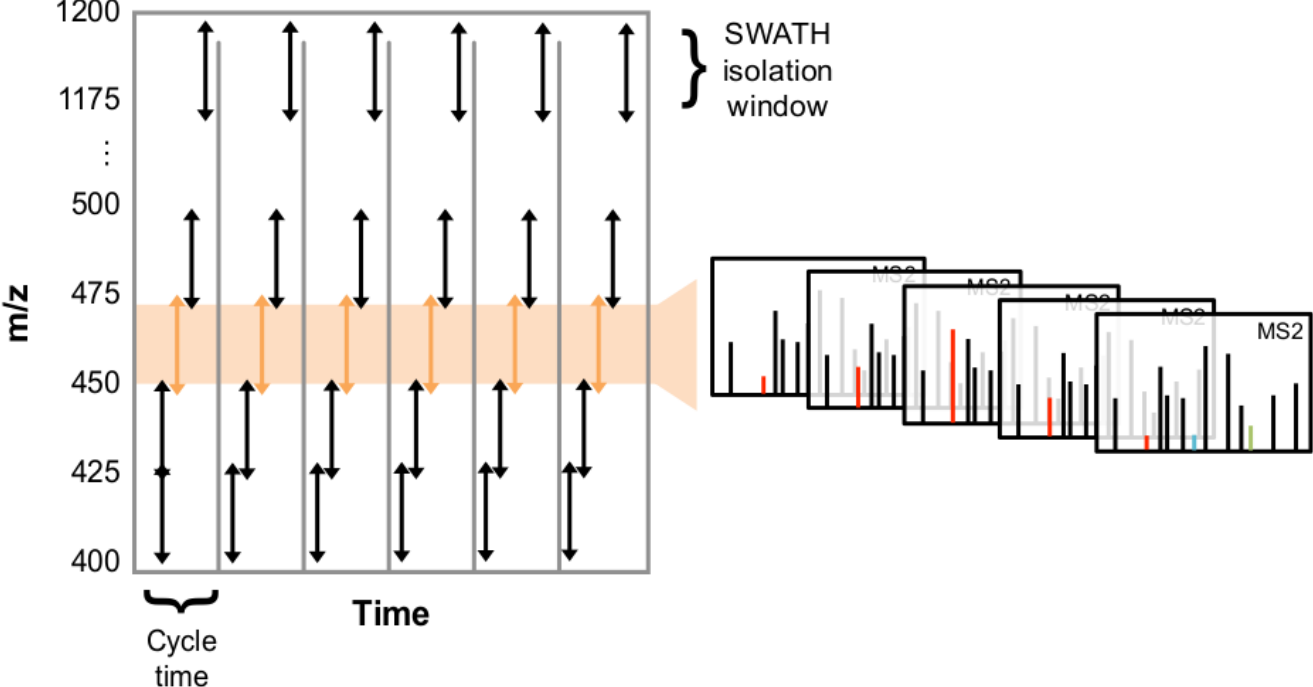


Data Independent Acquisition: Expanding the Scope of DIA Strategies for Quantitative Mass Spectrometry



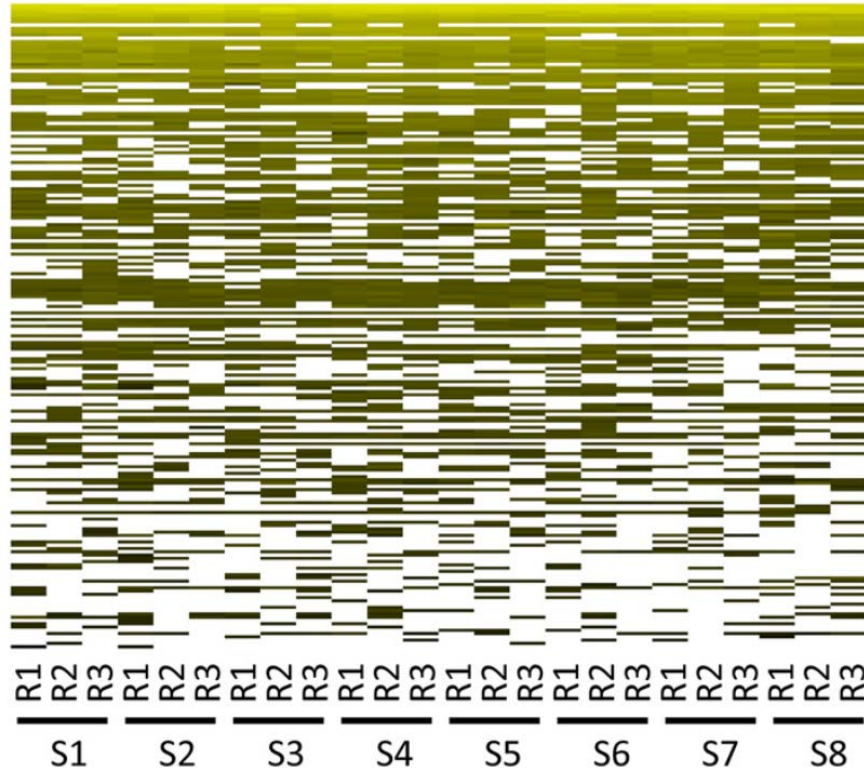
We're going to try to get some real time feedback from you...

<https://goo.gl/Cho7iT>

Just answer question 1 to start

DIA addresses missing data problem

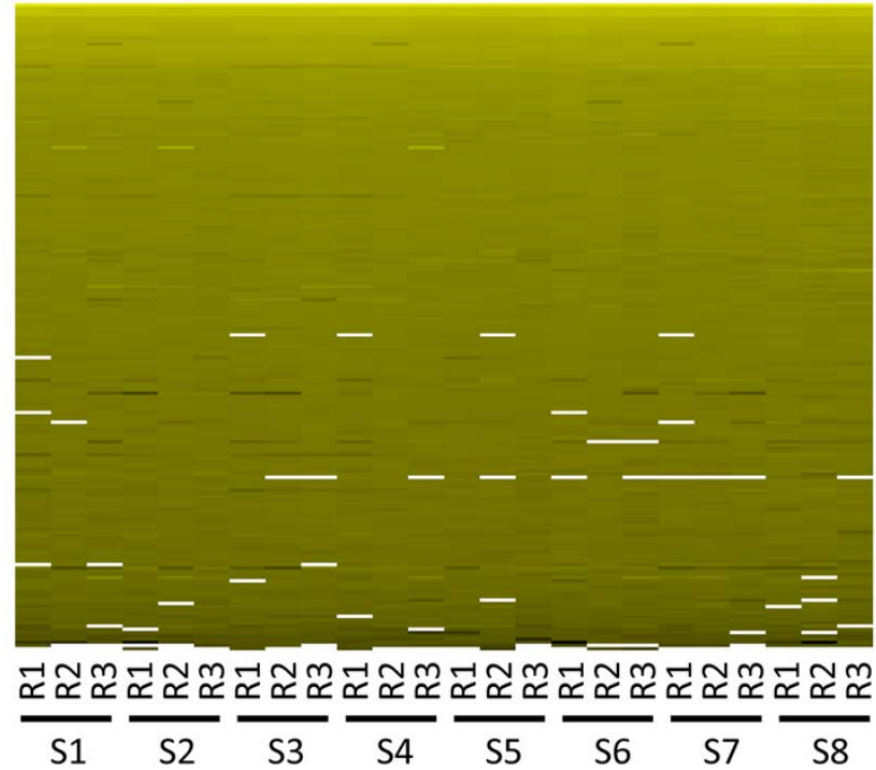
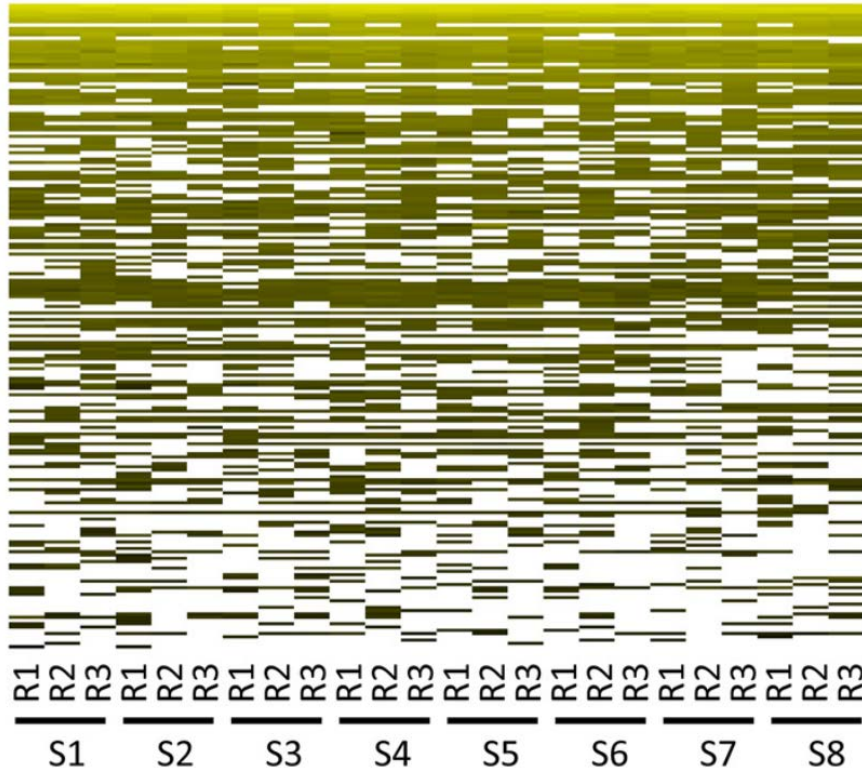
How to go from here



DIA addresses missing data problem

How to go from here

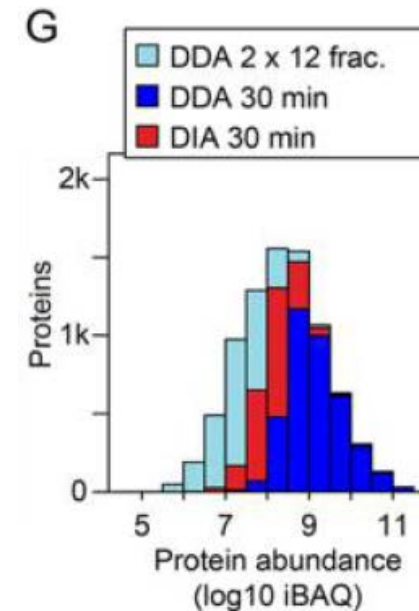
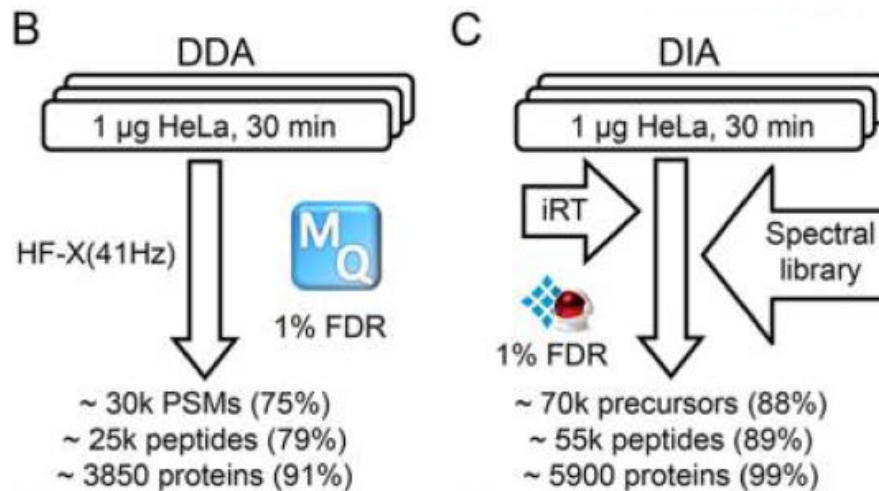
to here?



Many more traditionally DDA focused groups are adopting DIA...

Performance Evaluation of the Q Exactive HF-X for Shotgun Proteomics

Christian D. Kelstrup,[†] Dorte B. Bekker-Jensen,[†] Tabiwang N. Arrey,[‡] Alexander Hogrebe,[†] Alexander Harder,[‡] and Jesper V. Olsen^{*,†}



DIA: Beyond peptide quantification

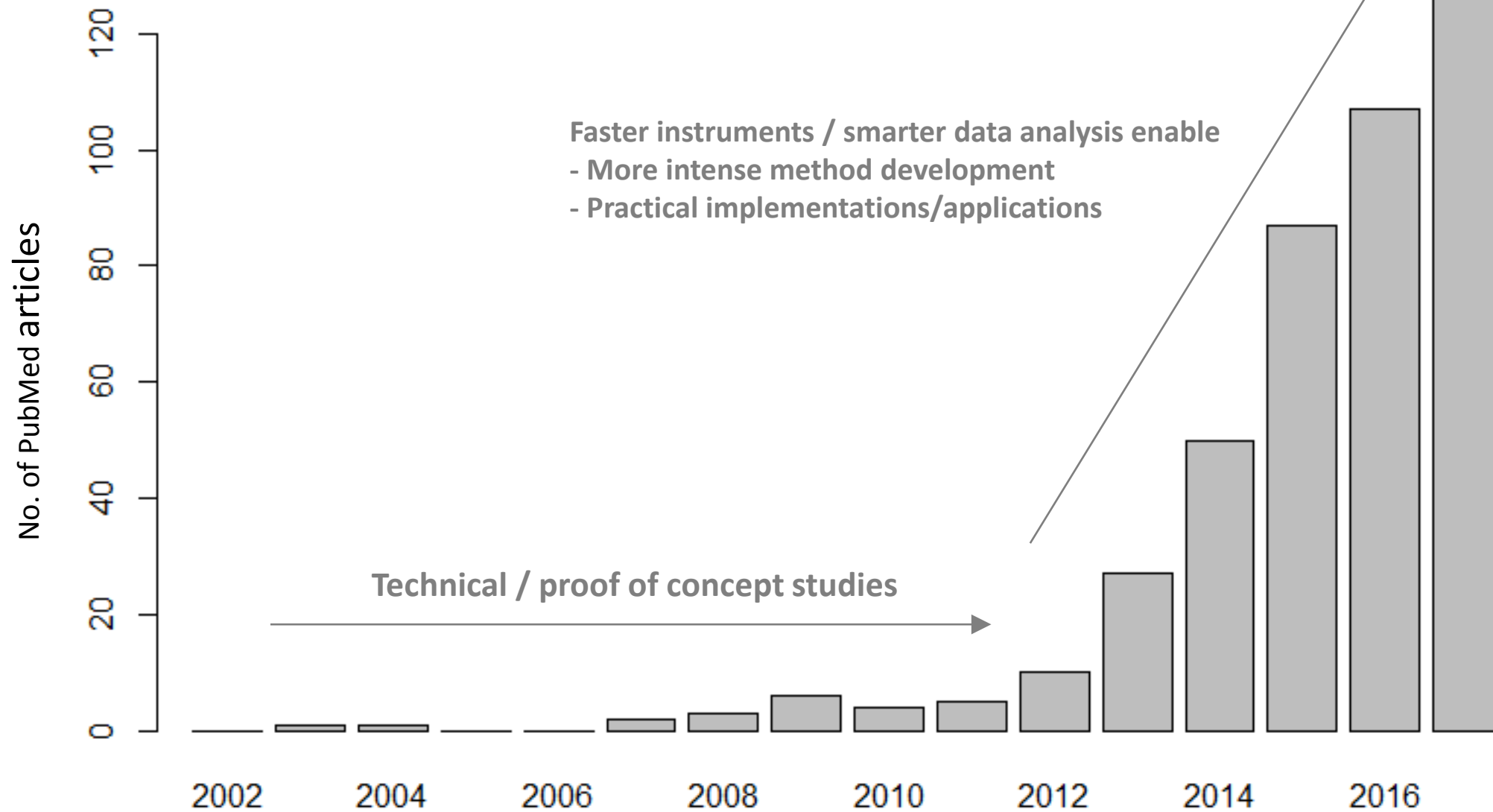
- Gérard Hopfgartner: Small molecules
- Brian Searle: PTM
- Birgit Schilling: PTM
- Isabell Bludau: Protein Complexes

- Switch presentations

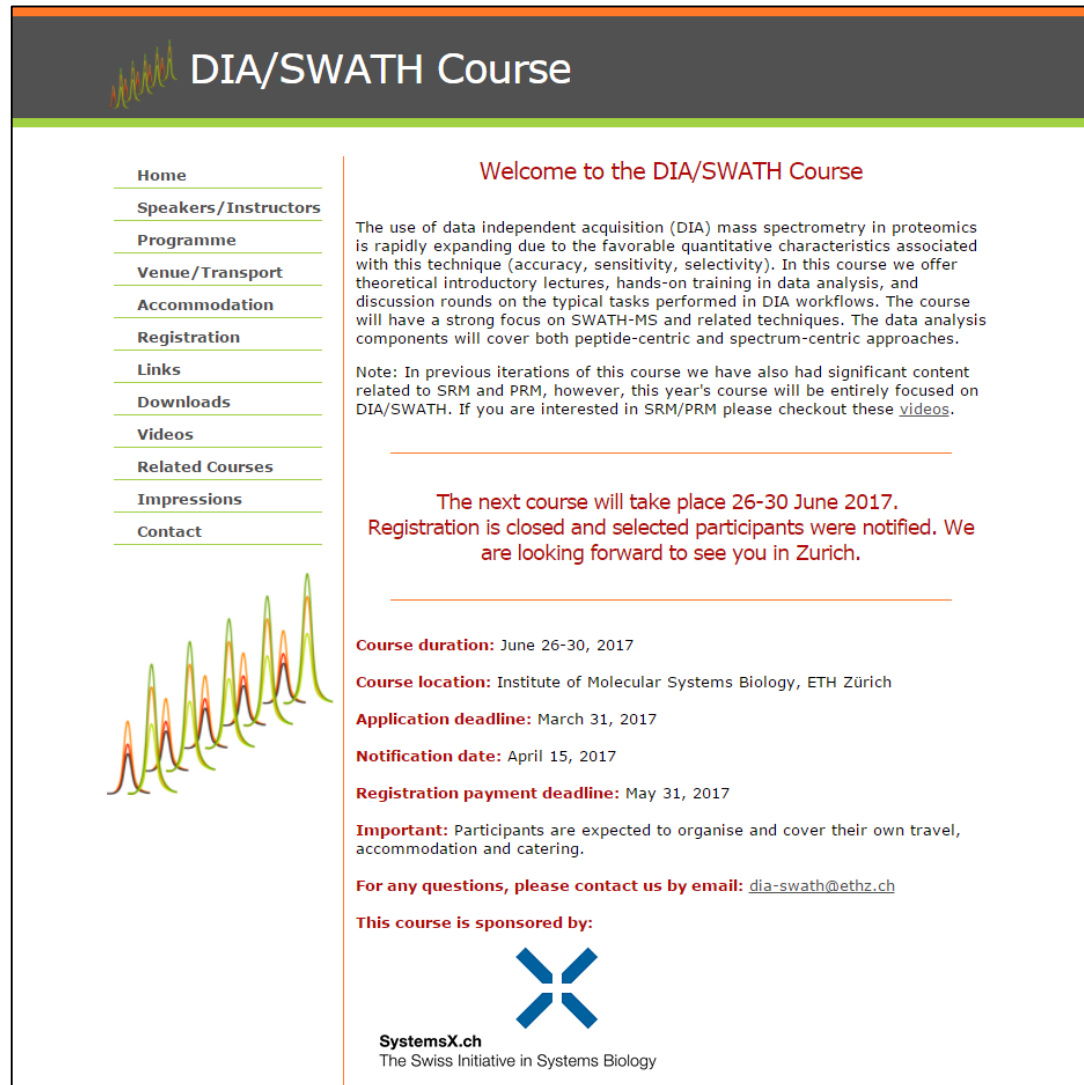
Development and application rate of DIA/SWATH

PubMed query:

((proteom* OR protein) OR peptide) AND ("data independent acquisition" OR SWATH)



If you want learn more about DIA/SWATH



The screenshot shows the homepage of the DIA/SWATH Course website. The header features the course title and a logo. A navigation menu on the left lists various sections. The main content area includes a welcome message, a detailed description of the course, and key dates and information.

DIA/SWATH Course

- Home
- Speakers/Instructors
- Programme
- Venue/Transport
- Accommodation
- Registration
- Links
- Downloads
- Videos
- Related Courses
- Impressions
- Contact

Welcome to the DIA/SWATH Course

The use of data independent acquisition (DIA) mass spectrometry in proteomics is rapidly expanding due to the favorable quantitative characteristics associated with this technique (accuracy, sensitivity, selectivity). In this course we offer theoretical introductory lectures, hands-on training in data analysis, and discussion rounds on the typical tasks performed in DIA workflows. The course will have a strong focus on SWATH-MS and related techniques. The data analysis components will cover both peptide-centric and spectrum-centric approaches.

Note: In previous iterations of this course we have also had significant content related to SRM and PRM, however, this year's course will be entirely focused on DIA/SWATH. If you are interested in SRM/PRM please checkout these [videos](#).


The next course will take place 26-30 June 2017.
Registration is closed and selected participants were notified. We are looking forward to see you in Zurich.

Course duration: June 26-30, 2017
Course location: Institute of Molecular Systems Biology, ETH Zürich
Application deadline: March 31, 2017
Notification date: April 15, 2017
Registration payment deadline: May 31, 2017

Important: Participants are expected to organise and cover their own travel, accommodation and catering.

For any questions, please contact us by email: dia-swath@ethz.ch

This course is sponsored by:

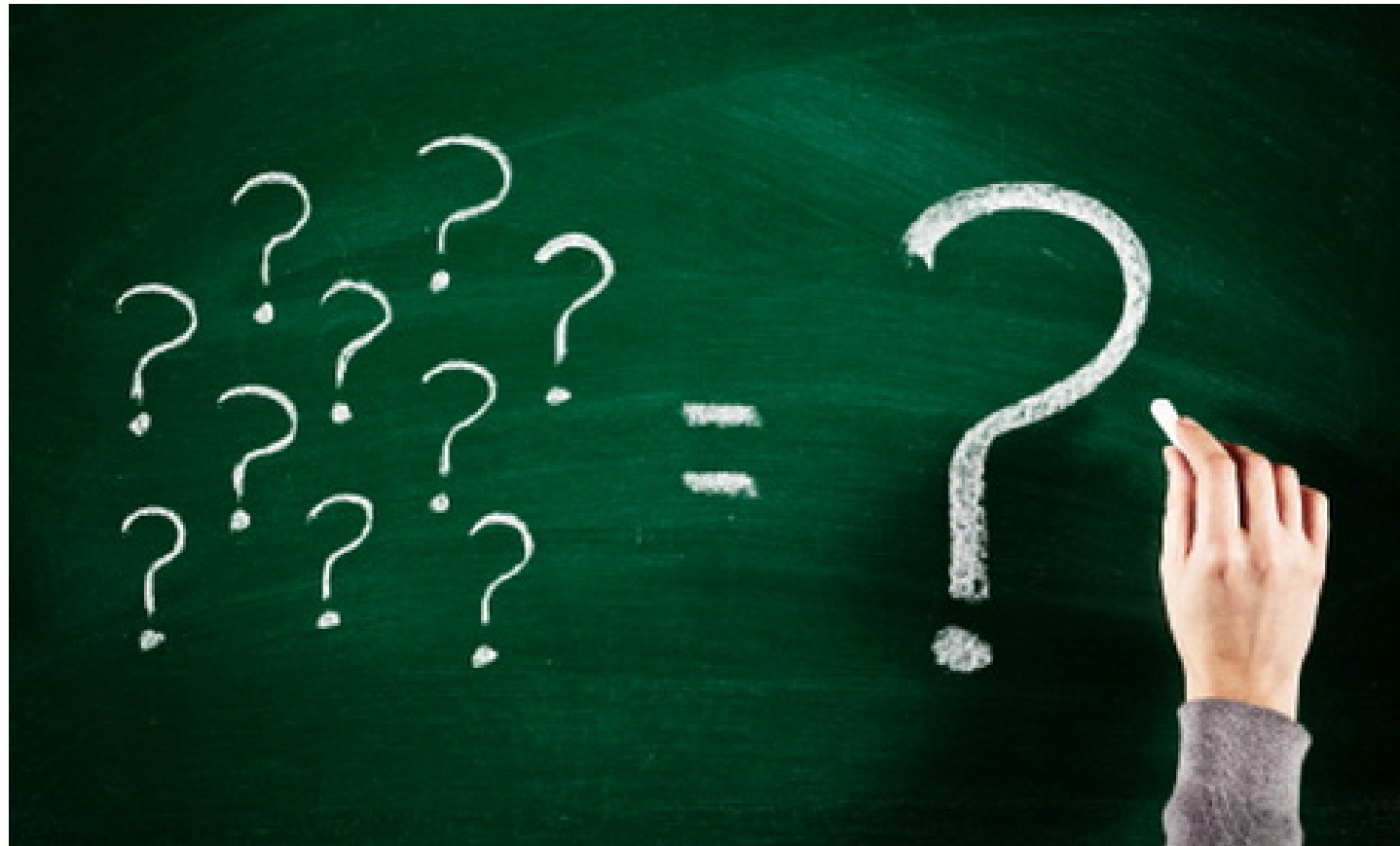


SystemsX.ch
The Swiss Initiative in Systems Biology

dia-swath-course.ethz.ch

Registration for this year is closed
but lecture videos will be
posted **late July 2018**

Comments? Questions?



Analyzing PTMs with DIA

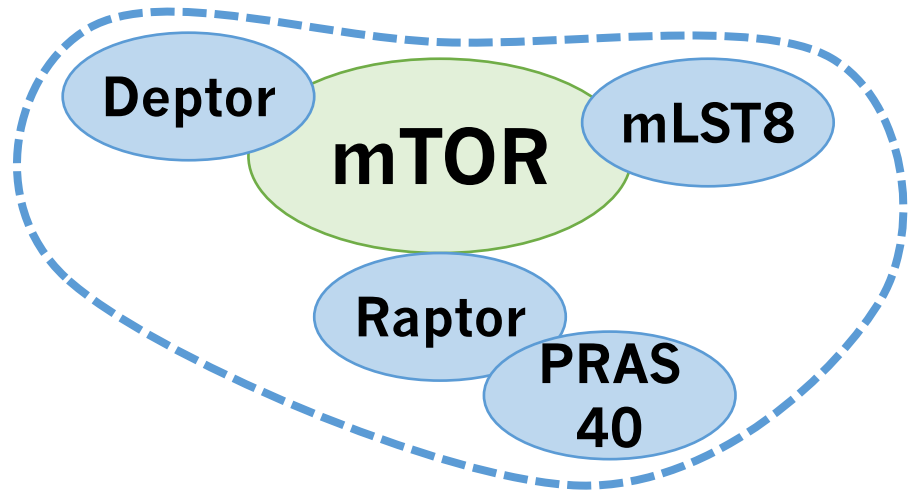
Brian C. Searle^{1, 2}

¹University of Washington, Seattle, WA

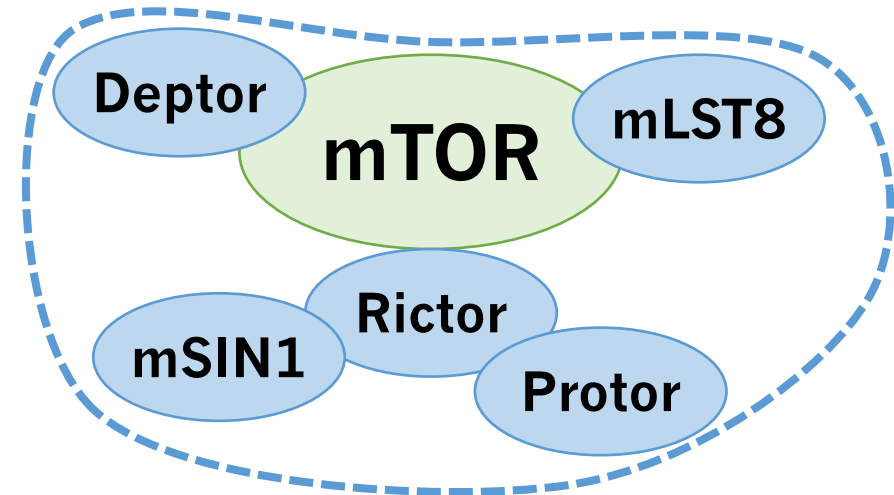
²Proteome Software Inc., Portland, OR

searleb@uw.edu / brian.searle@proteomesoftware.com

MTORC1 vs MTORC2



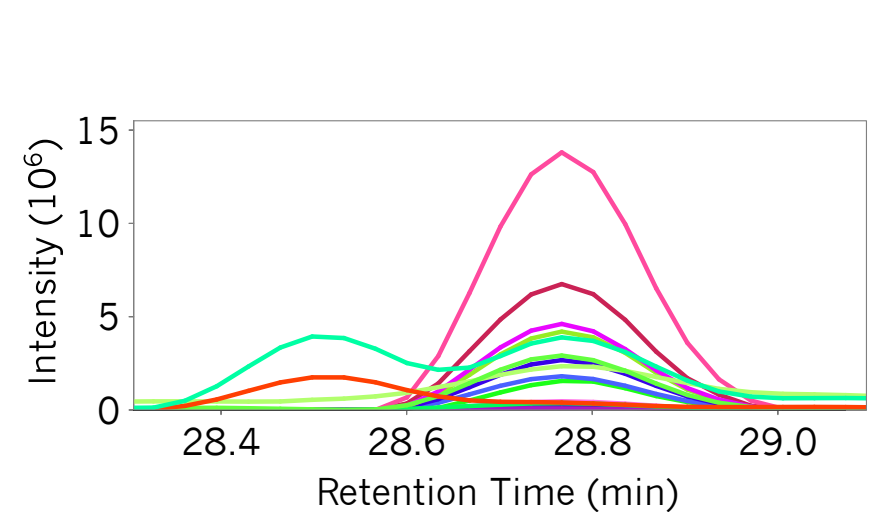
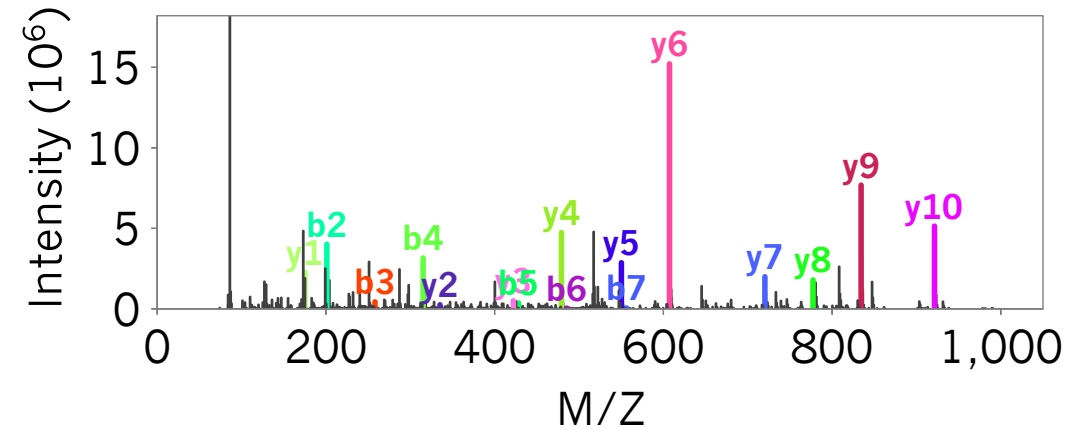
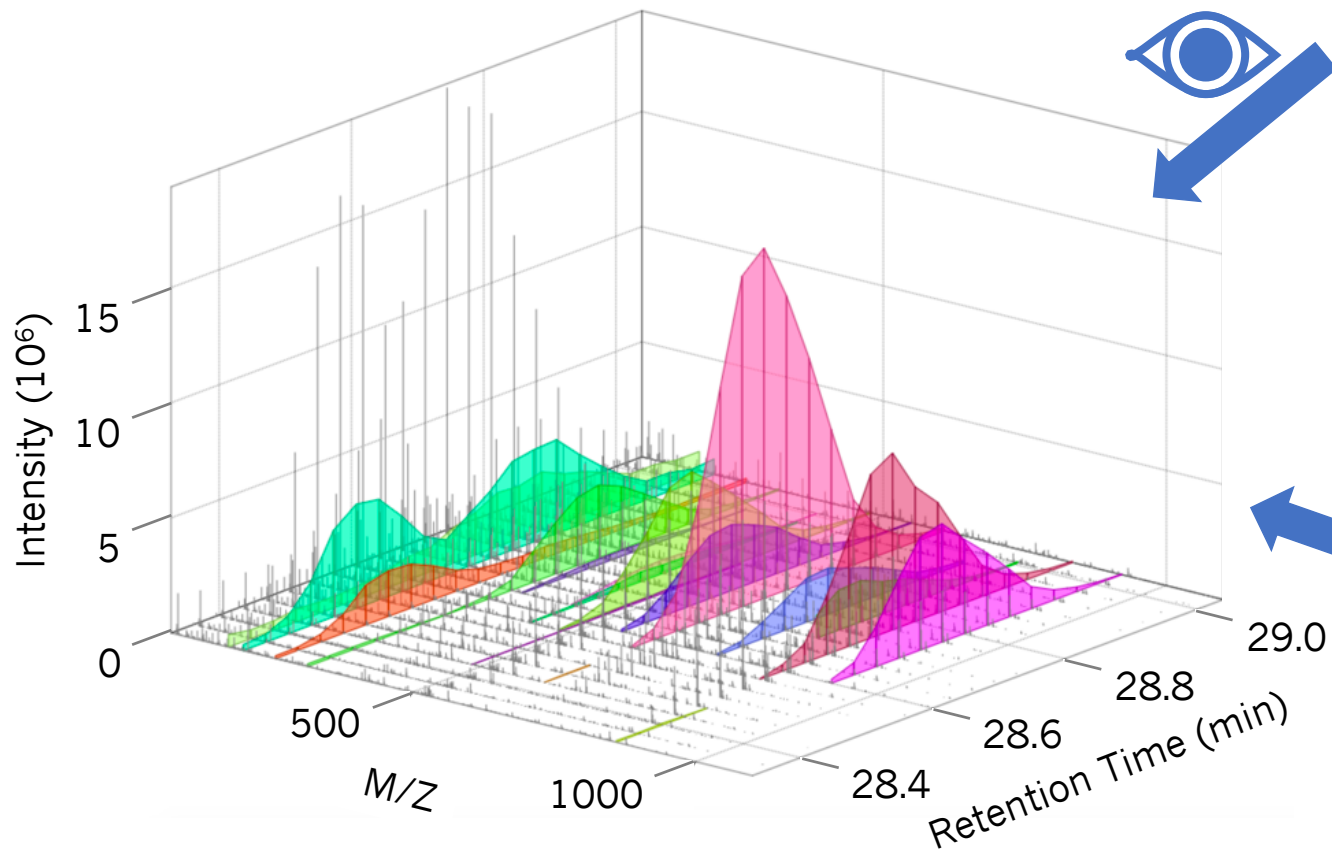
MTORC1



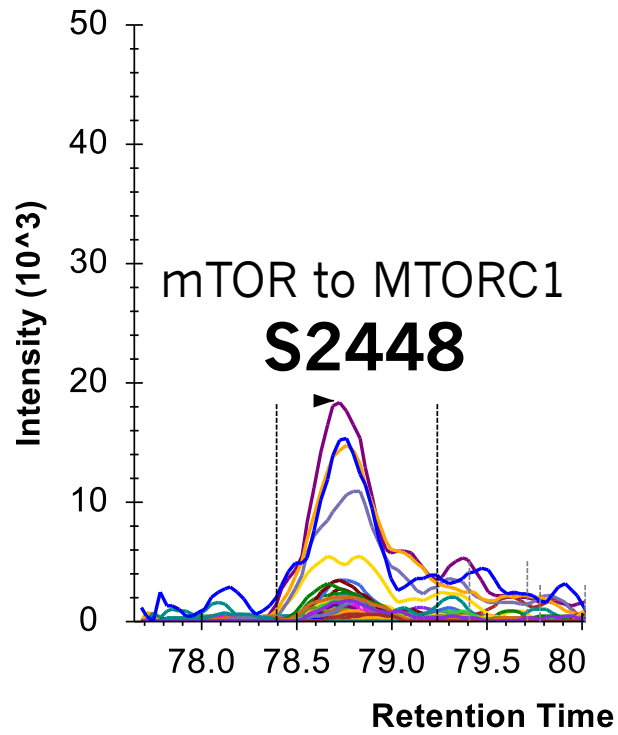
MTORC2

- **MTOR:S2448** TDpS_YSAGQSVEILDGVELGEPAHKK (**MTORC1**)
- **MTOR:S2481** KTGTTVPESIHpS_FFIGDGLVKPEALNK (**MTORC2**)

MS/MS can be considered from multiple views

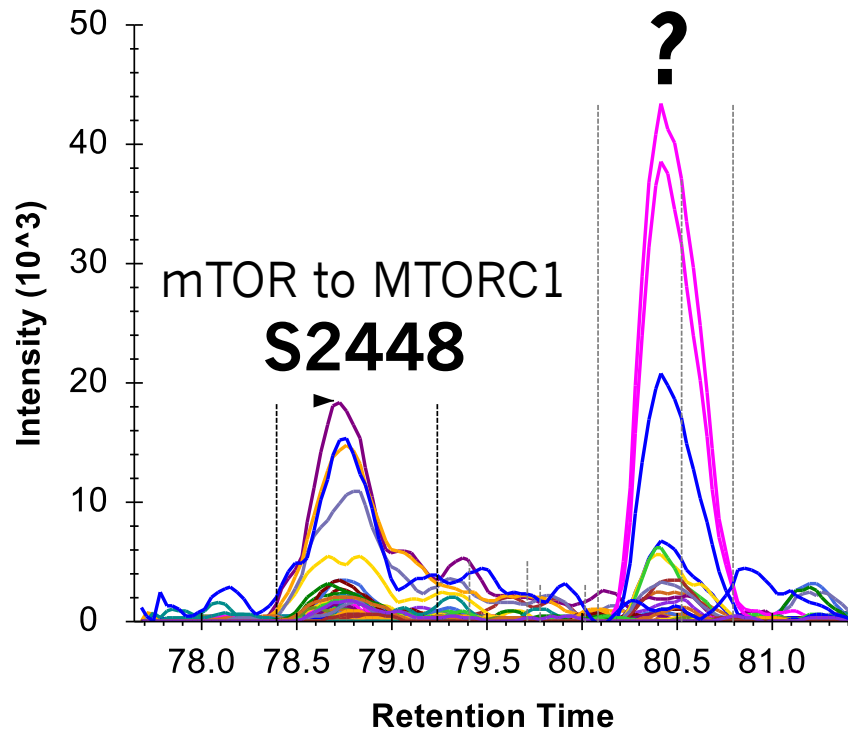


mTOR phosphorylation state is complex

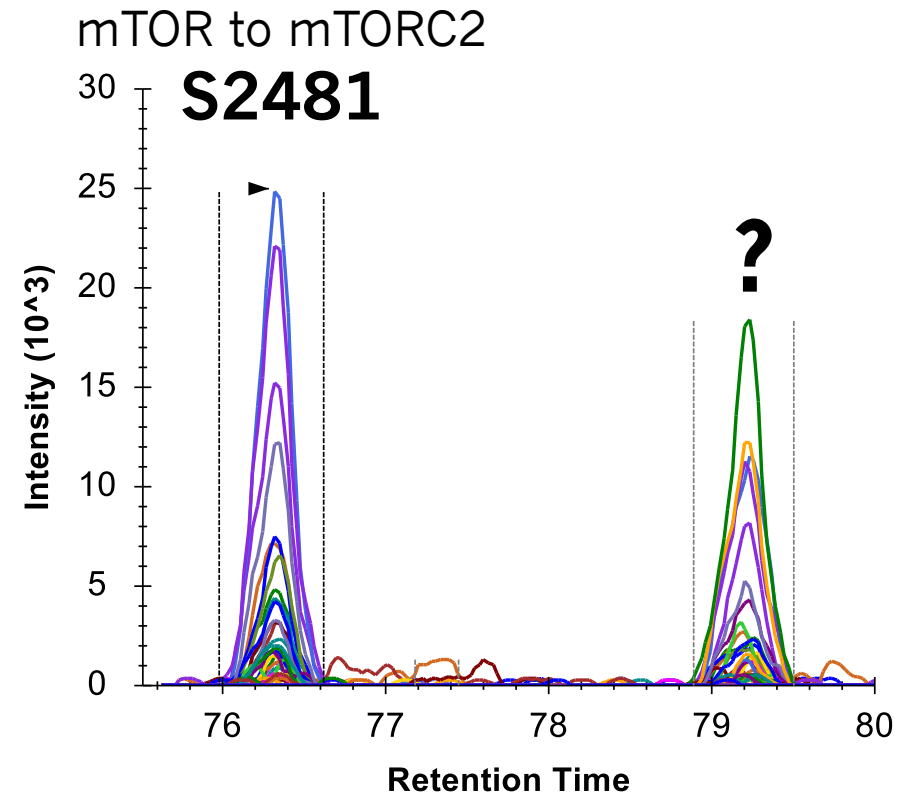


TDpSYSAGQSVEILDGVELGEPAAHKK

mTOR phosphorylation state is complex

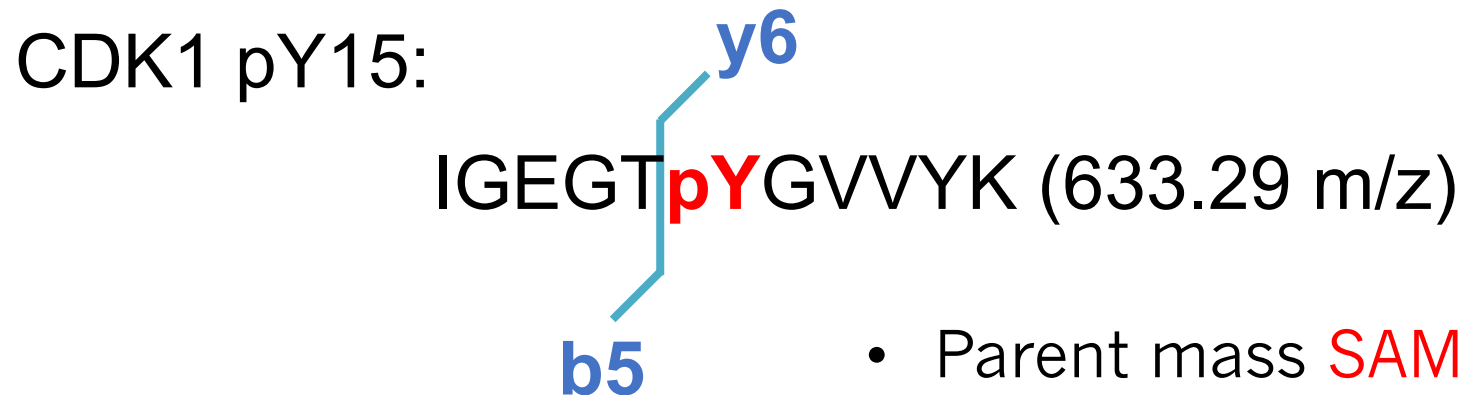
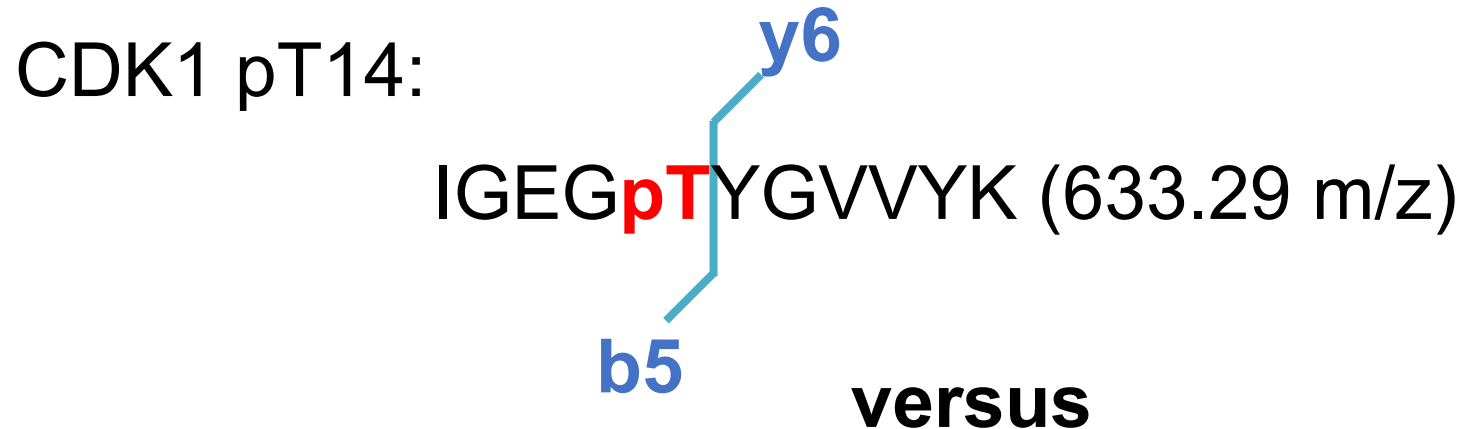


TDpSYSAGQSVEILDGVELGEPAAHKK



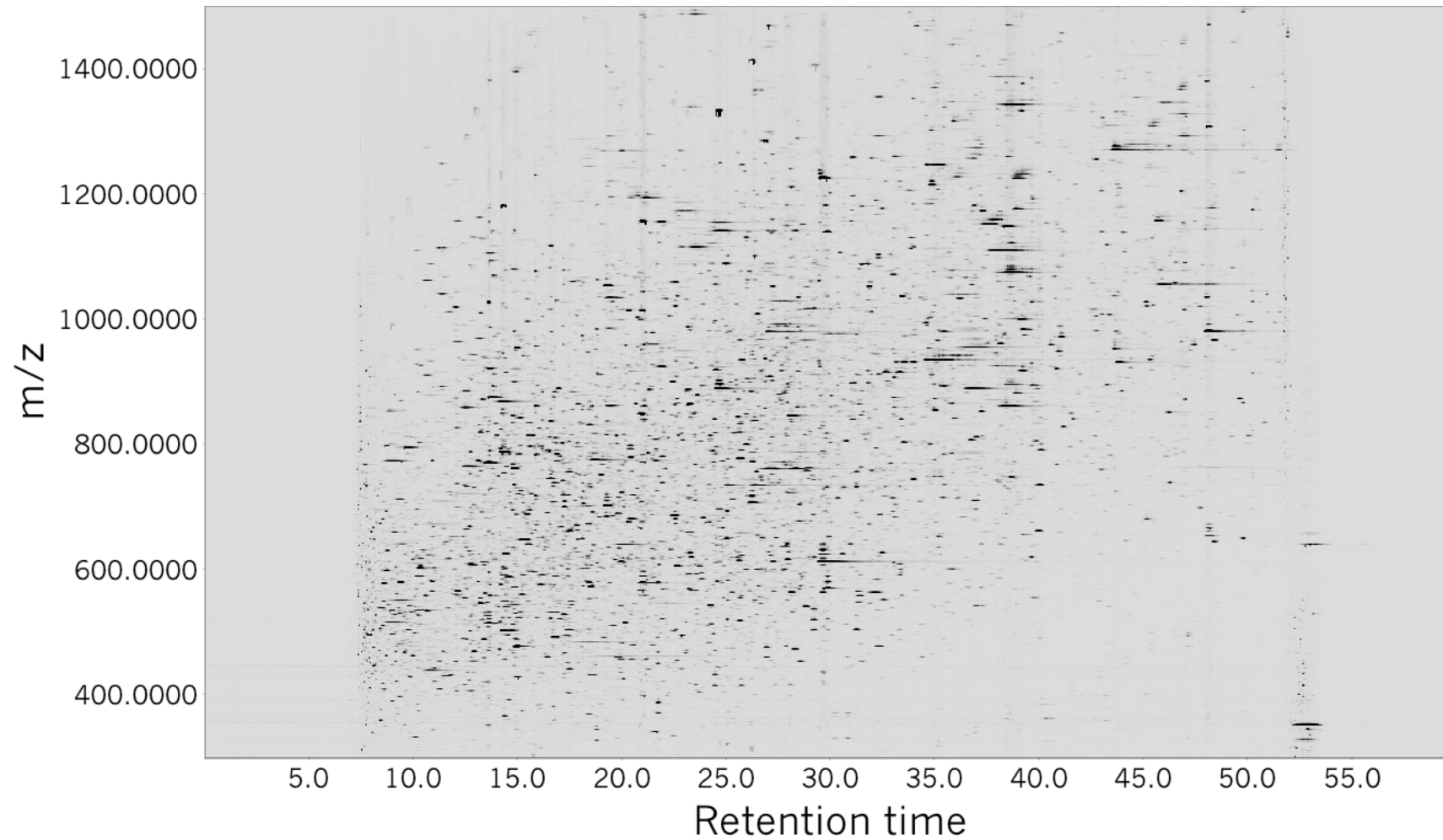
KTGTTVPESIHpSFIGDGLVKPEALNK

What is a positional isomer?

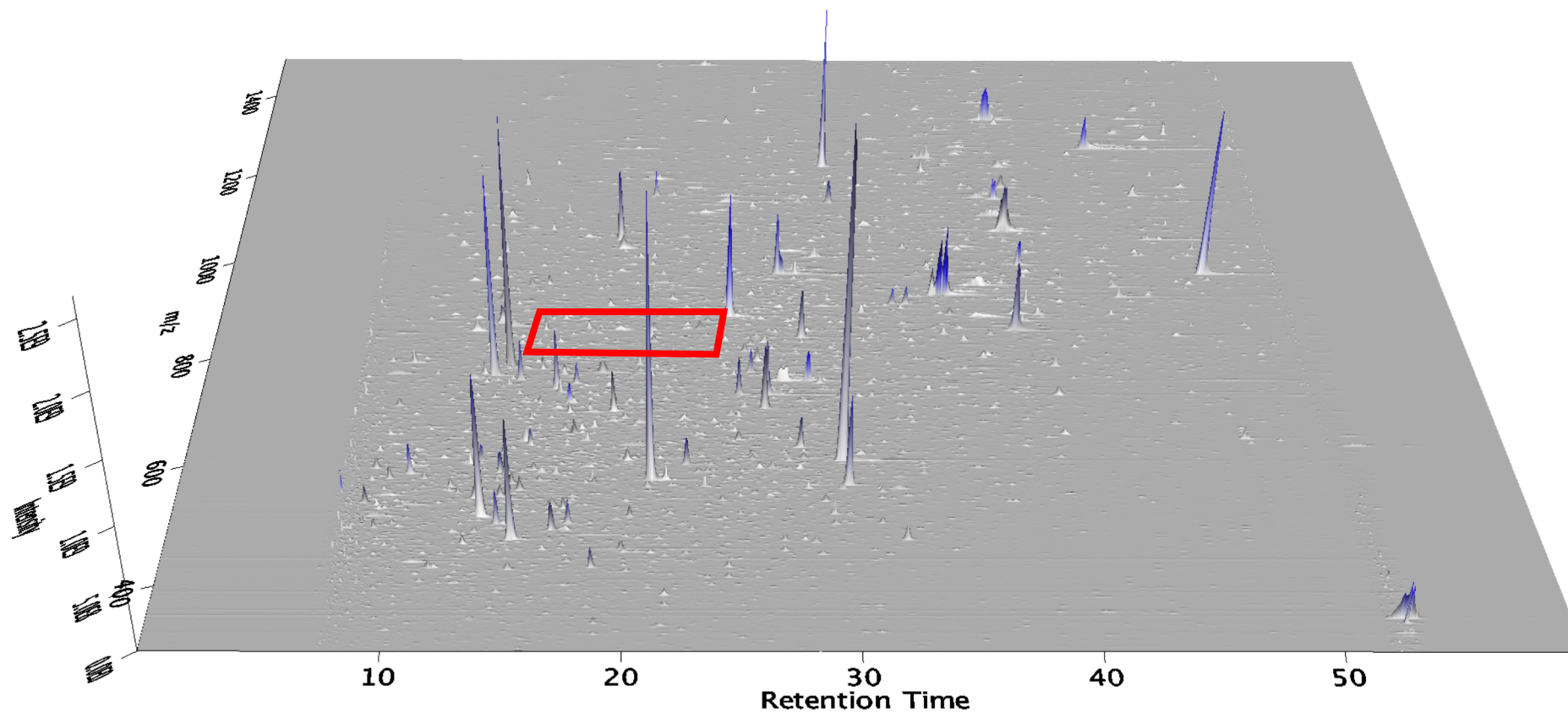


- Parent mass **SAME**
- **A FEW** fragmentation masses differ
- HPLC retention times **MAY** differ

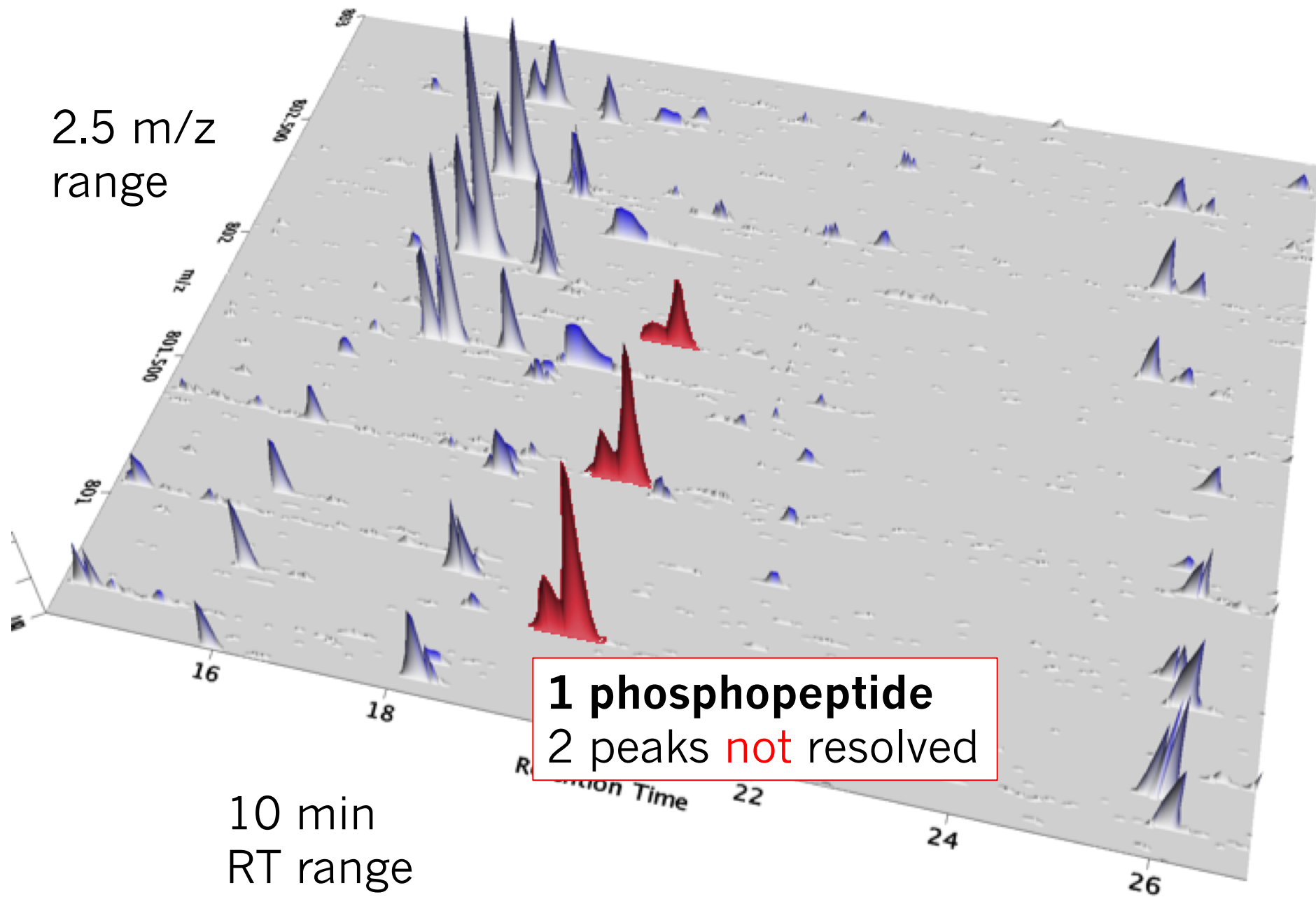
Zoomed out view of a phosphoproteome



Zoomed out view of a phosphoproteome

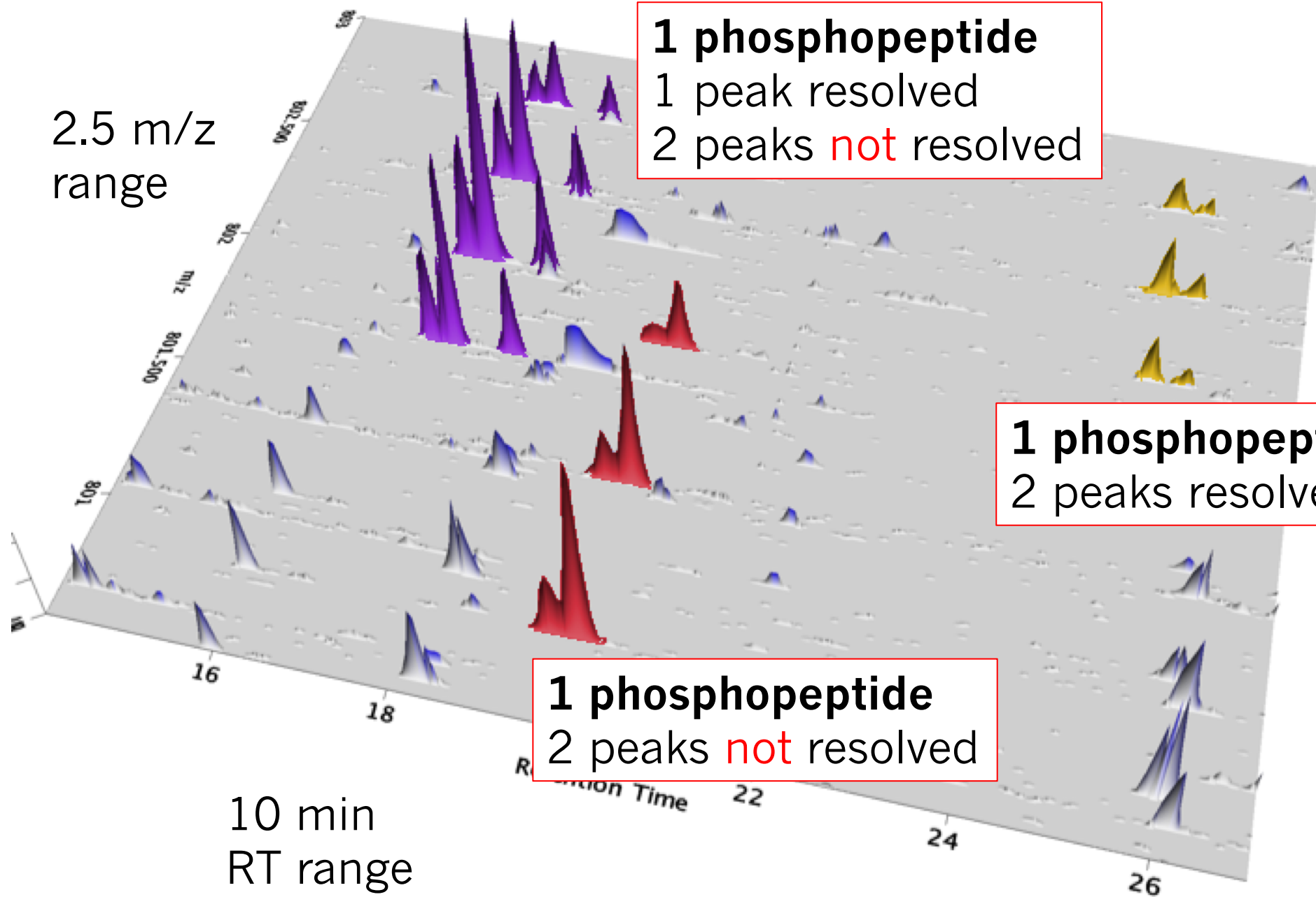


2.5 m/z
range

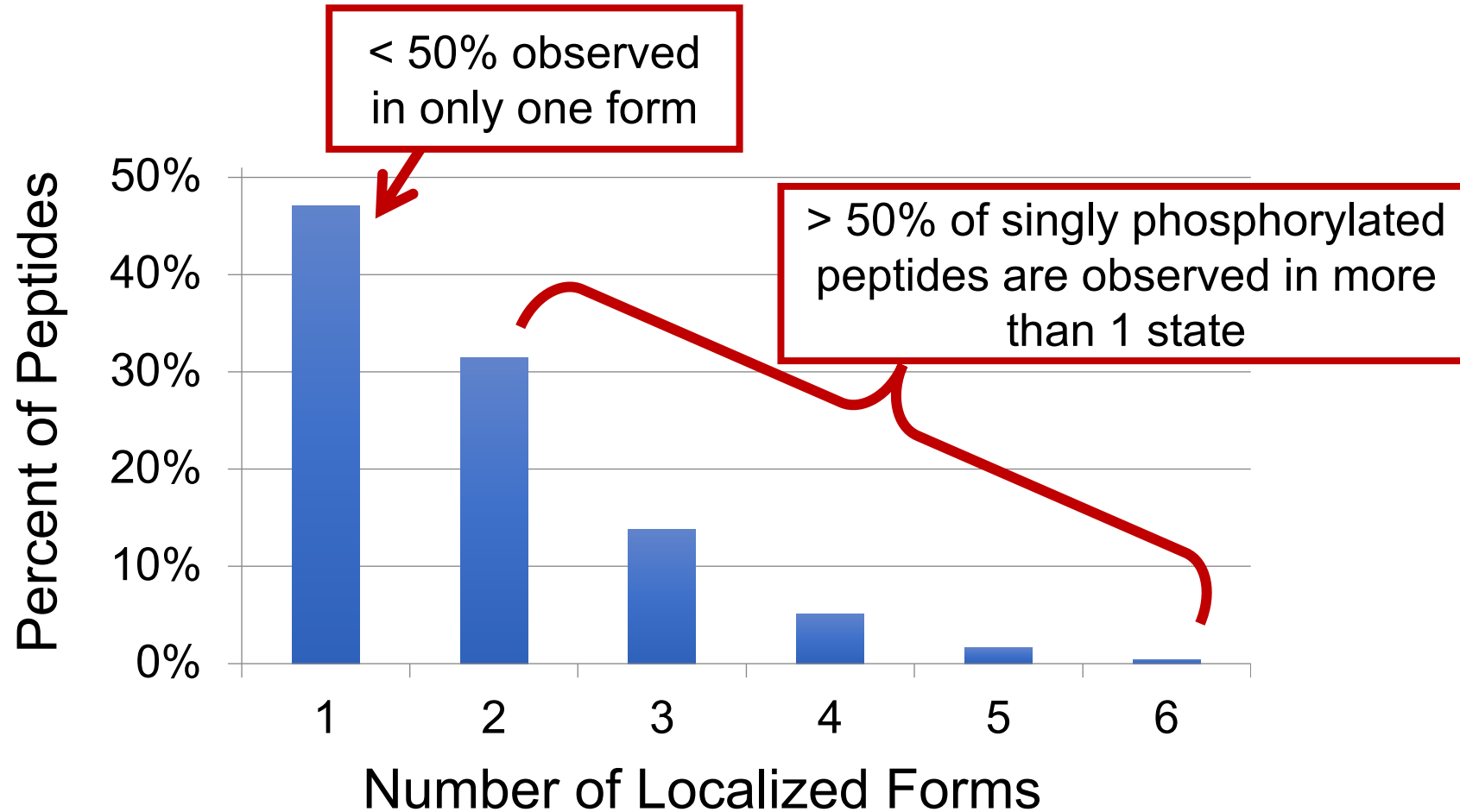


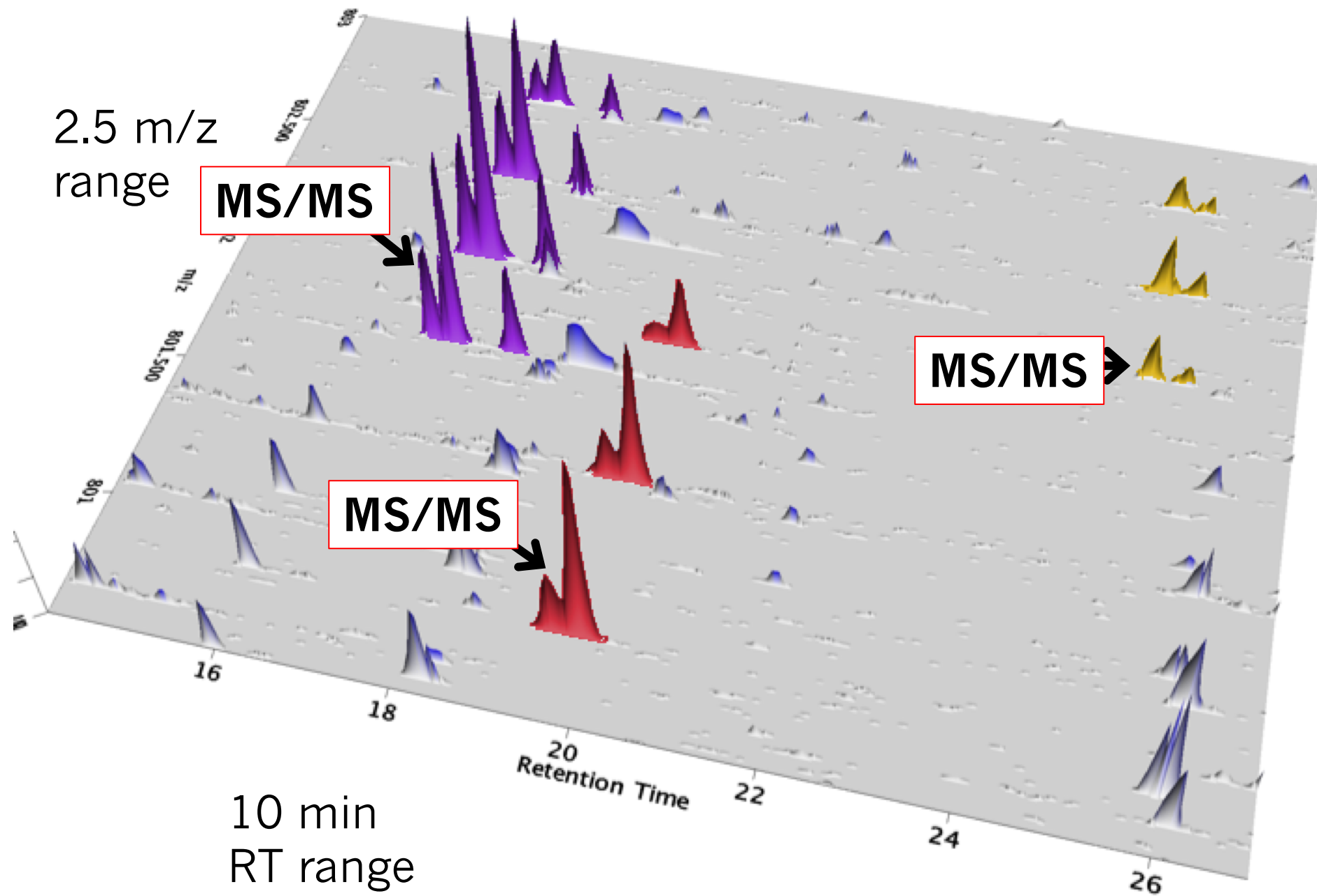
10 min
RT range

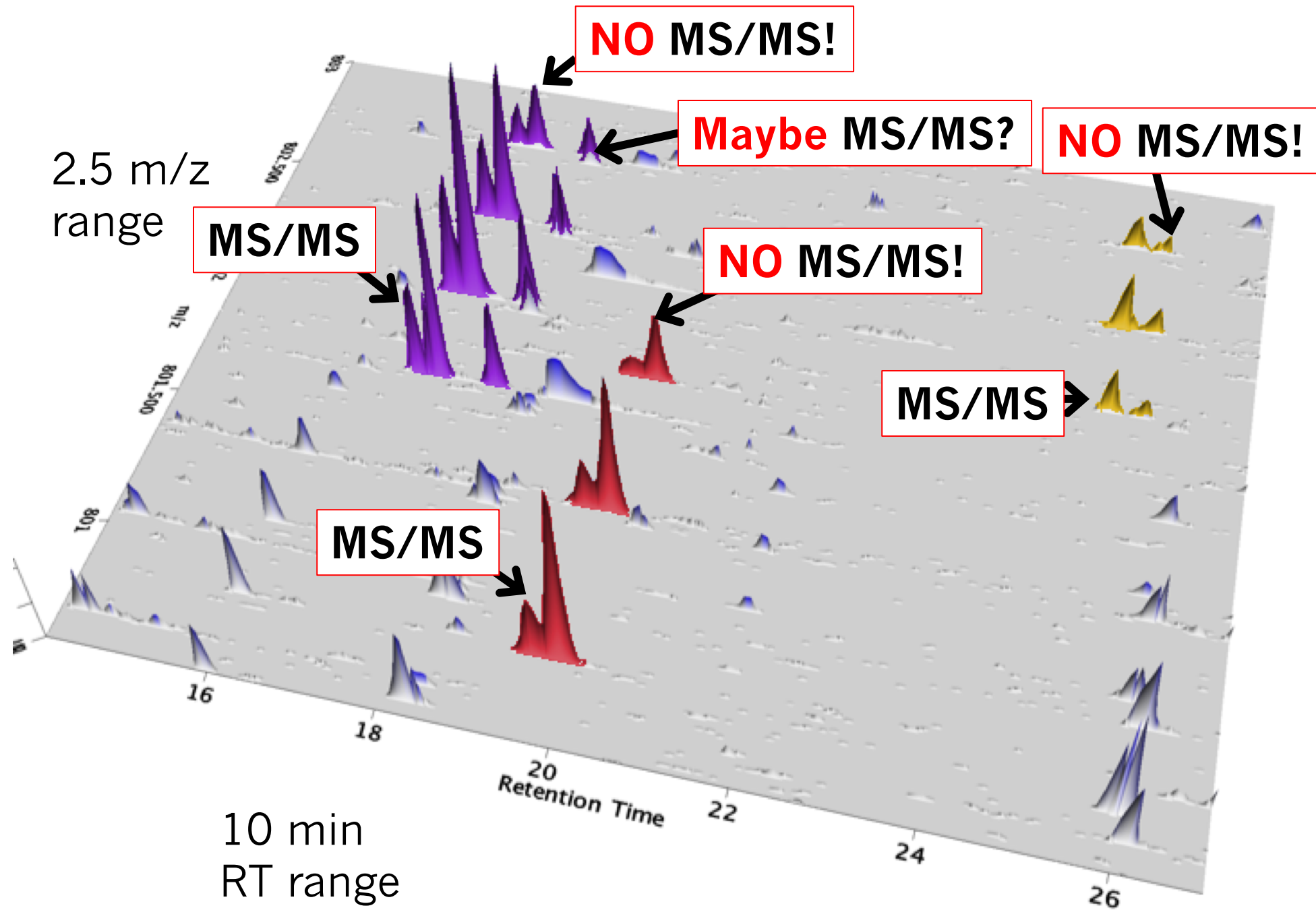
2.5 m/z
range

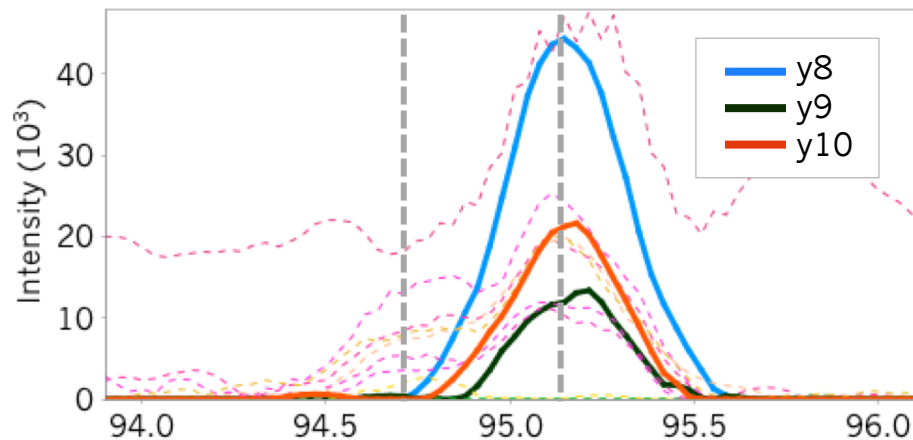
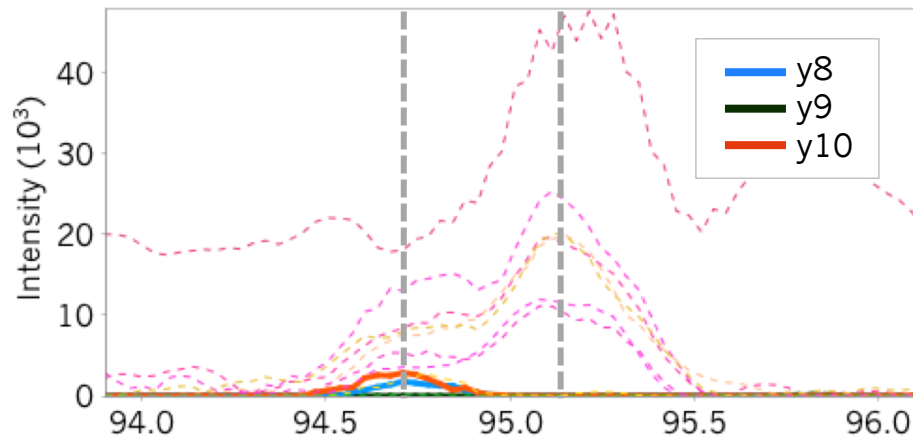
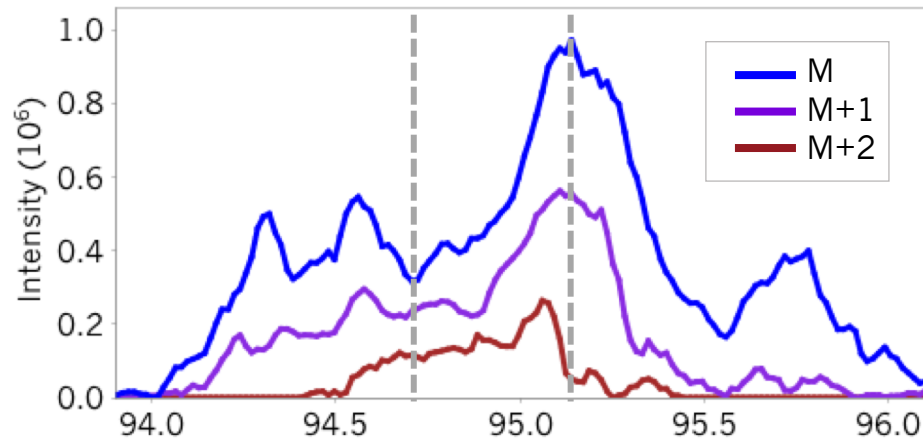


Most phosphopeptides exist in multiple localization states









Precursor Signal for
AITGASLADIMAK + (p)

y^{10}
 y^9
 y^8
 AIT

T

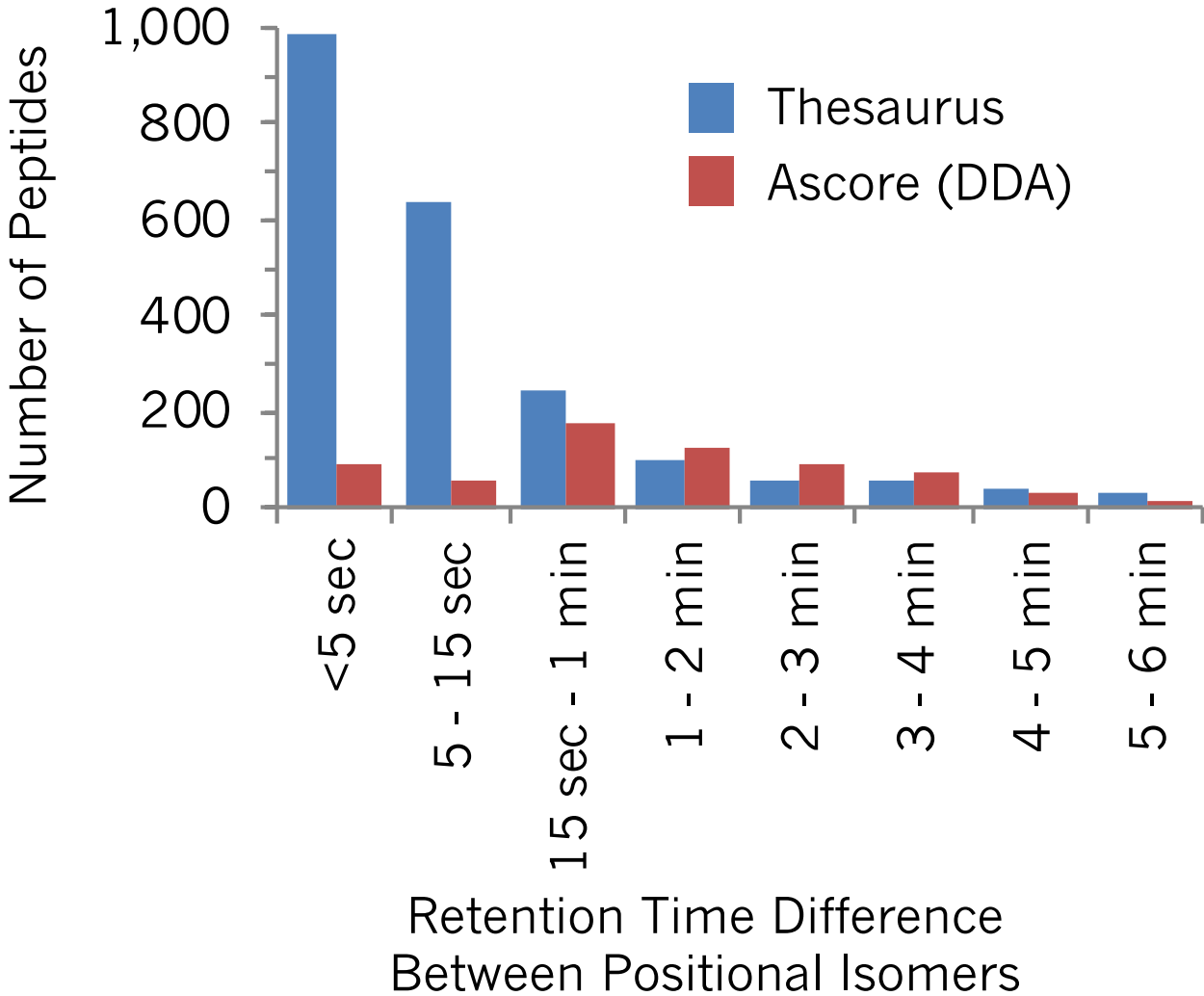
GASLADIMAK

y^{10}
 y^9
 y^8
 AITGAP

S

LADIMAK

Many positional isomers elute within 60 seconds of each other

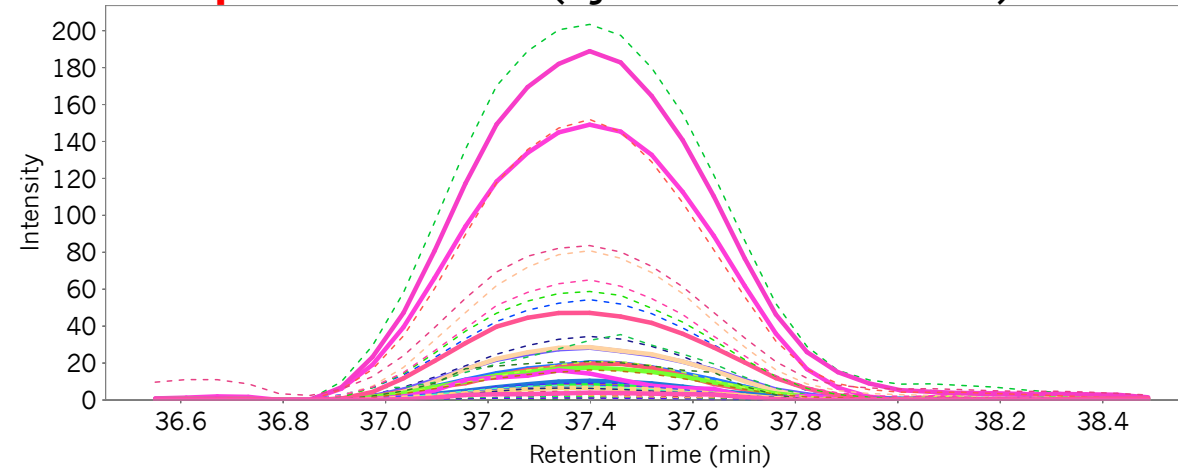


Some peptides rearrange in the gas phase

GIRP

S

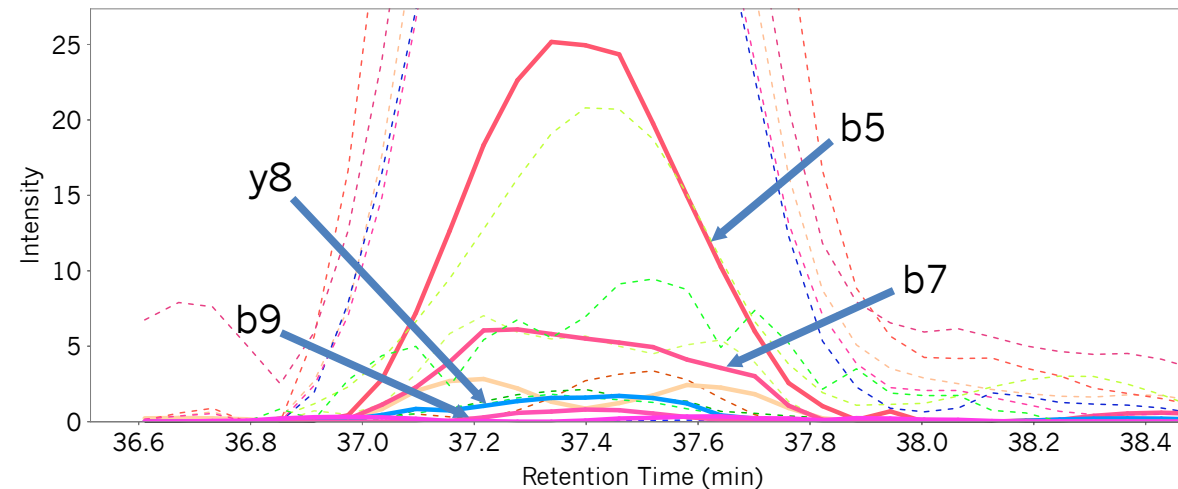
PLENSLHR (synthetic standard)



GIRPSPLEN

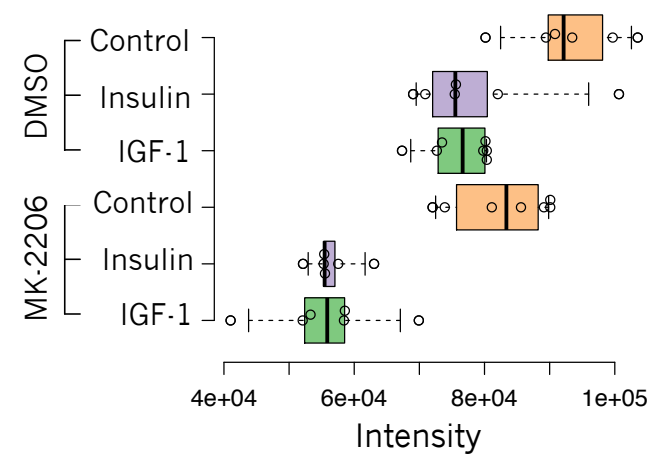
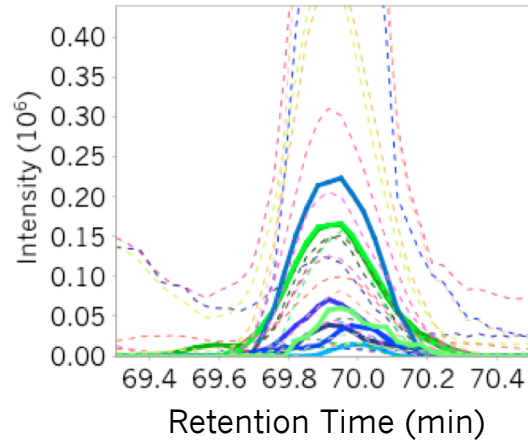
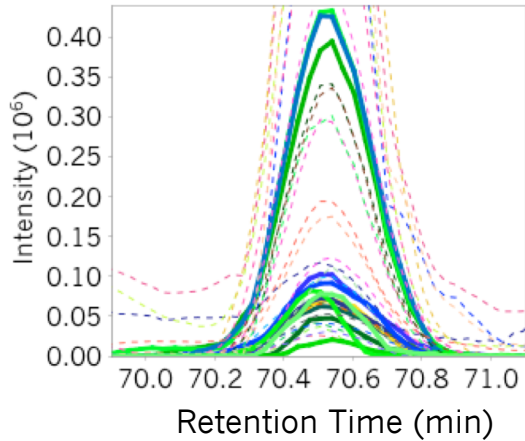
S

LHR (gas phase rearrangement)

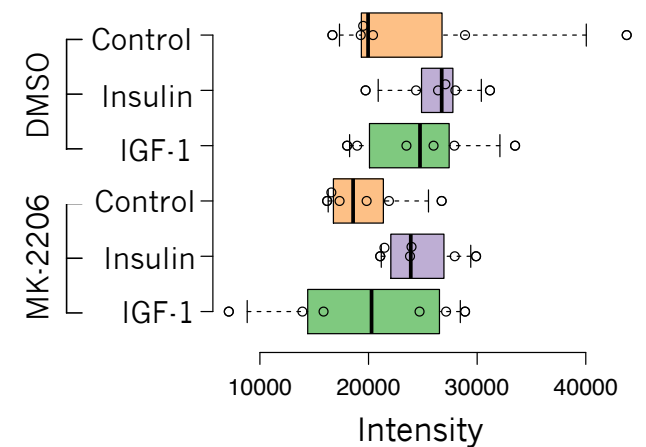
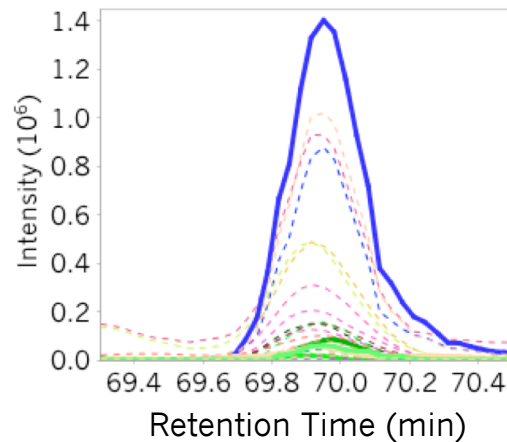
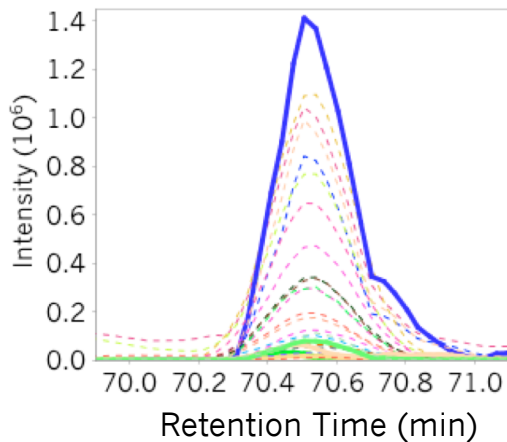


Some phosphopeptides don't resolve chromatographically

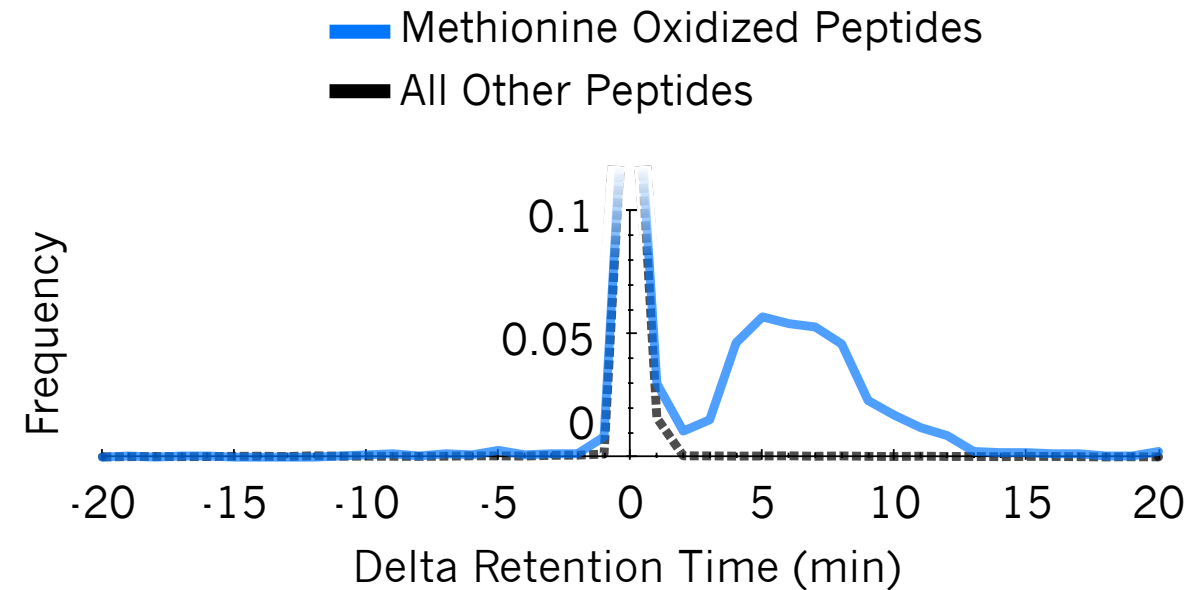
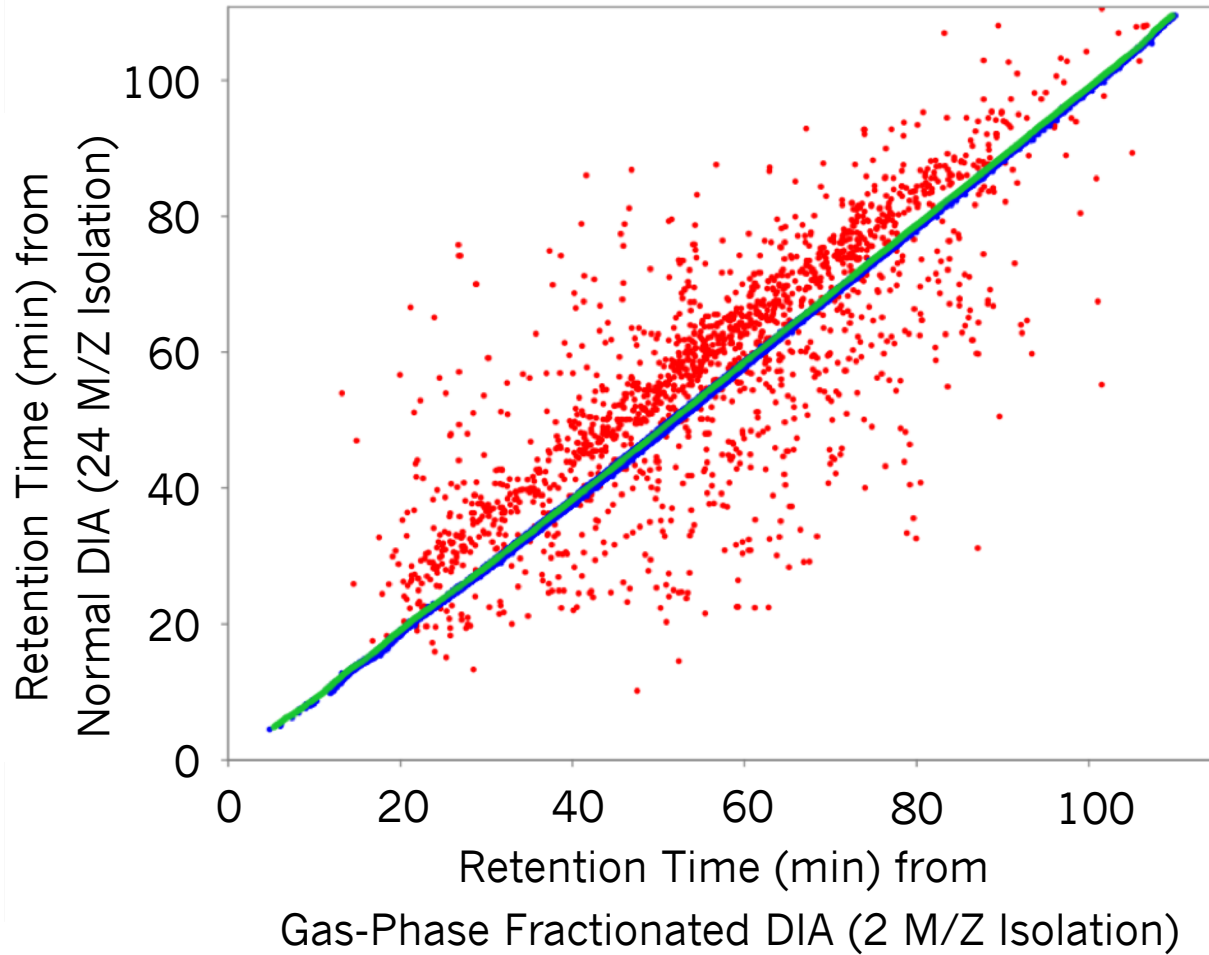
MARK3 S469 GIAPA**pS**PMLGNASNPKNKADIPER (FDR=3.6e-7)



MARK3 S476 GIAPASPMLGNA**pS**NPNKADIPER (FDR=0.5)

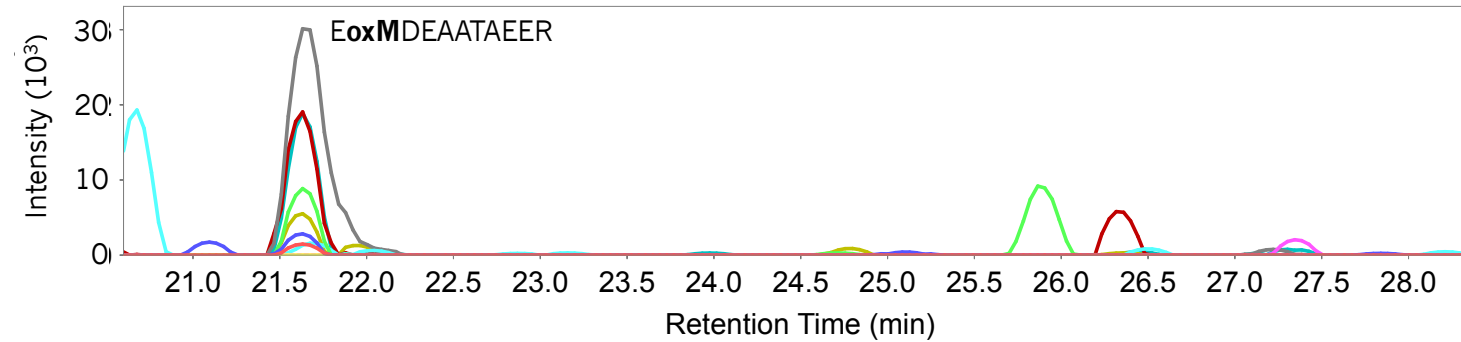


Unmodified peptides can masquerade as modified peptides

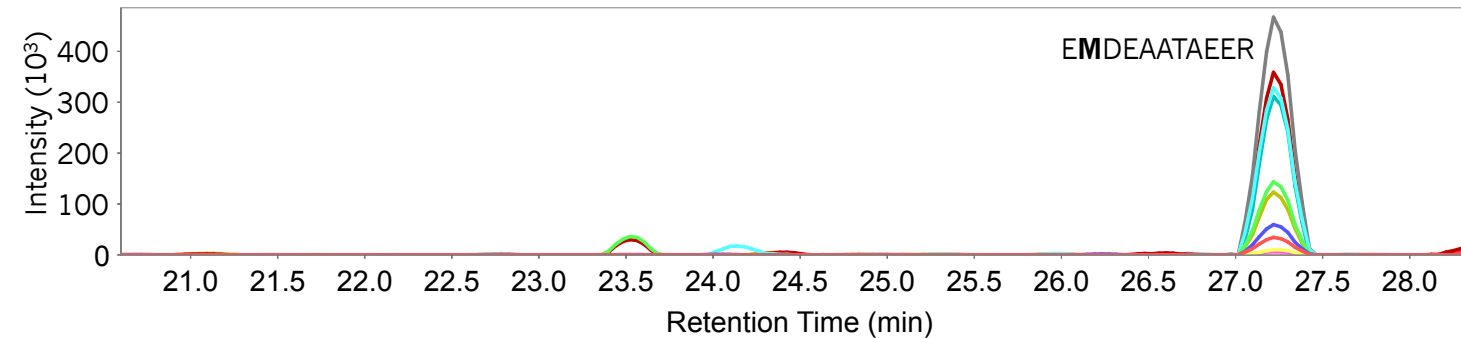


Modified peptides share fragment ions

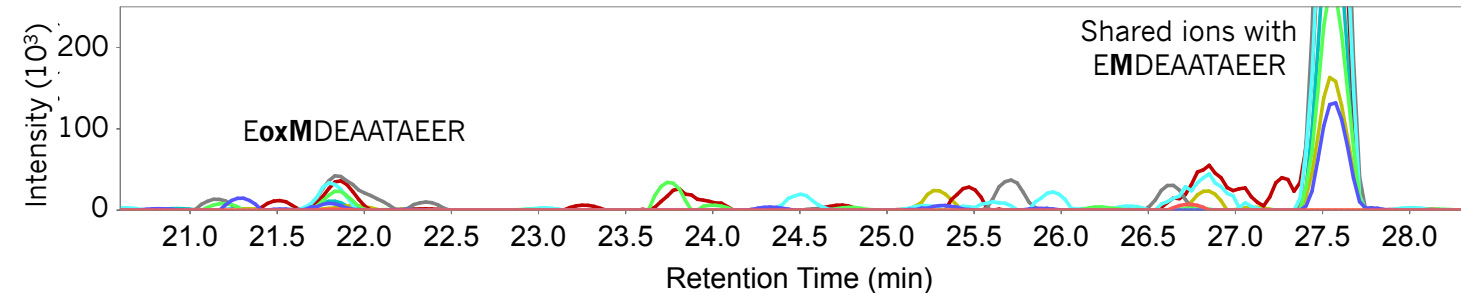
Ions assigned to EoxMDEAATAEER with 2 m/z precursor isolation



Ions assigned to EMDEAATAEER with 2 m/z precursor isolation

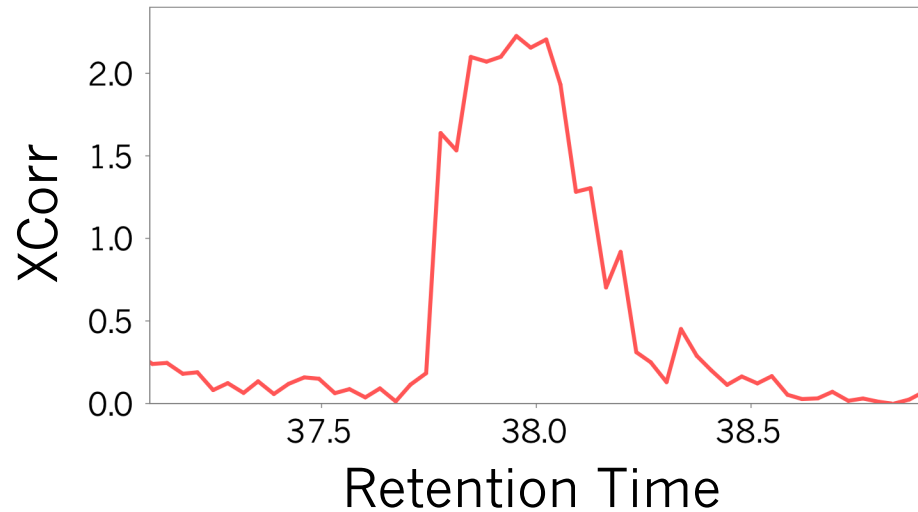
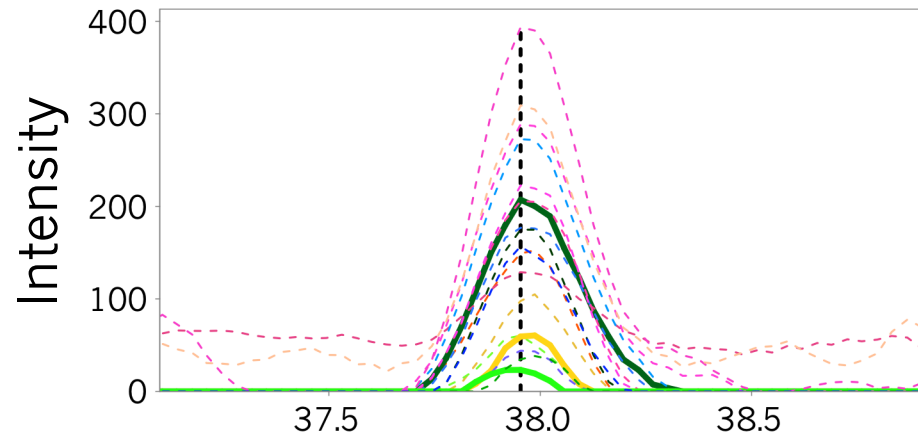


Ions assigned to EoxMDEAATAEER with 24 m/z precursor isolation

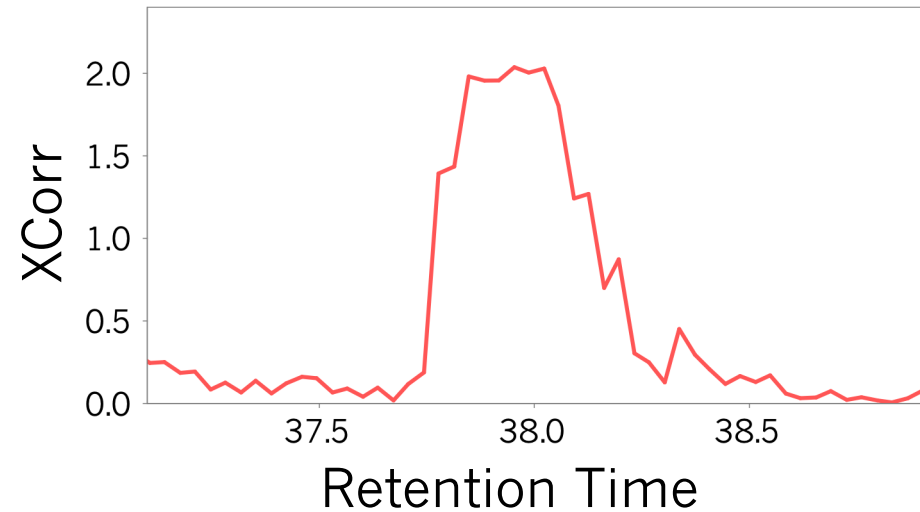
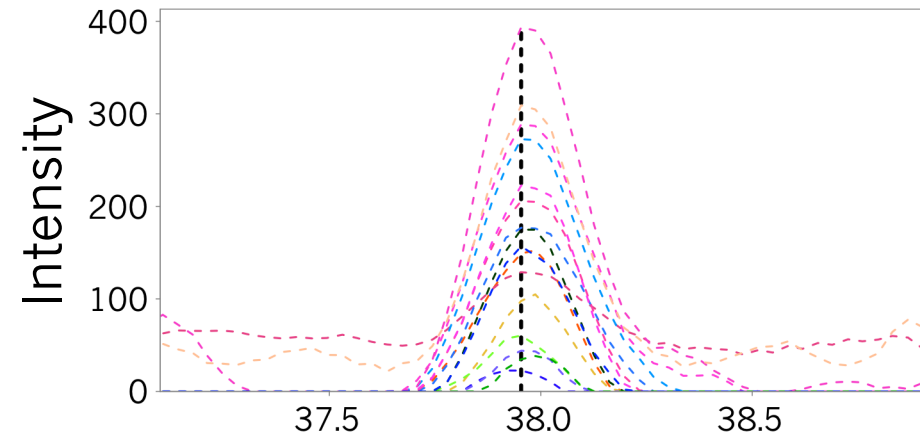


“Localization” is a problem for homologous peptides too

VAP**E**EH**P**VLLTEAPLNPK

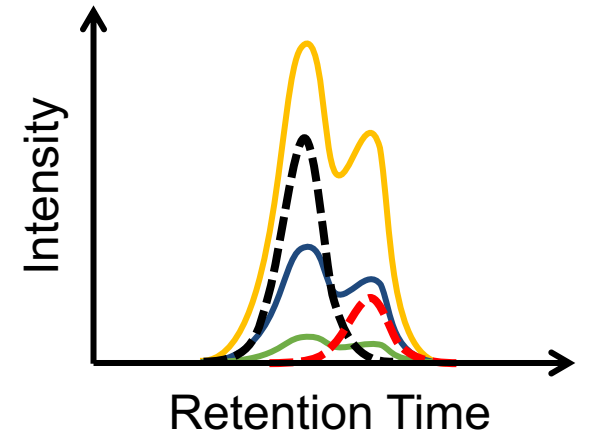
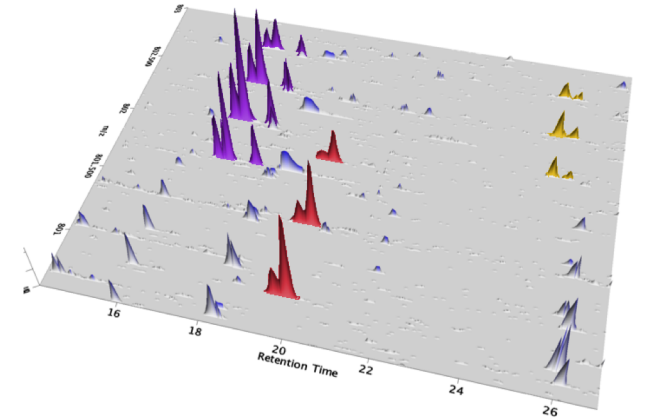


VAP**D**EH**P**I LLTEAPLNPK



Conclusions

- Phosphopeptide positional isomers are surprisingly common
- DIA may be the only way to reproducibly analyze phosphopeptides
- Modifications (and sequence variants) with small mass shifts can cause false positives and serious over reporting in DIA



Acknowledgements

Genome Sciences UW

Mike MacCoss

Jarrett Egertson

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Sonia Ting

Han-Yin Yang

Judit Villén

Rob Lawrence

Ariadna Llovet

Proteome Software

Susan Ludwigsen

Phillip Seitzer

Seth Just



Funding
F31:GM 119273

DIA PTM II : Identification and Quantification

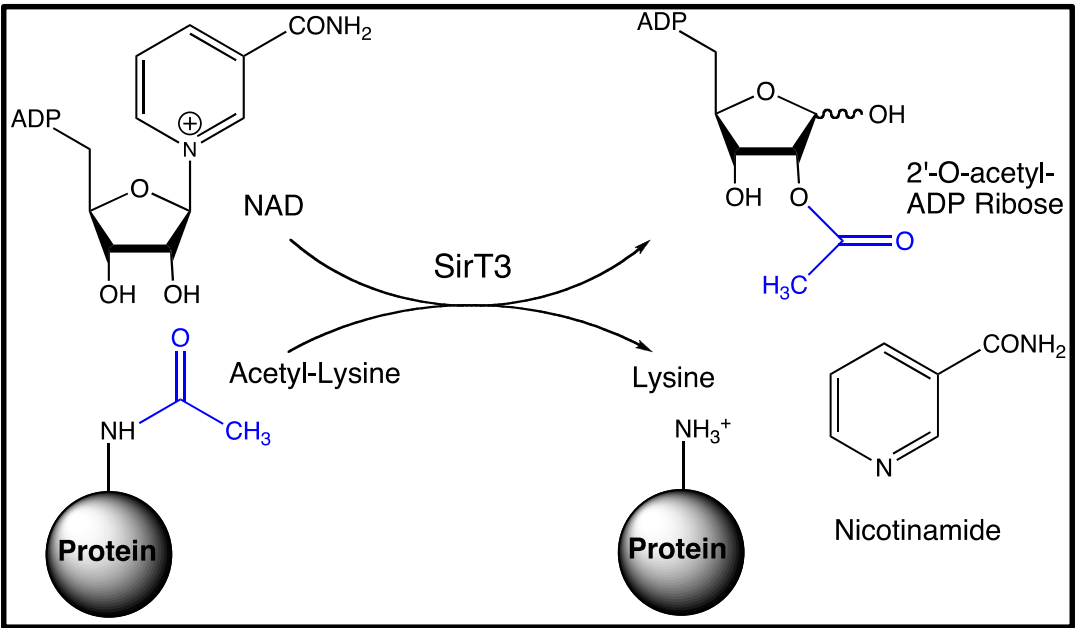
Birgit Schilling
June 4th, 2018



Data-independent acquisitions (DIA) and PTM Challenge


- PTM-Isoform chromatography - co-elution ?
- Typical DDA workflows use dynamic exclusion of precursor ions
- Site Localization within PTM-containing peptide
- Chimeric MS/MS spectra of isoforms
- PTM Crosstalk and DIA
- PTM site occupancy is often low, particularly for minor isoforms and some species may never be selected for MS/MS in DDA workflows

Mitochondrial Sirtuins - Deacetylases, Acetyltransferases and Metabolic Regulation



Diet stress: Diabetes, Metabolic Syndrome and Fatty Liver Disease

6 dietary regimens \times [acute (2 wk) + chronic (10 wk)] = 12 conditions



Mice	Water (2 wk)	Water (10 wk)	Water + Glc (2 wk)	Water + Glc (10 wk)	Water + Fru (2 wk)	Water + Fru (10 wk)
Chow	5 mice	5 mice	5 mice	5 mice	5 mice	5 mice
HFD Chow	5 mice	5 mice	5 mice	5 mice	5 mice	5 mice

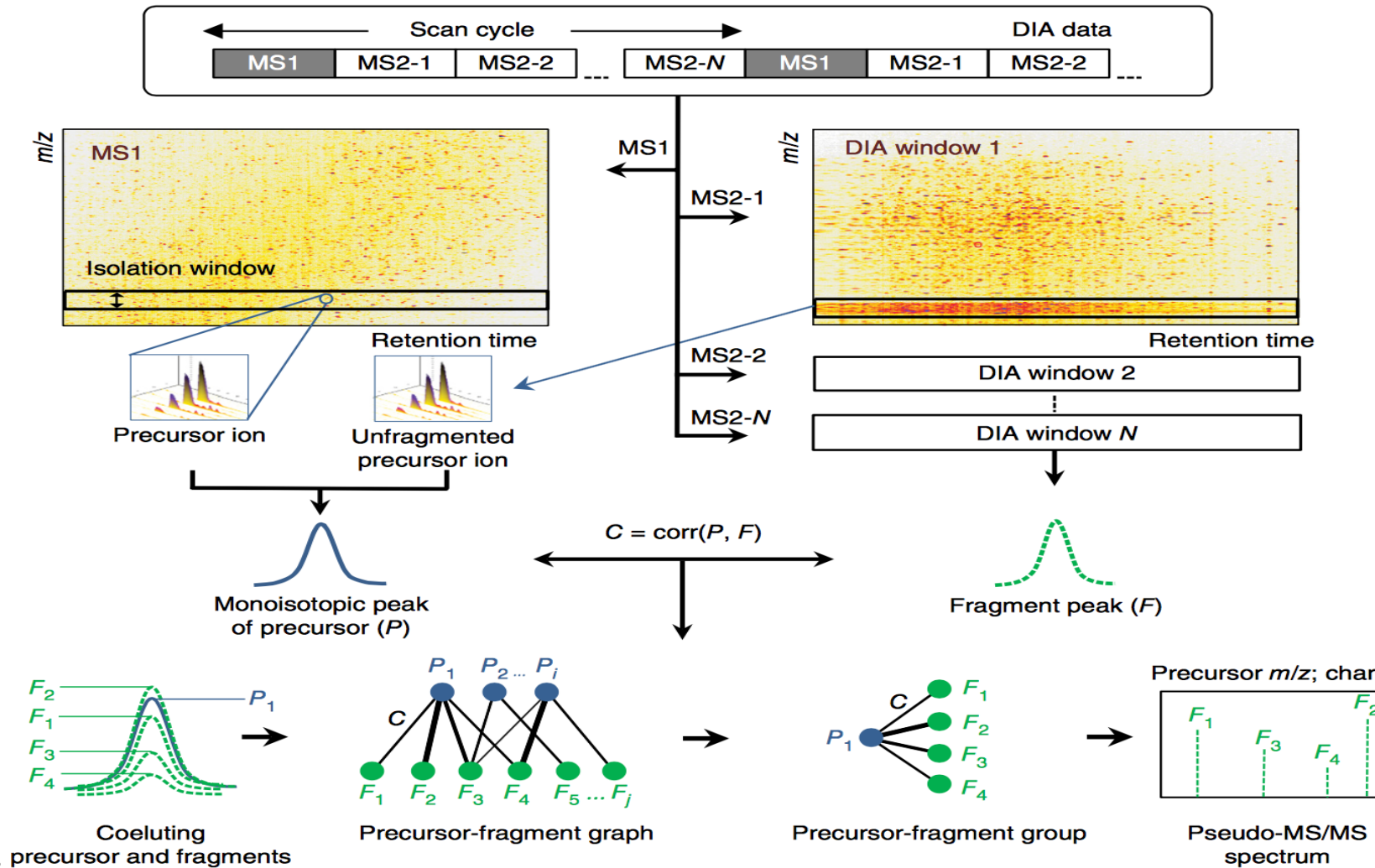
Remodeling of the Mitochondrial Acyl-Proteome

- Protein expression changes
- Acetylation (after enrichment)
- Succinylation (after enrichment)

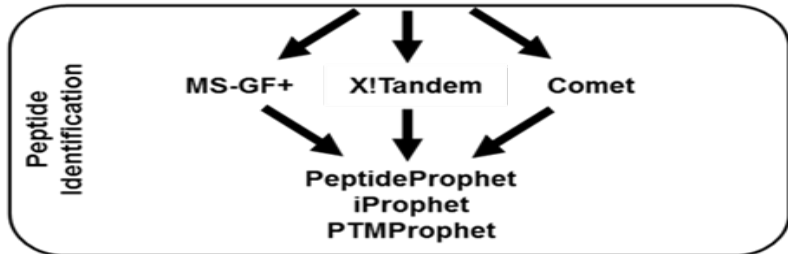
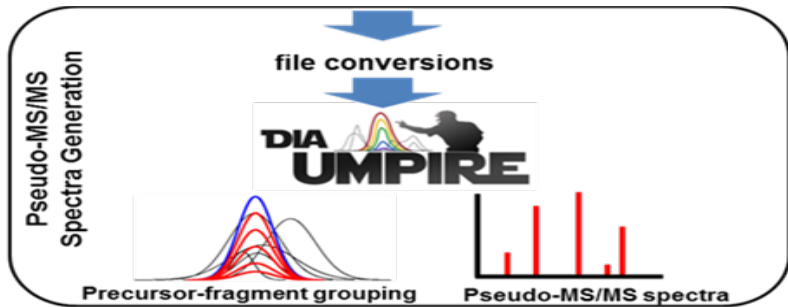
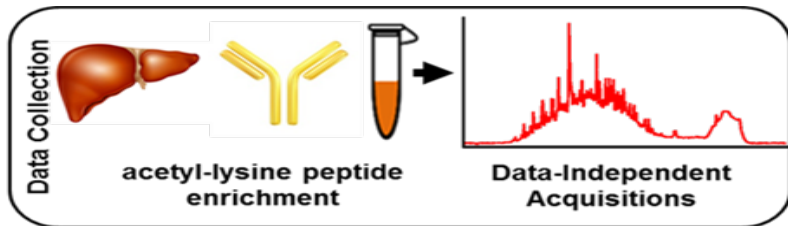


Challenge: How can we quickly, accurately, and comprehensively quantify changes in protein acylation?

DIA-Umpire

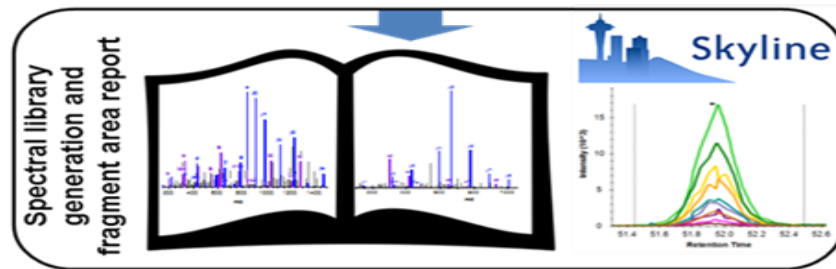


PIQED: Identification & Quantification of PTM using exclusively DIA



“PIQED: Automated PTM Identification and Quantification using Exclusively Data-Independent Acquisition”

Meyer et al.,
Nature Methods, 2017



Workflow for fast & accurate Quantification of PTM

PIQED Graphical User Interface

Get Input

DIA_UMPIRE

General Info

Enter input directory (only wiff files)

Enter output directory (all other files)

Enter number of threads

Enter amount of ram

Click to run

Path to AB_SCIEX_MS_Converter.exe

Path to msconvert.exe

Path to indexmzXML.exe

Path to DIA_Umpire_SE.jar

Path to DIA-Umpire signal extraction .params

DATABASE SEARCH

Click to run

MSGFSearch Info

Path to msgfplus.jar

Path to .fasta file

Enter ##ppm

Enter enzyme (-e)

Path to mods .txt file

Enter ntt (-ntt)

Enter ti (-ti)

Click to run

Path to tandem.exe

Path to tandem2xml.exe

Path to _params.xml file

Path to the taxonomy.txt (or .xml)

Click to run

Path to .fasta file

Path to comet.exe

Path to .params file

PROPHET + SKYLINE

Click to run

Peptide Prophet and IProphet Info

Path to xinteract.exe

Path to InterProphetParser.exe

Click to run

Path to PTMProphetParser.exe

Enter the masses in this format: [residue:mass,residue:mass]

Enter fragment mass tolerance (Da)

Enter minimum iProphet score (MINPROB)

Click to run

Path to SkylineRunner.exe

Enter a report name to use in Skyline

Path to Skyline .fasta file

Path to Skyline template document

Path to PTMprophet pep.xml file (leave empty for default)

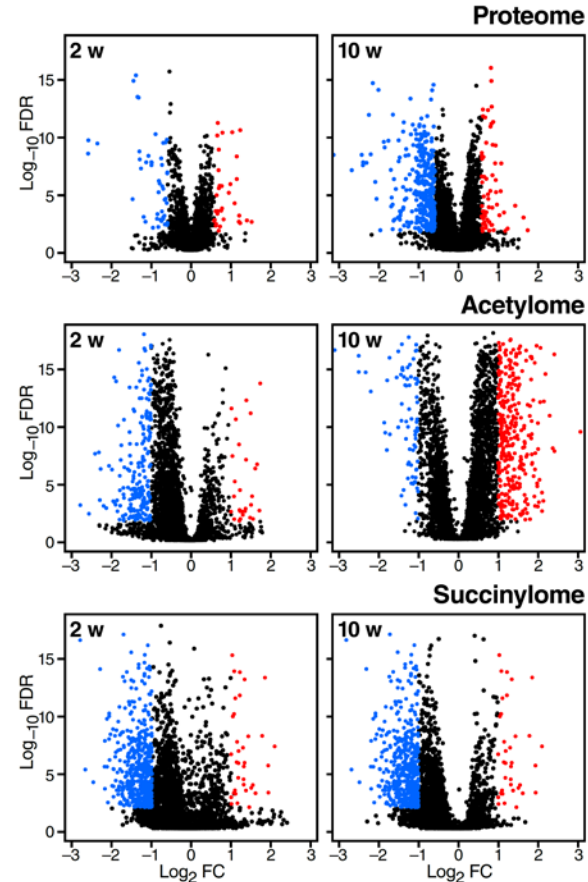
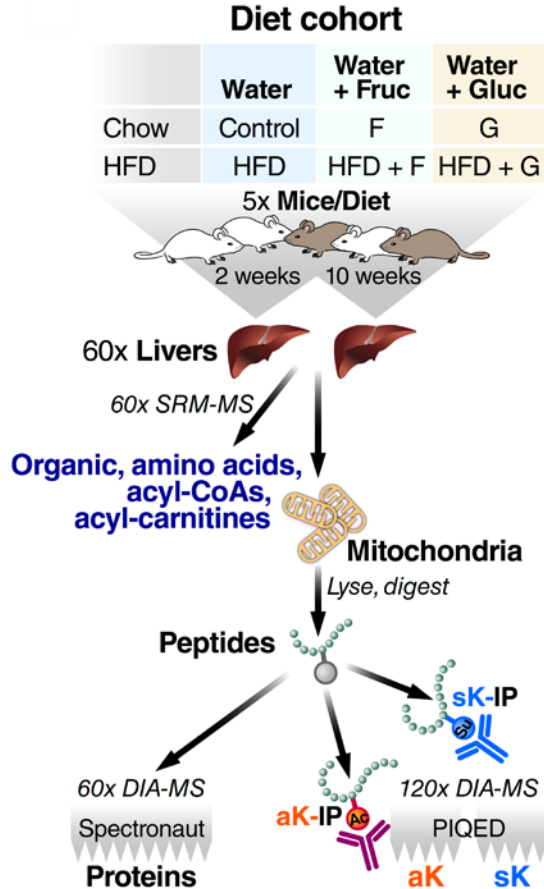
Click to run

Enter the labels each separated by a comma

Clear Default Browse Load Save

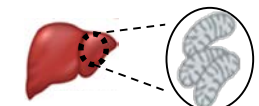
Exit Finished

Mouse Diet Study - Workflow and Data Overview

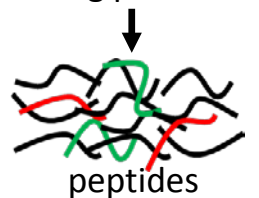


MULTIPLE-PTM Affinity Enrichment – One Pot for PTM cross-talk

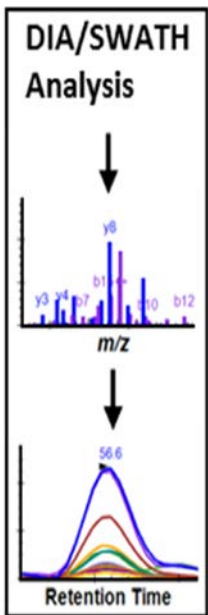
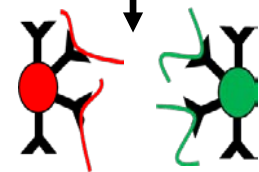
mito isolation
trypsin digestion



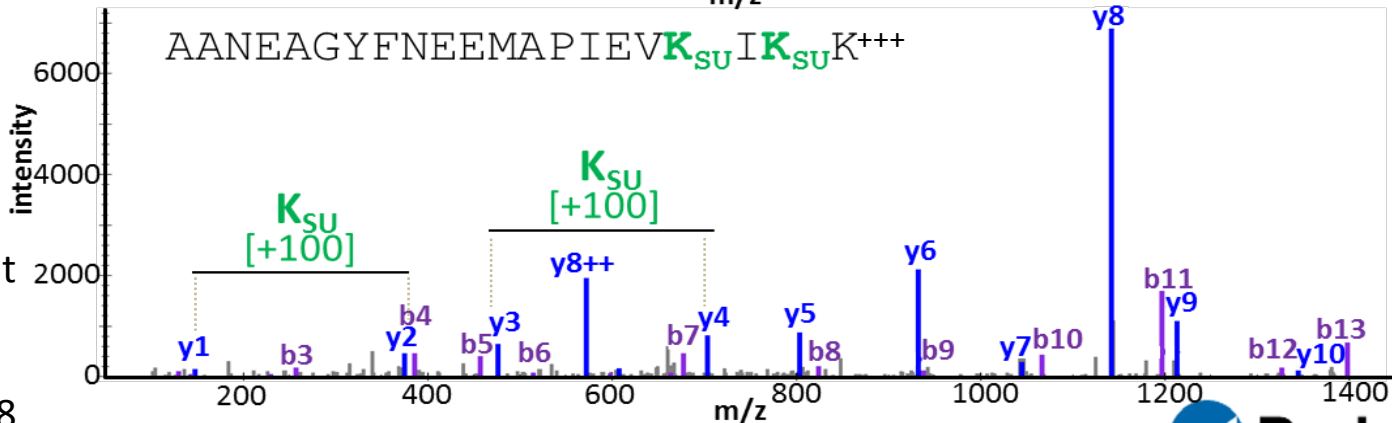
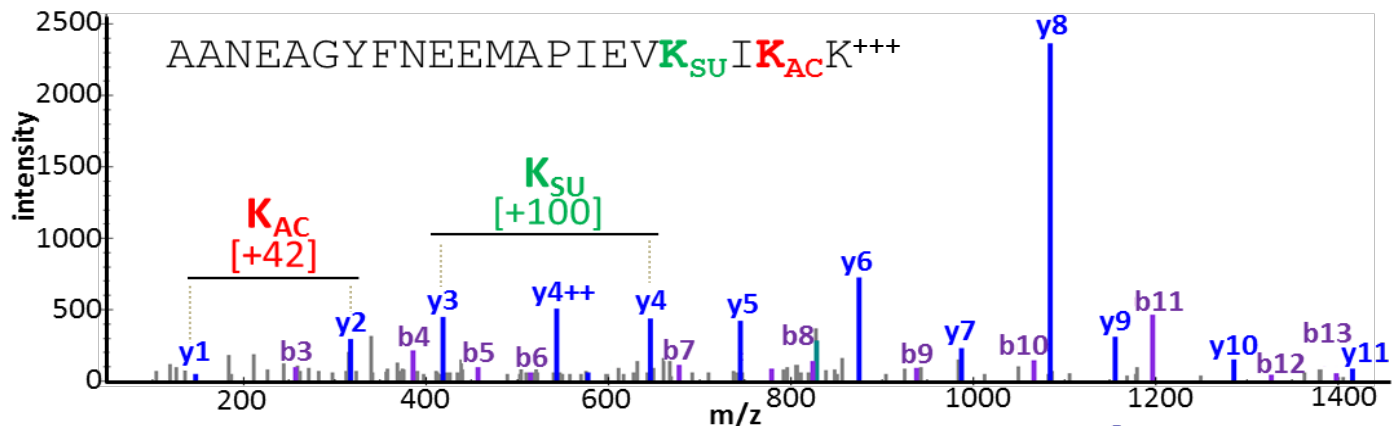
1mg protein



peptides



3-ketoacyl-CoA thiolase, mitochondrial



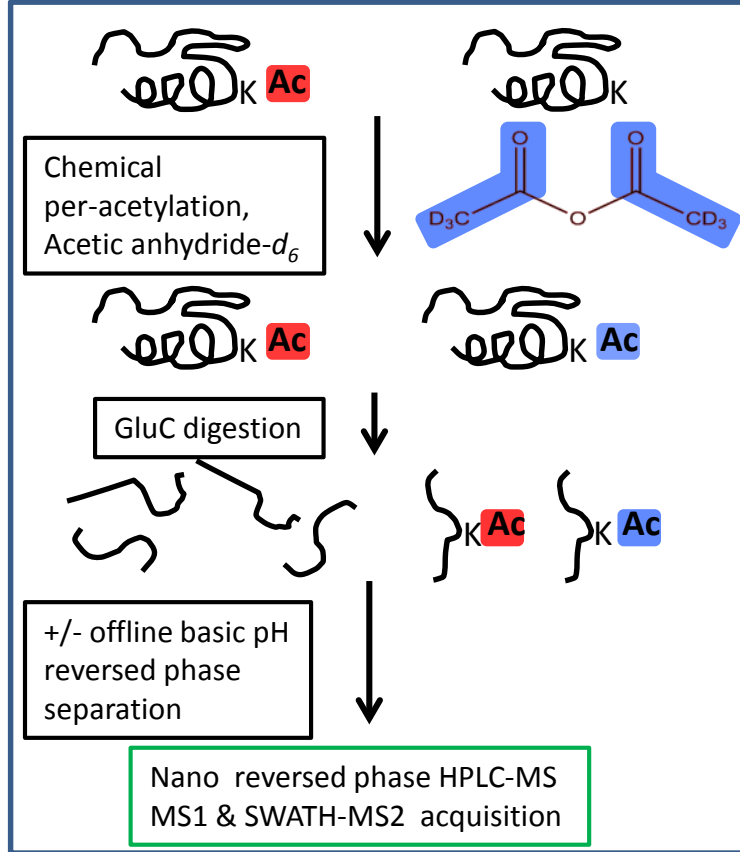
Combined affinity enrichment

Combination with PIQED

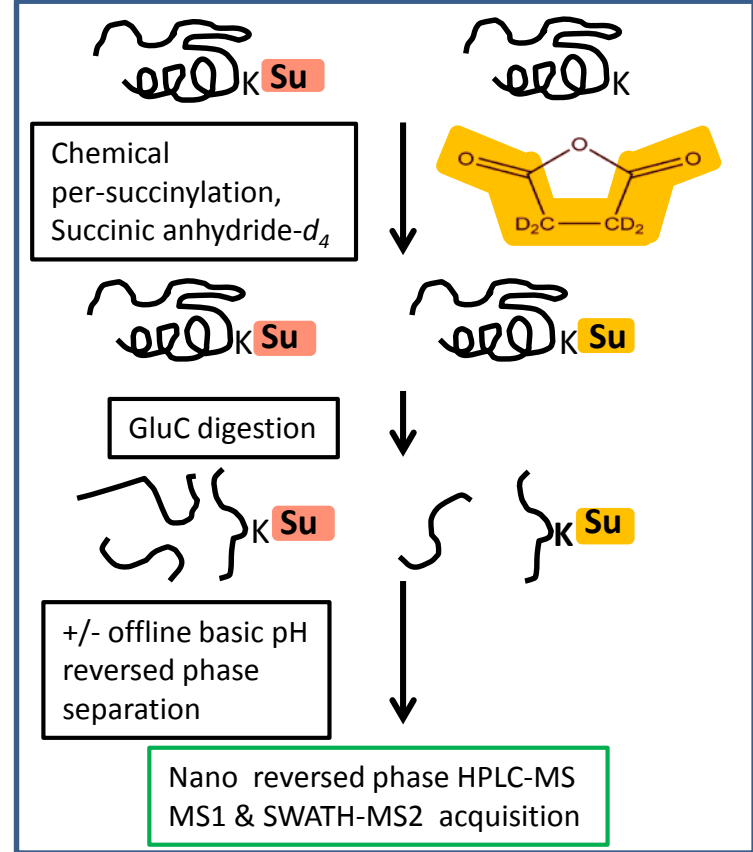
Basty et al., in revision 2018

Stoichiometry chemical labeling strategy and workflow

A

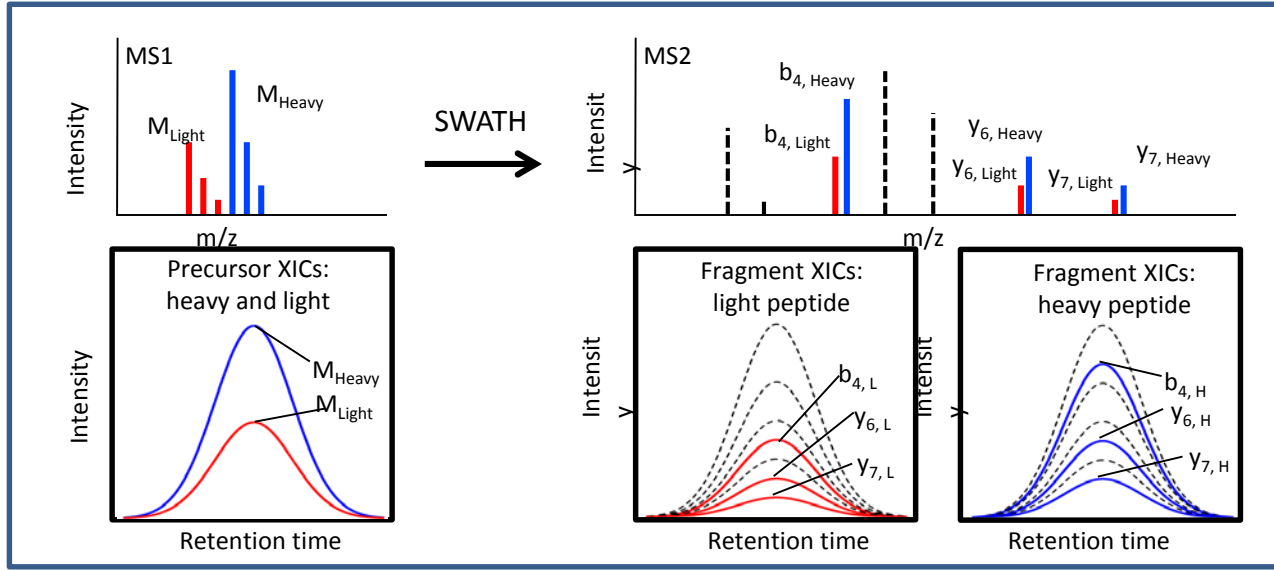


B



Stoichiometry chemical labeling strategy and workflow

C



Only differentiating MS2 ions are used for occupancy calculations

- MS1 light: **red**
- MS1 heavy: **blue**

- MS2 differentiating ions for **light** peptide **in red**
- MS2 differentiating ions for **heavy** peptide form **in blue**
- MS2 ions that are common in **light** and **heavy** peptide pair are **in black** (broken line)

A large, modern, curved building with a light-colored stone facade and a prominent triangular roof section. The building has multiple rows of windows and a large glass entrance area. The foreground shows a paved area and some landscaping.

Schilling Lab

J.G Meyer, N. Basisty, L. Wei, T. Payne, A. Holtz

Alexey Nesvizhskii (U Michigan); Hanno Steen (Harvard)

R. Kahn, S. Softic (Harvard, Joslin Diabetes)

E. Verdin (Buck), C. Newgard (Duke)

Christie Hunter (SCIEX), Brendan MacLean (UW)

Financial Support from:

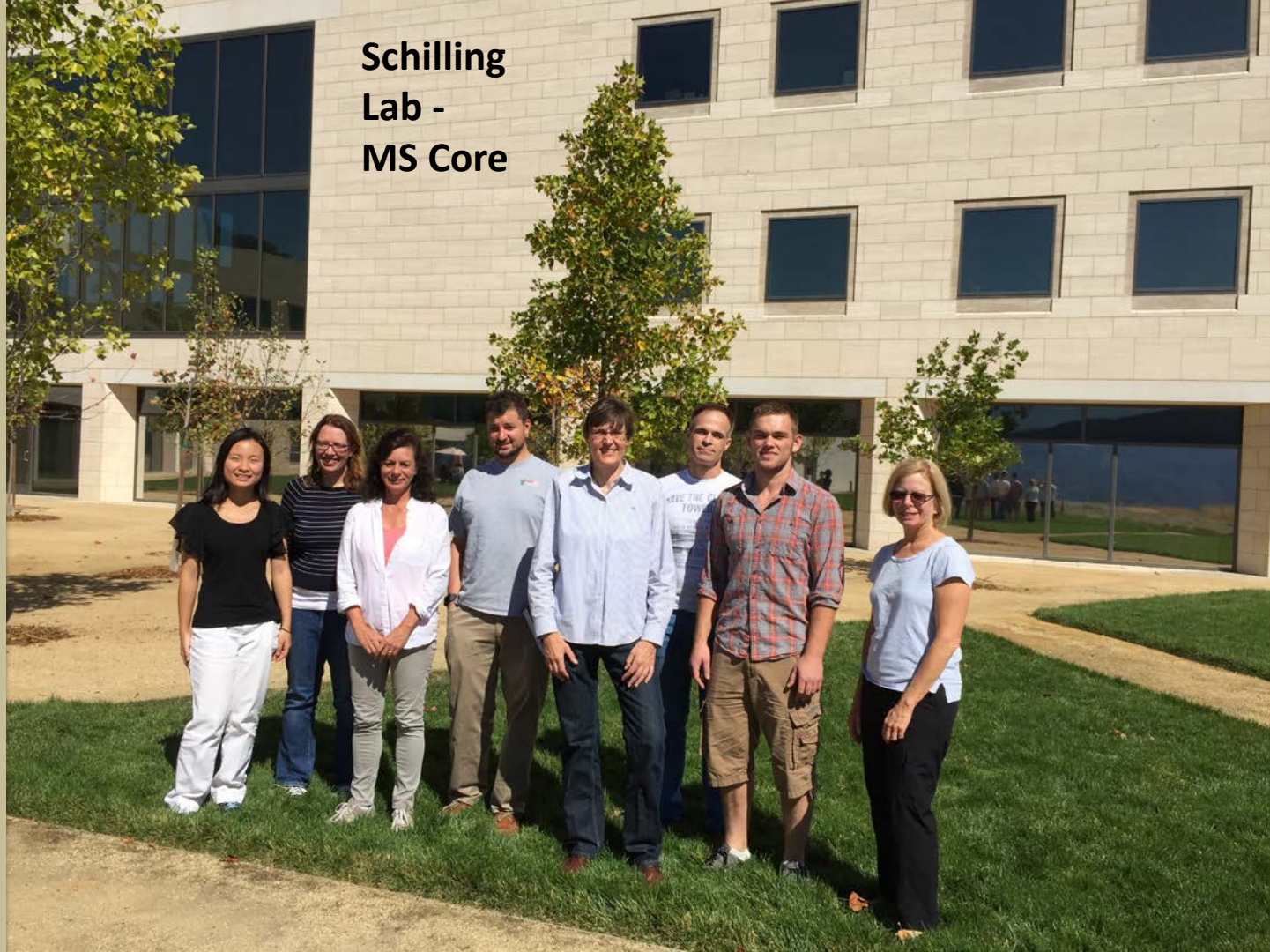
Buck Institute; NIH and NIA

U. Washington, Seattle, Pilot Award Shock Center



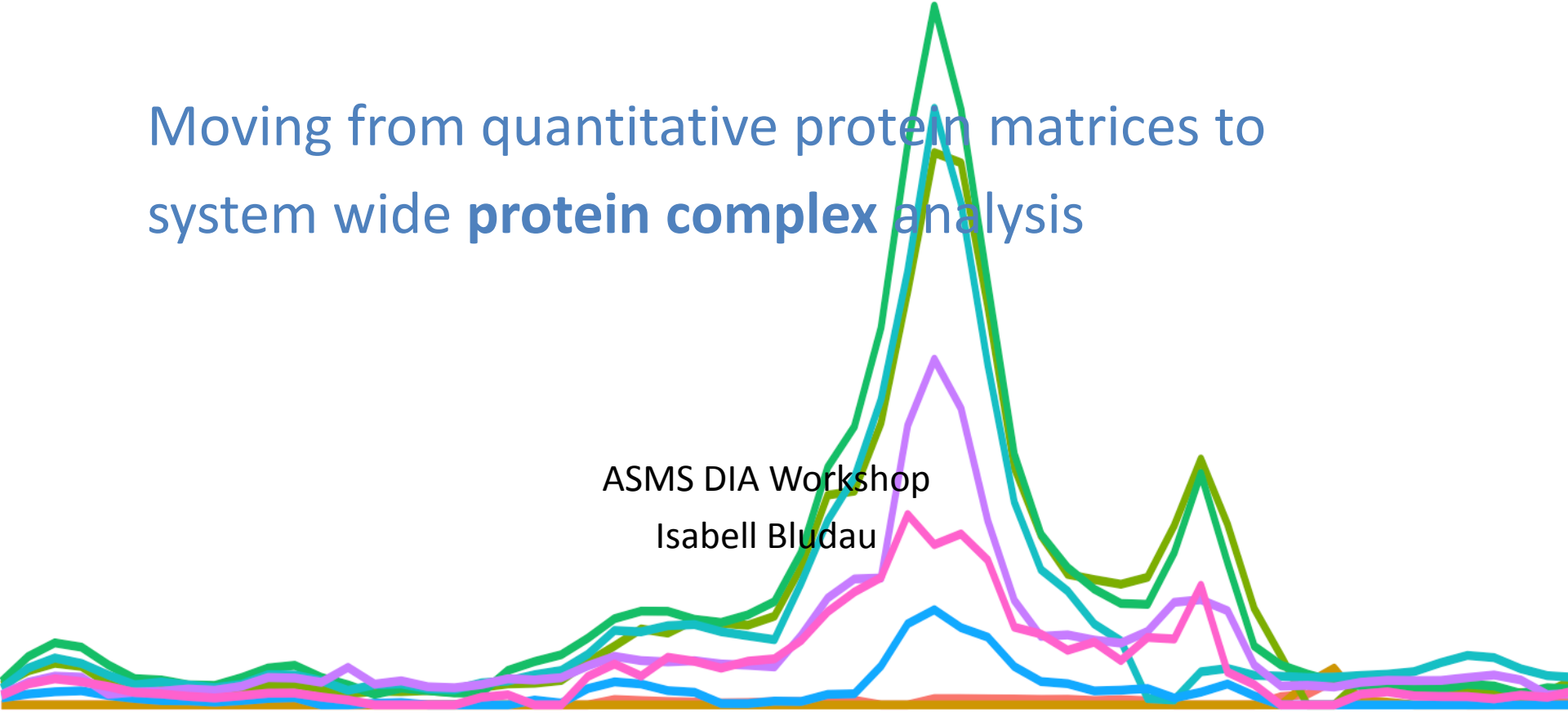
Live better longer

**Schilling
Lab -
MS Core**



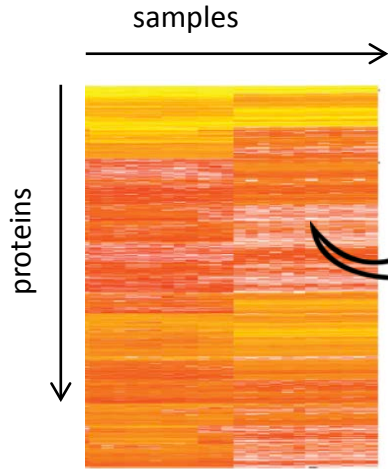
Moving from quantitative protein matrices to system wide **protein complex** analysis

ASMS DIA Workshop
Isabell Bludau

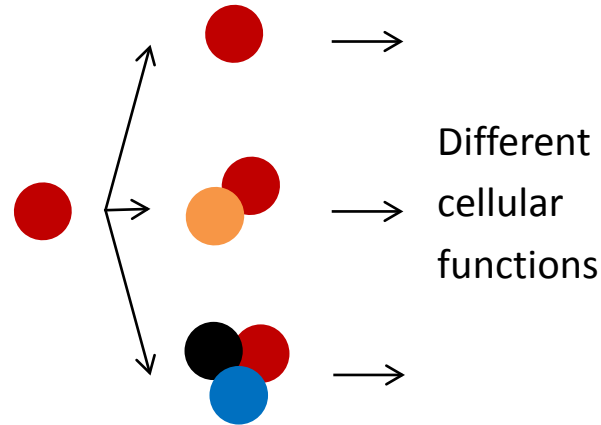


Proteins form multi-protein complexes

Protein identification and quantification



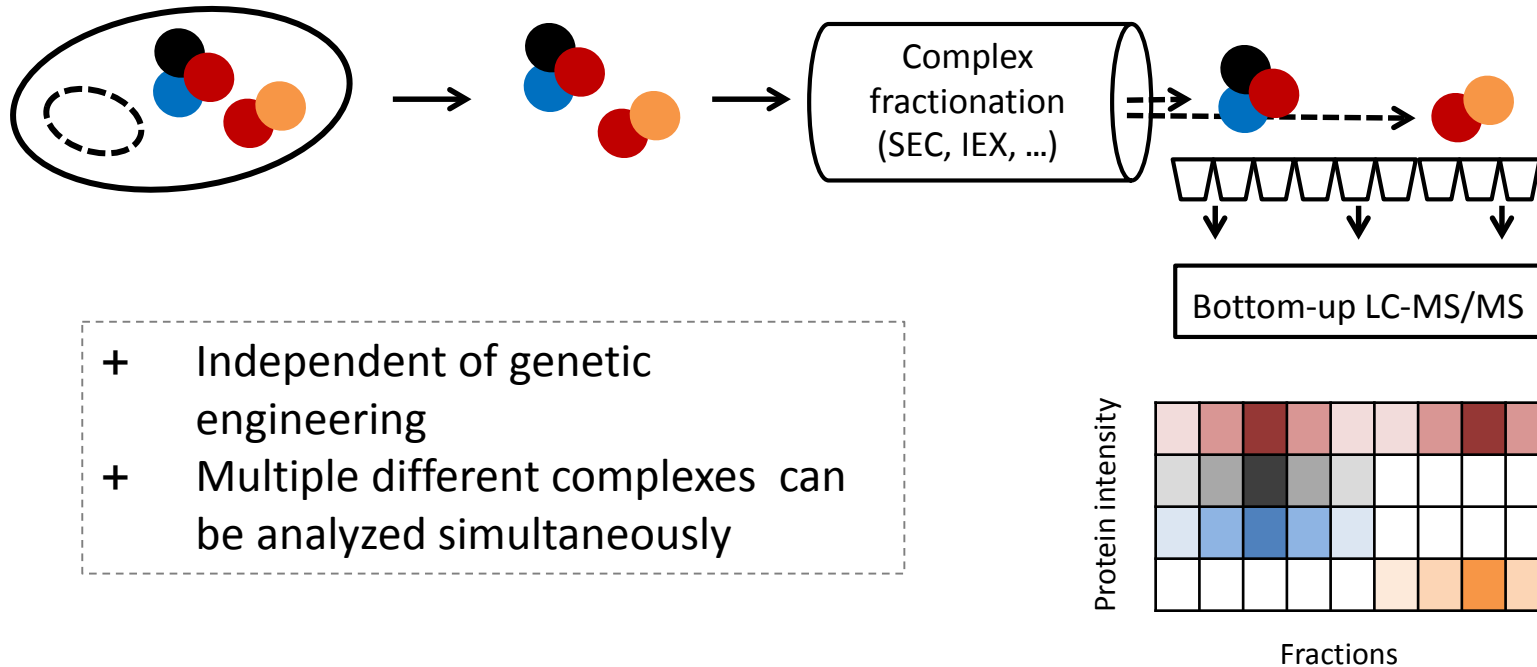
Many proteins interact to perform specific functions



Protein complex identification and quantification

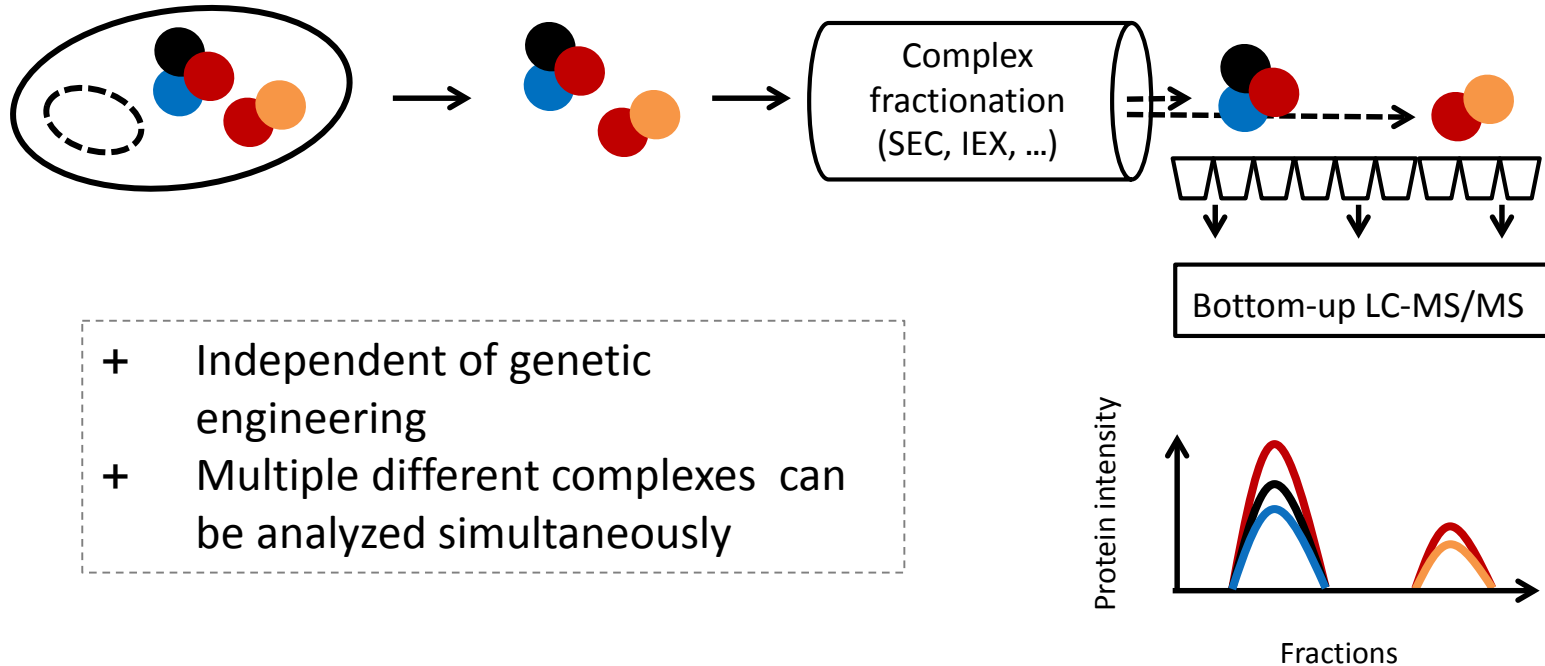
- Functional Complementation
- CO-IP & Western-Blotting
- Affinity Purification & Mass Spectrometry
- **Protein Correlation Profiling**

Protein correlation profiling

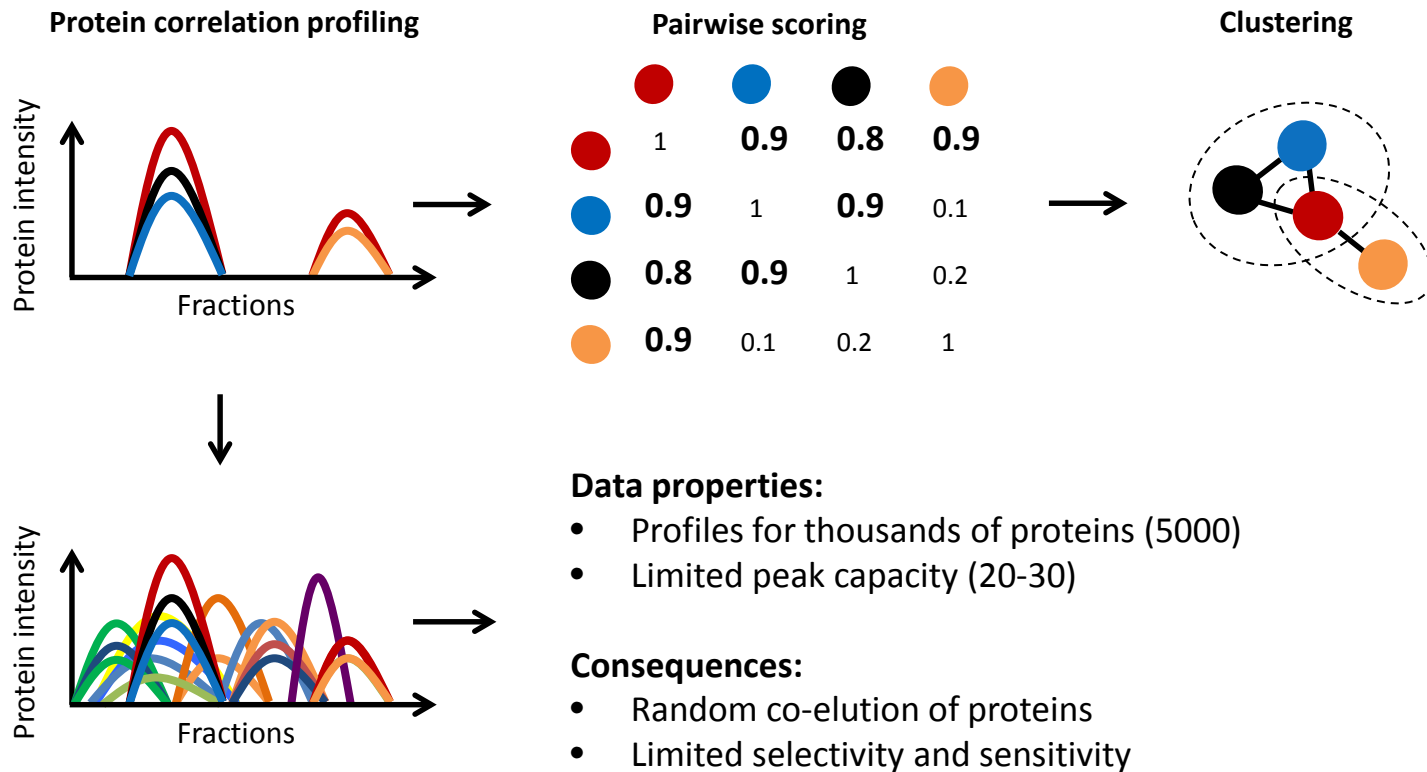


- + Independent of genetic engineering
- + Multiple different complexes can be analyzed simultaneously

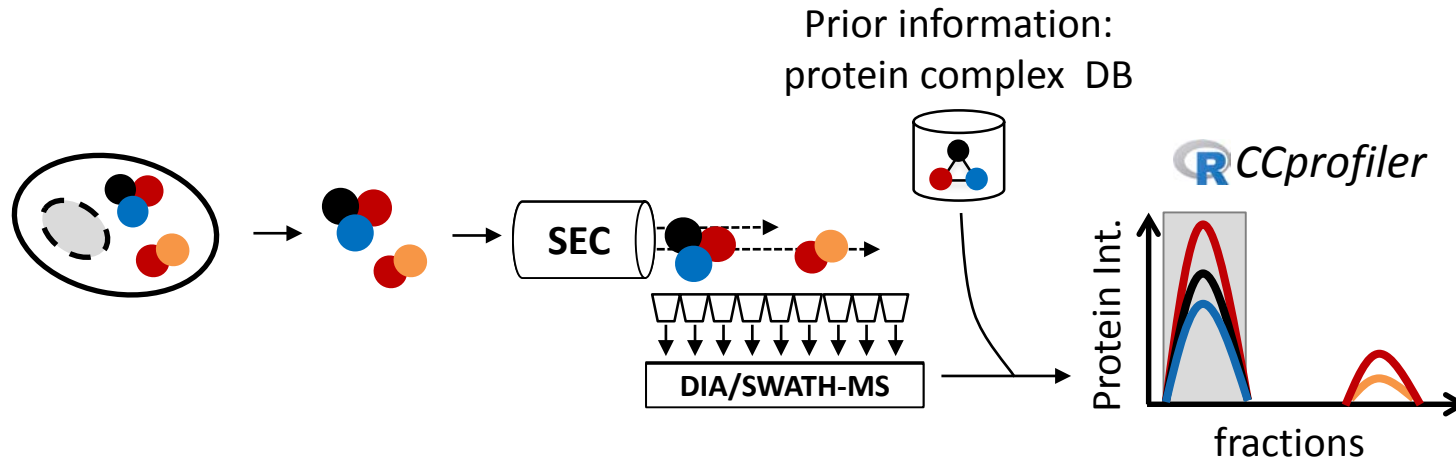
Protein correlation profiling



Established analysis strategy



Complex-centric proteome profiling by SEC-SWATH-MS



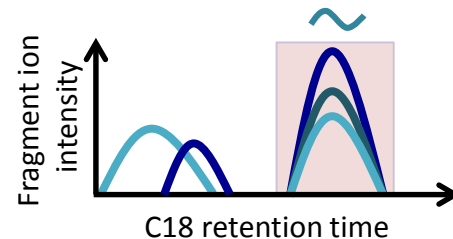
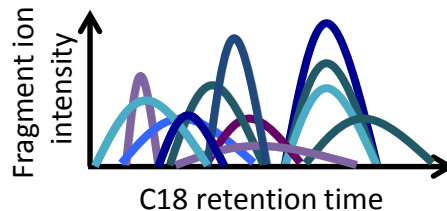
- ✓ **SEC** high resolution, 60-80 fractions, molecular weight calibration
- ✓ **DIA/SWATH-MS** highly complete quantitative matrix
- ✓ **Complex-centric analysis** increased sensitivity and selectivity in protein complex detection

Complex-centric proteome profiling

an extension of peptide-centric DIA analysis

Peptide-centric
DIA analysis

Spectral library with
specific peptide query
parameters

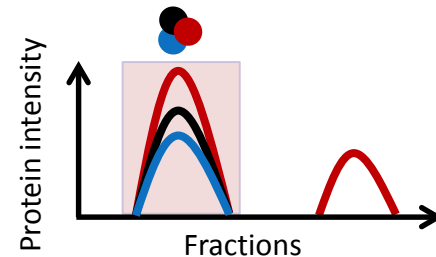
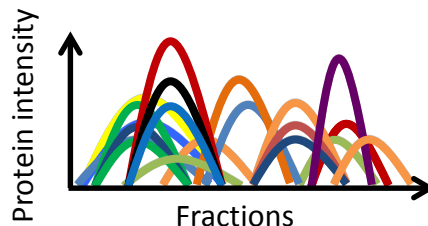


Complex-centric
proteome profiling

Prior knowledge



e.g. complex database
(CORUM), PPI network
(BioPlex, StringDB)



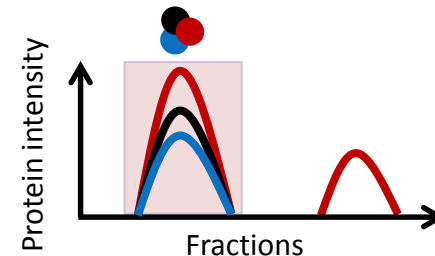
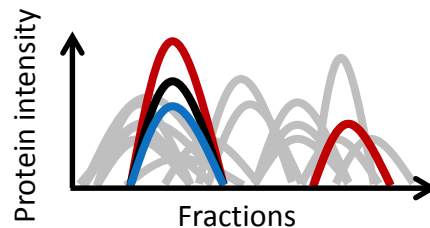
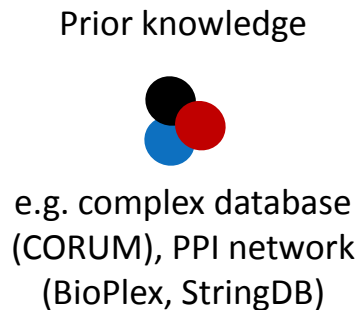
Complex-centric proteome profiling

an extension of peptide-centric DIA analysis

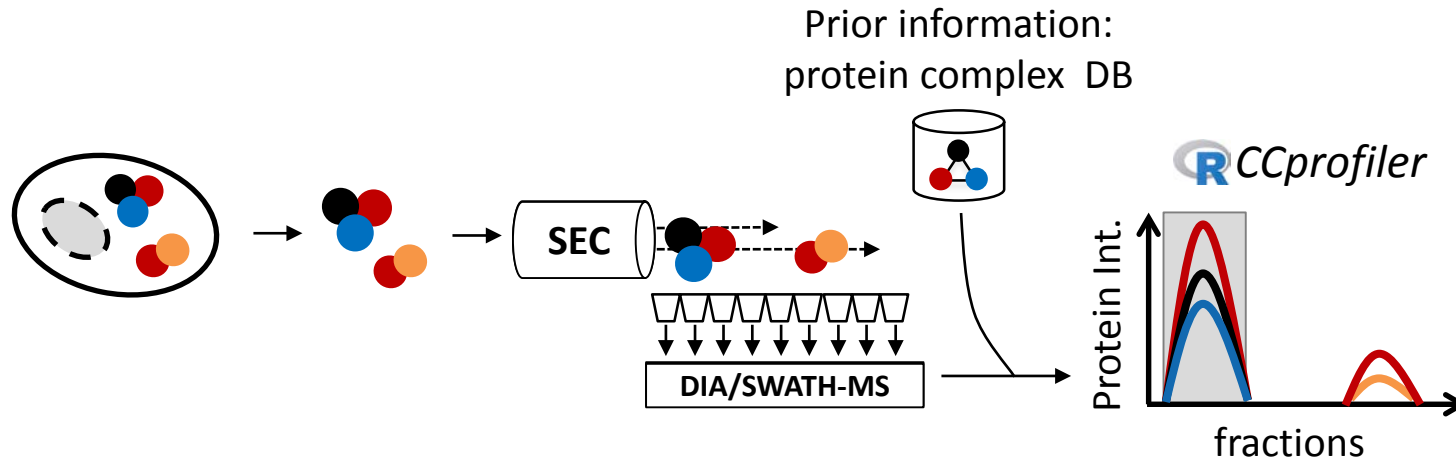


<https://github.com/CCprofiler/CCprofiler/>

Complex-centric
proteome profiling



Complex-centric proteome profiling by SEC-SWATH-MS

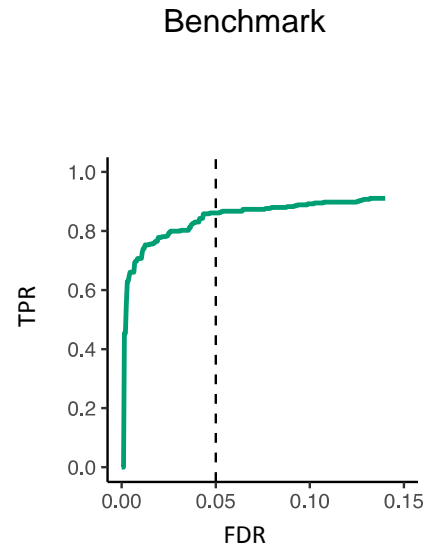


- ✓ **SEC** high resolution, 60-80 fractions, molecular weight calibration
- ✓ **DIA/SWATH-MS** highly complete quantitative matrix
- ✓ **Complex-centric analysis** increased sensitivity and selectivity in protein complex detection

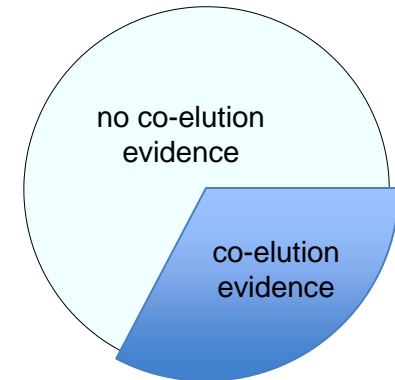
Complex-centric proteome profiling by SEC-SWATH-MS

- ✓ **Detect and quantify hundreds of protein complexes**

HEK293 soluble proteome



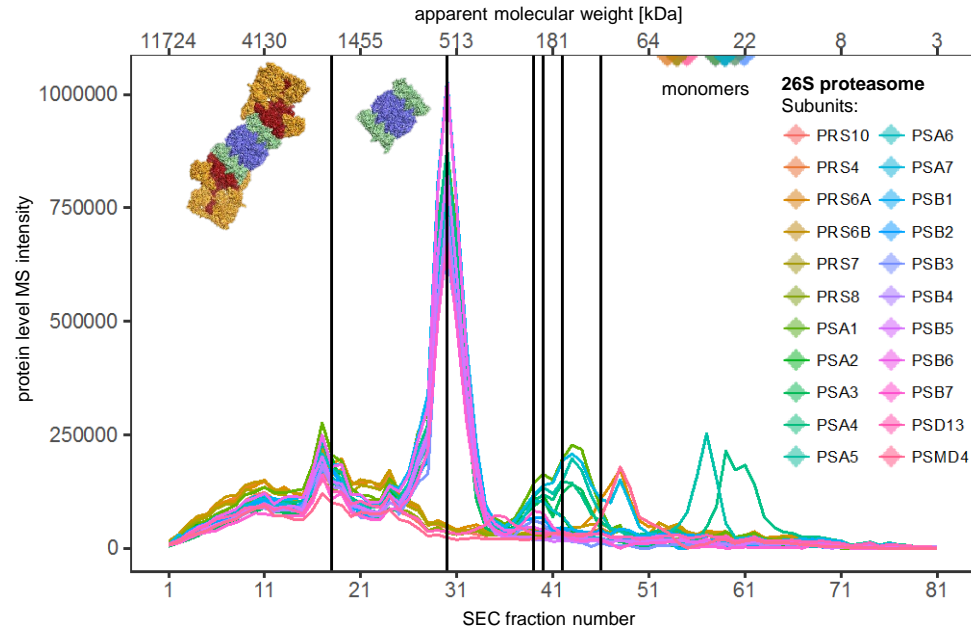
Evidence for 572 out of 1753 protein complex queries from CORUM (5% FDR)



Complex-centric proteome profiling by SEC-SWATH-MS

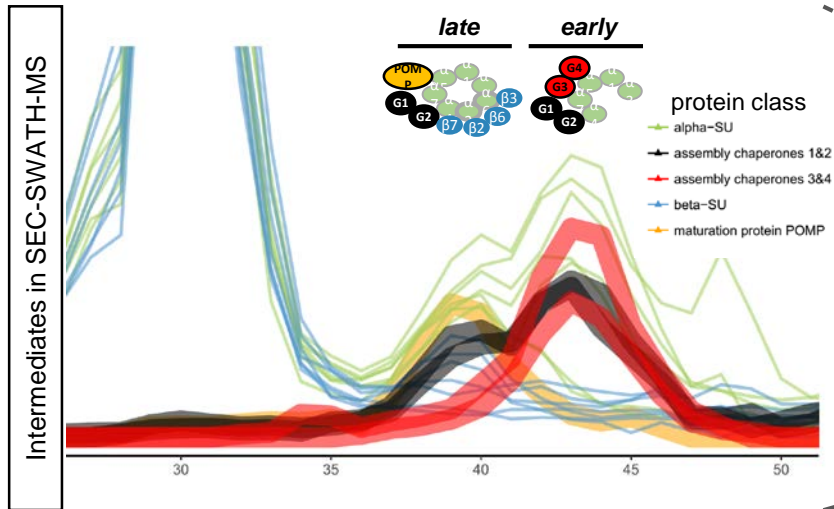
- ✓ Detect and quantify hundreds of protein complexes
- ✓ Investigate proteome modularity and assembly intermediates

HEK293 soluble proteome Proteasome assembly

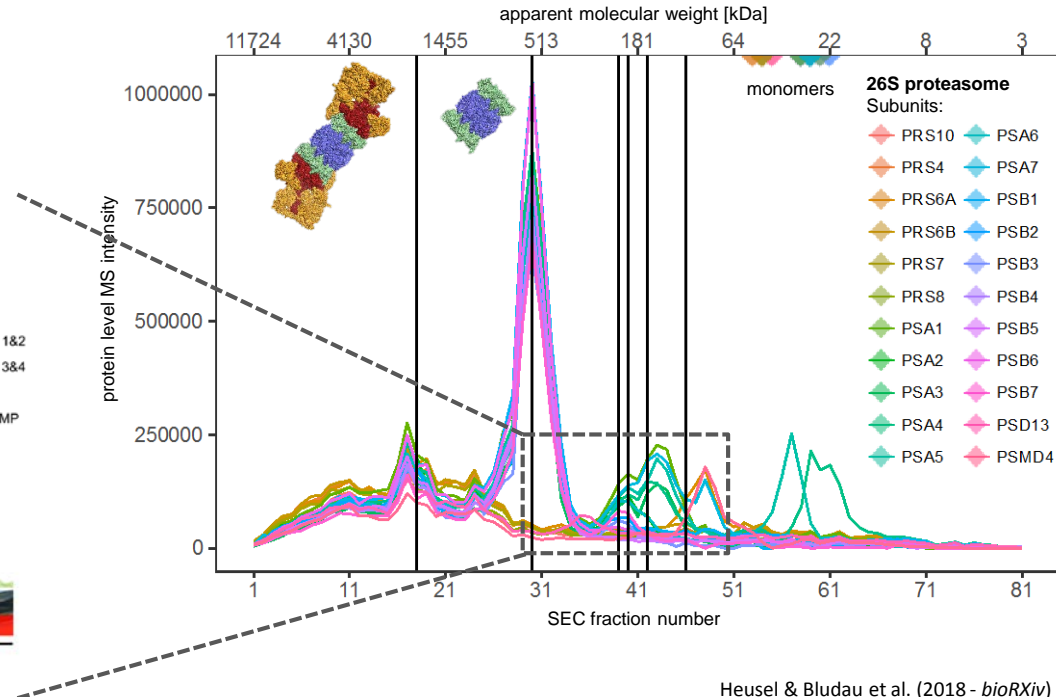


Complex-centric proteome profiling by SEC-SWATH-MS

- ✓ Detect and quantify hundreds of protein complexes
- ✓ Investigate proteome modularity and assembly intermediates

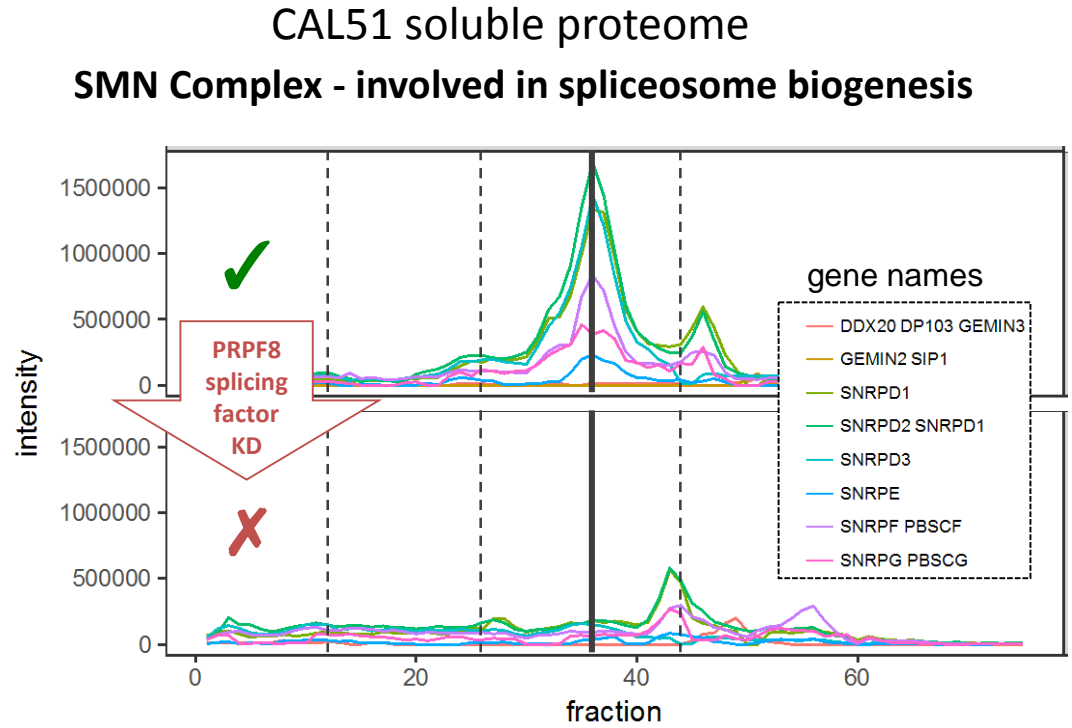


HEK293 soluble proteome Proteasome assembly



Complex-centric proteome profiling by SEC-SWATH-MS

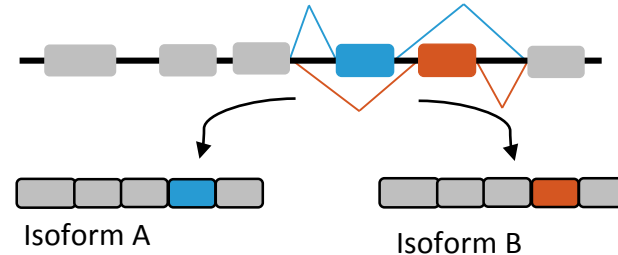
- ✓ Detect and quantify hundreds of protein complexes
- ✓ Investigate proteome modularity and assembly intermediates
- ✓ **Detect and quantify dynamic changes in the complexome**



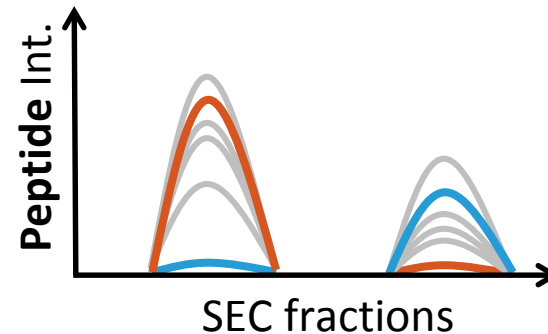
Complex-centric proteome profiling by SEC-SWATH-MS

- ✓ Detect and quantify hundreds of protein complexes
- ✓ Investigate proteome modularity and assembly intermediates
- ✓ Detect and quantify dynamic changes in the complexome
- ✓ **Investigate proteoform specific complex assembly**

RNAseq based isoform information

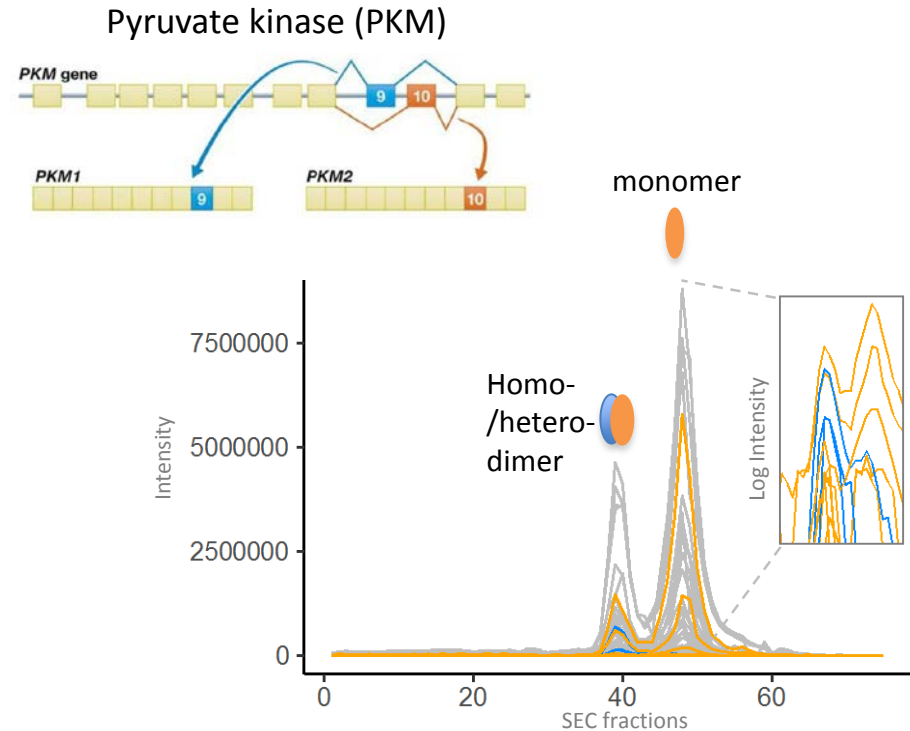


Test distinct isoform integration into protein / complex elution features



Complex-centric proteome profiling by SEC-SWATH-MS

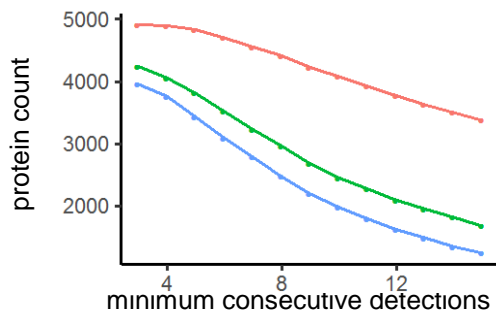
- ✓ Detect and quantify hundreds of protein complexes
- ✓ Investigate proteome modularity and assembly intermediates
- ✓ Detect and quantify dynamic changes in the complexome
- ✓ **Investigate proteoform specific complex assembly**



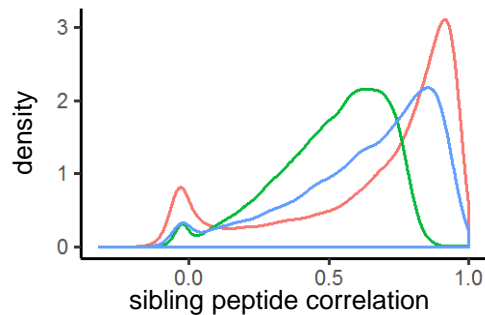
Complex-centric proteome profiling by SEC-SWATH-MS

Why DIA?

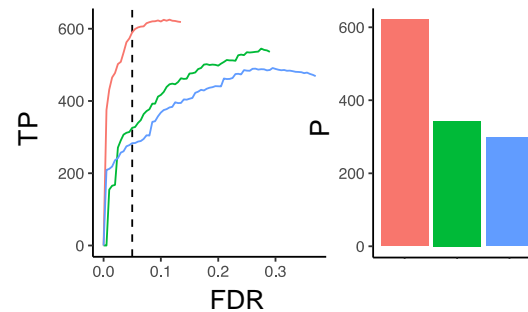
Consistency of quantification



Precision of quantification



Performance in complex-centric profiling



■ DIA/SWATH-MS2 ■ DDA-MS1 ■ DDA-SpectralCounts

Side-by-side DDA and DIA acquisition (AB Sciex TripleTOF 5600+)

Complex-centric proteome profiling by SEC-SWATH-MS



Challenges?

- Many fractions required for high resolution SEC profiles: machine time and work expensive
- Each fraction contains different subset of the proteome: highly heterogeneous dataset requiring cautious DIA scoring



Opportunities?

- Dynamic complex rewiring on proteome wide scale
- Analysis of isoform crosstalk between different splice variants and PTMs

Thank you for your attention!

Aebersold lab

- Ruedi Aebersold
- **Moritz Heusel**
- **Max Frank**
- Robin Hafen
- Ludovic Gillet
- George Rosenberger
- Matthias Gsteiger
- Ben Collins

External collaborators

- Vihandha Wickramasinghe
- Ashok Venkitaraman

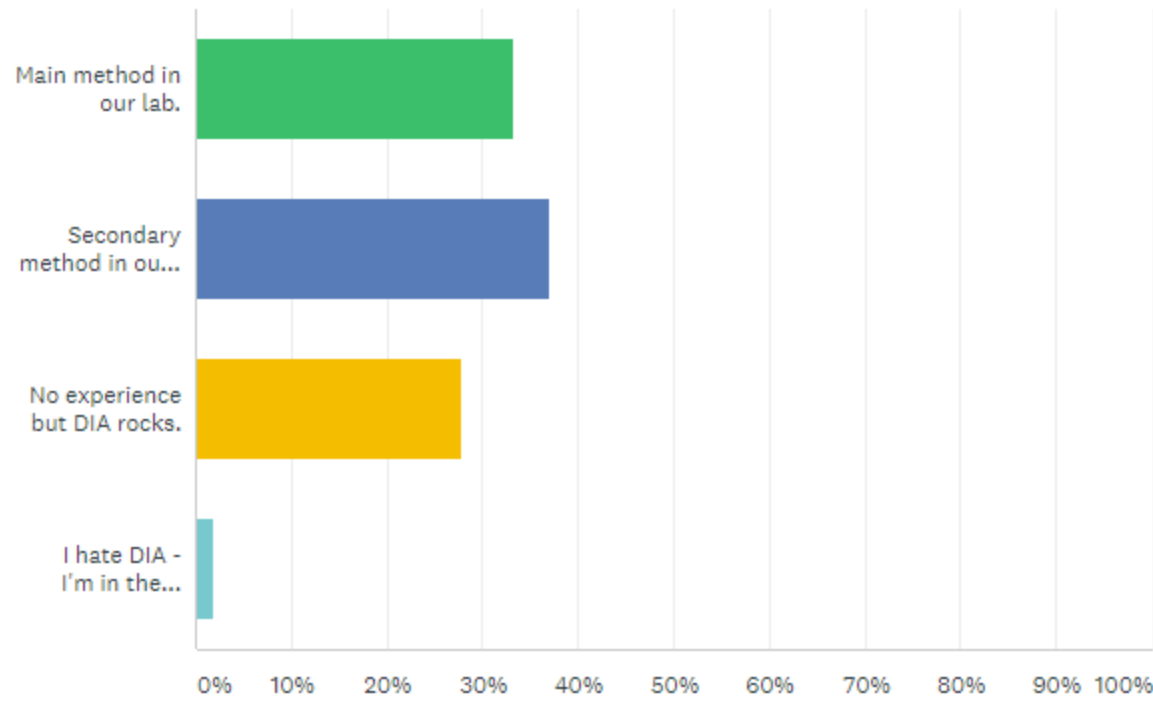


Q1

Customize Export

What's your experience with DIA?

Answered: 54 Skipped: 0



ANSWER CHOICES	RESPONSES
▼ Main method in our lab.	33.33% 18
▼ Secondary method in our lab.	37.04% 20
▼ No experience but DIA rocks.	27.78% 15
▼ I hate DIA - I'm in the wrong room.	1.85% 1
TOTAL	54

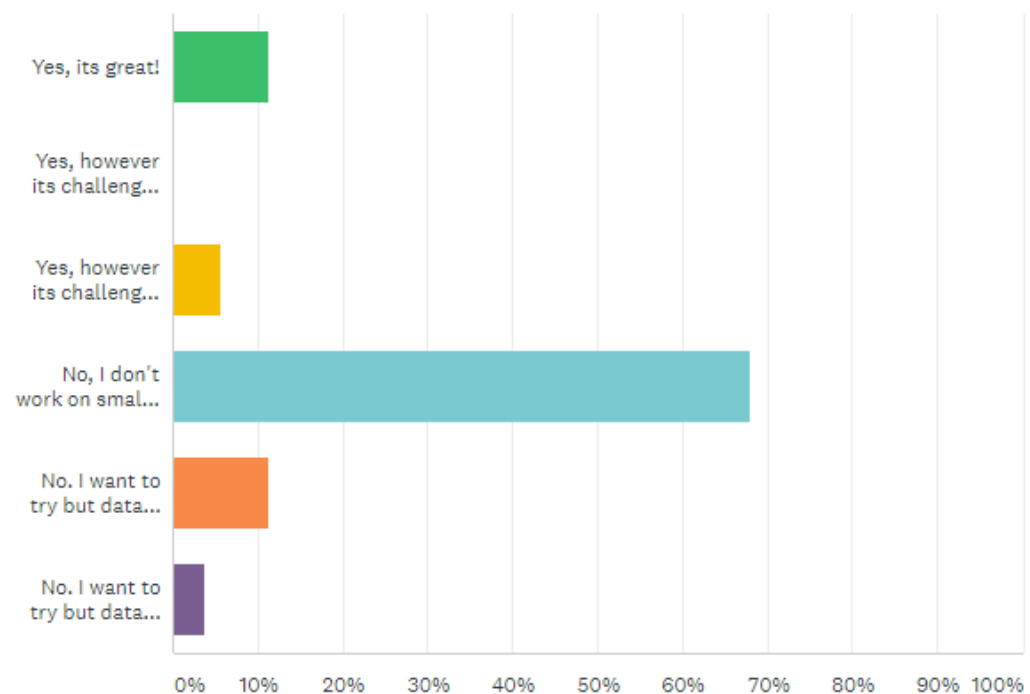
Q2

Customize

Export ▾

Have you tried small molecule DIA?

Answered: 53 Skipped: 1



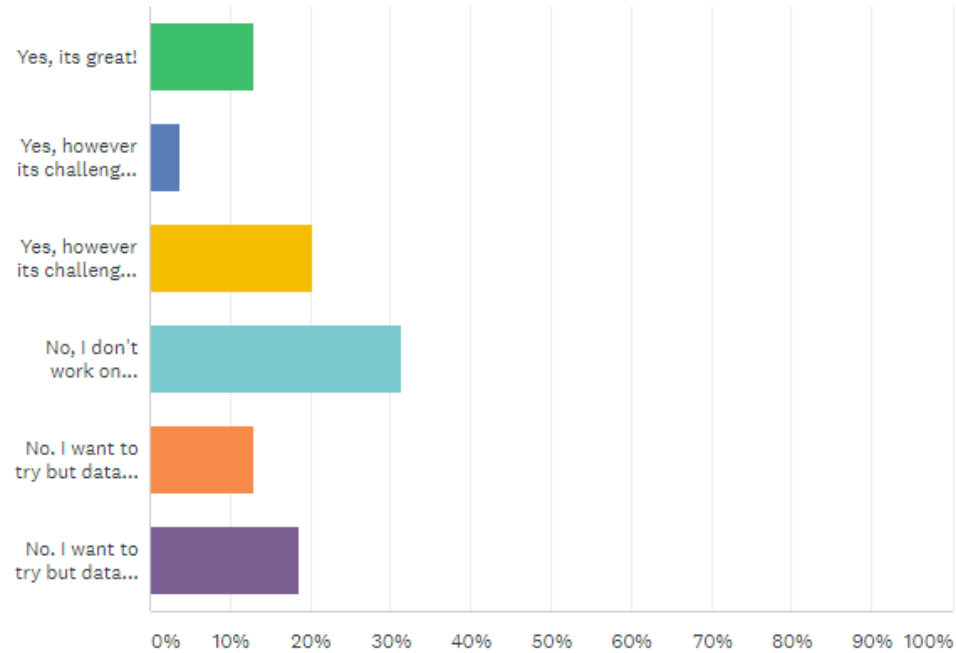
ANSWER CHOICES	RESPONSES
▼ Yes, its great!	11.32% 6
▼ Yes, however its challenging for data acquisition reasons.	0.00% 0
▼ Yes, however its challenging for data analysis reasons.	5.66% 3
▼ No, I don't work on small molecules!	67.92% 36
▼ No. I want to try but data acquisition is the main barrier.	11.32% 6
▼ No. I want to try but data analysis is the main barrier.	3.77% 2
TOTAL	53

Q3

Customize Export

Have you tried PTM (or similarly SAV) analysis by DIA?

Answered: 54 Skipped: 0



ANSWER CHOICES	RESPONSES
Yes, its great!	12.96% 7
Yes, however its challenging for data acquisition reasons.	3.70% 2
Yes, however its challenging for data analysis reasons.	20.37% 11
No, I don't work on PTMs/SAVs!	31.48% 17
No. I want to try but data acquisition is the main barrier.	12.96% 7
No. I want to try but data analysis is the main barrier.	18.52% 10
TOTAL	54

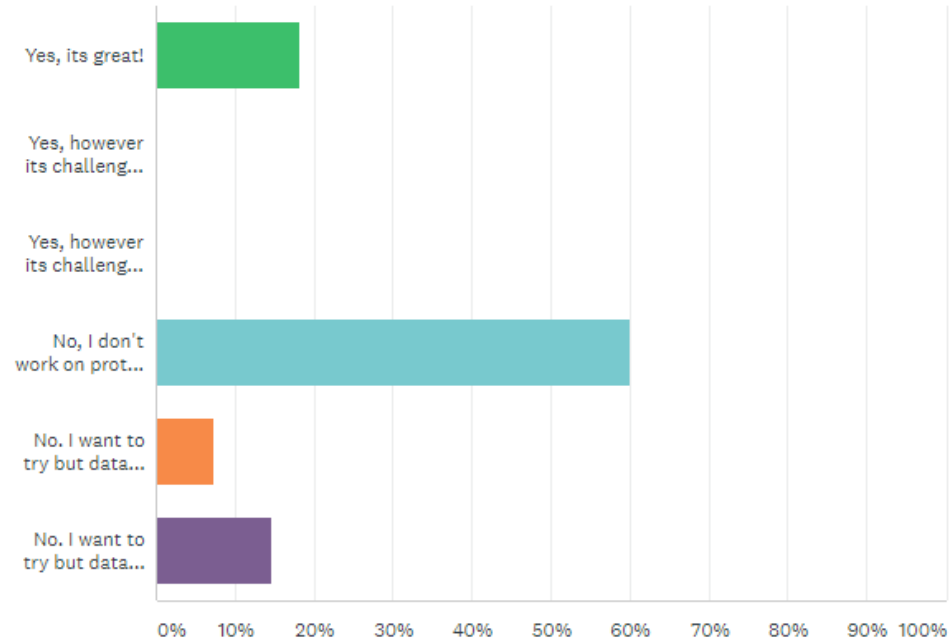
Q4

Customize

Export ▼

Have you tried protein complex analysis using DIA?

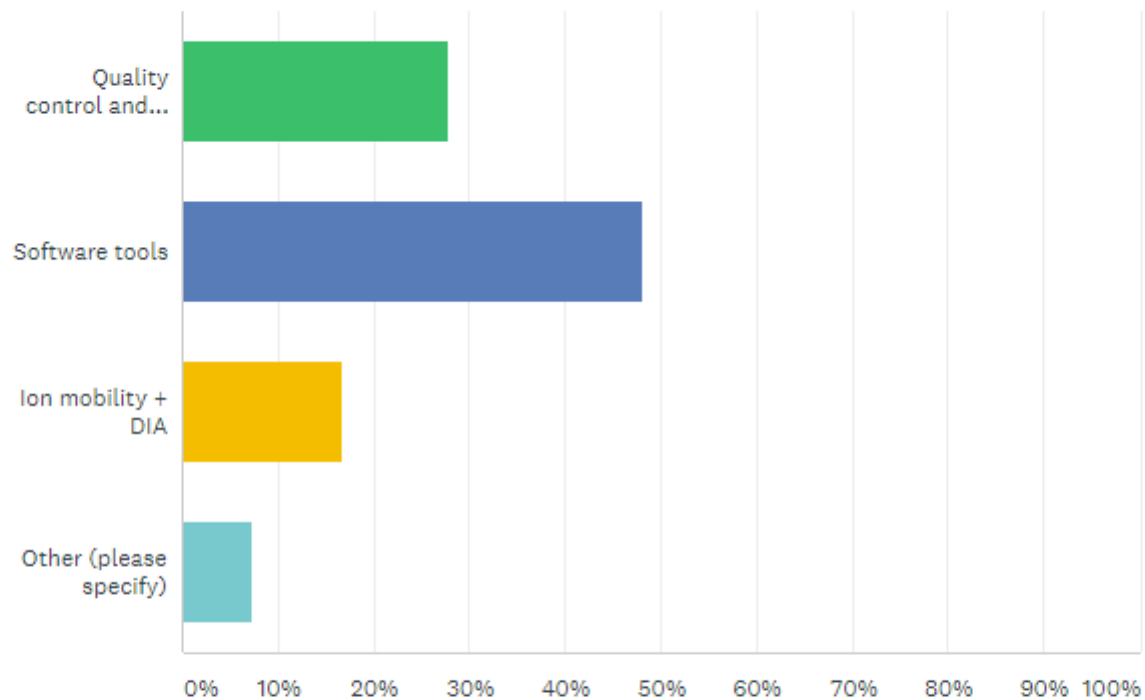
Answered: 55 Skipped: 0



ANSWER CHOICES	RESPONSES
▼ Yes, its great!	18.18% 10
▼ Yes, however its challenging for data acquisition reasons.	0.00% 0
▼ Yes, however its challenging for data analysis reasons.	0.00% 0
▼ No, I don't work on protein complexes!	60.00% 33
▼ No. I want to try but data acquisition is the main barrier.	7.27% 4
▼ No. I want to try but data analysis is the main barrier.	14.55% 8
TOTAL	55

What would you like to discuss next year in the DIA workshop?

Answered: 54 Skipped: 1



ANSWER CHOICES	RESPONSES
▼ Quality control and batch effects	27.78% 15
▼ Software tools	48.15% 26
▼ Ion mobility + DIA	16.67% 9
▼ Other (please specify) Responses	7.41% 4

Other (please specify)	Responses	7.41%	4
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RESPONSES (4)

TEXT ANALYSIS

TAGS (0)

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Search responses



Showing 4 responses

FDR

6/5/2018 3:17 AM

[View respondent's answers](#)

All of the above!

6/5/2018 2:52 AM

[View respondent's answers](#)

Experimental designs/workflows

6/5/2018 2:51 AM

[View respondent's answers](#)

Decoventlutoon/Identification

6/5/2018 2:50 AM

[View respondent's answers](#)