+03	Secondary
613.317	1055.520	24	11.65	23.65	+ 1.000e+03	Secondary
521.793	451.266	22	21.33	23.33	+ 1.000e+03	Secondary
521.793	588.325	22	21.33	23.33	+ 1.000e+03	Secondary
521.793	659.362	22	21.33	23.33	+ 1.000e+03	Secondary
521.793	730.399	22	21.33	23.33	+ 1.000e+03	Secondary
521.793	801.436	22	21.33	23.33	+ 1.000e+03	Primary
521.793	914.520	22	21.33	23.33	+ 1.000e+03	Primary
521.793	985.557	22	21.33	23.33	+ 1.000e+03	Secondary
526.797	461.274	22	21.33	23.33	+ 1.000e+03	Secondary
526.797	598.333	22	21.33	23.33	+ 1.000e+03	Secondary
526.797	669.370	22	21.33	23.33	+ 1.000e+03	Secondary
526.797	740.407	22	21.33	23.33	+ 1.000e+03	Secondary
526.797	811.444	22	21.33	23.33	+ 1.000e+03	Reference
526.797	924.528	22	21.33	23.33	+ 1.000e+03	Reference
526.797	995.565	22	21.33	23.33	+ 1.000e+03	Secondary
769.888	376.219	30	25.75	27.75	+ 1.000e+03	Secondary
769.888	489.303	30	25.75	27.75	+ 1.000e+03	Secondary
769.888	652.366	30	25.75	27.75	+ 1.000e+03	Secondary
769.888	753.414	30	25.75	27.75	+ 1.000e+03	Secondary
769.888	868.441	30	25.75	27.75	+ 1.000e+03	Primary
769.888	967.509	30	25.75	27.75	+ 1.000e+03	Secondary
769.888	1024.530	30	25.75	27.75	+ 1.000e+03	Primary
769.888	1111.562	30	25.75	27.75	+ 1.000e+03	Secondary
769.888	1296.642	30	25.75	27.75	+ 1.000e+03	Secondary
773.896	384.233	30	25.75	27.75	+ 1.000e+03	Secondary
773.896	497.317	30	25.75	27.75	+ 1.000e+03	Secondary
773.896	660.380	30	25.75	27.75	+ 1.000e+03	Secondary
773.896	761.428	30	25.75	27.75	+ 1.000e+03	Secondary
773.896	876.455	30	25.75	27.75	+ 1.000e+03	Primary
773.896	975.523	30	25.75	27.75	+ 1.000e+03	Secondary
773.896	1032.545	30	25.75	27.75	+ 1.000e+03	Primary
773.896	1119.577	30	25.75	27.75	+ 1.000e+03	Secondary
773.896	1304.657	30	25.75	27.75	+ 1.000e+03	Secondary
796.407	675.305	30	29.46	31.46	+ 1.000e+03	Secondary
796.407	762.337	30	29.46	31.46	+ 1.000e+03	Secondary
796.407	861.406	30	29.46	31.46	+ 1.000e+03	Secondary
796.407	918.427	30	29.46	31.46	+ 1.000e+03	Primary
796.407	1005.459	30	29.46	31.46	+ 1.000e+03	Secondary
796.407	1062.480	30	29.46	31.46	+ 1.000e+03	Primary
796.407	1161.549	30	29.46	31.46	+ 1.000e+03	Secondary

RT: 23.73 - 27.88



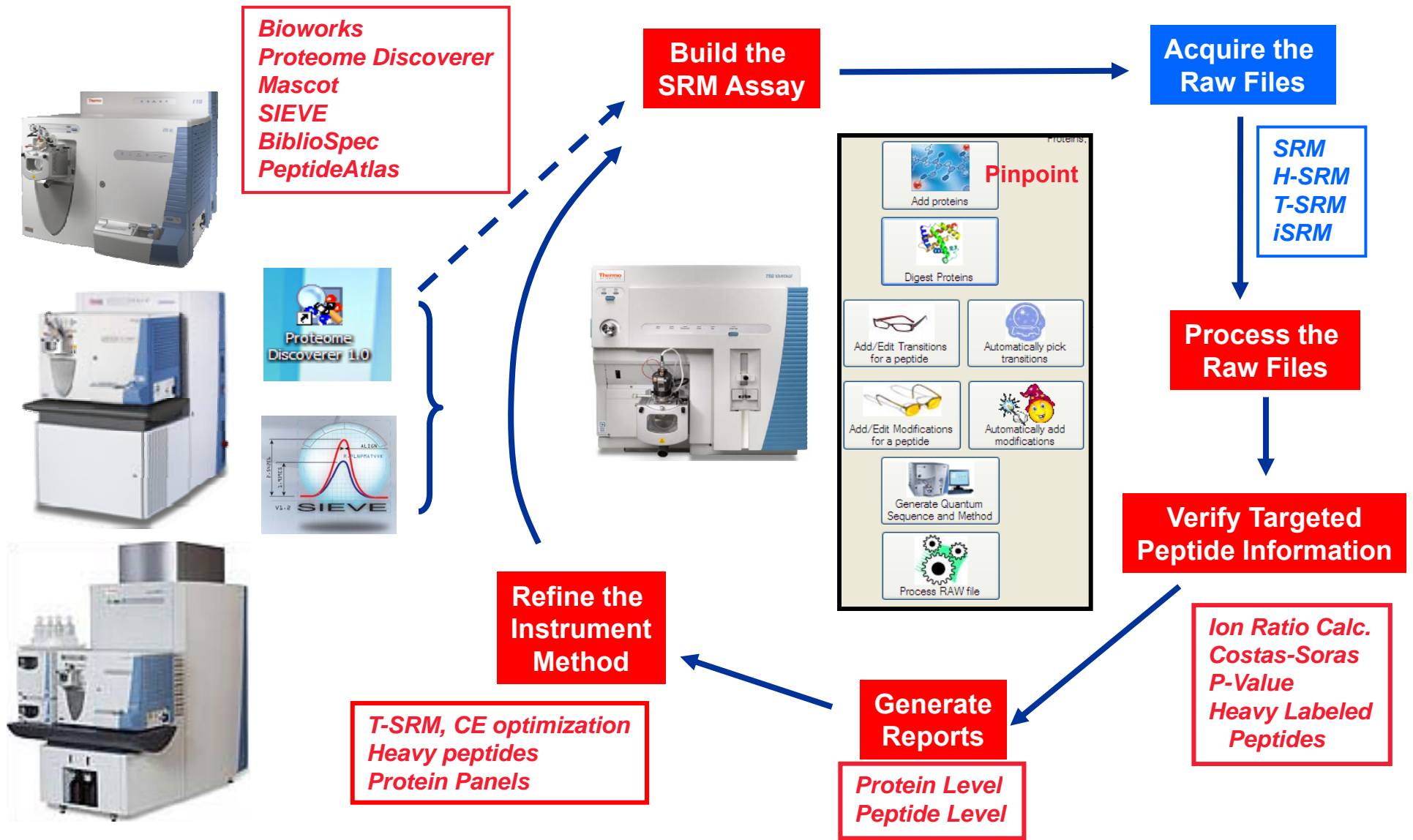
Scaling Options using iSRM and Pinpoint



And more...

- Single software to help iterative method building to go from protein list to absolute abundance
- Multi-threaded
- Extremely easy data and results sharing
- Customers can give video feedback
- Video help tutorials to get you started

Optimizing assays for specificity & sensitivity



Summary

- Targeted protein quantitation using triple quadrupole mass spectrometers is complementary to traditional western blots
- Thermo Fisher Scientific's workflow incorporates iSRM to facilitate rapid and robust method development for multiplexed assays.
- Pinpoint software allows initial SRM/iSRM assay to be designed automatically by using discovery MS/MS data or theoretical prediction. It also offers unique peptide verification scheme for SRM assay refinement.
- Thermo Fisher Scientific's workflow provides high-throughput peptide screens by simultaneously verifying and quantitating large number of peptides in a single HPLC MS/MS run.