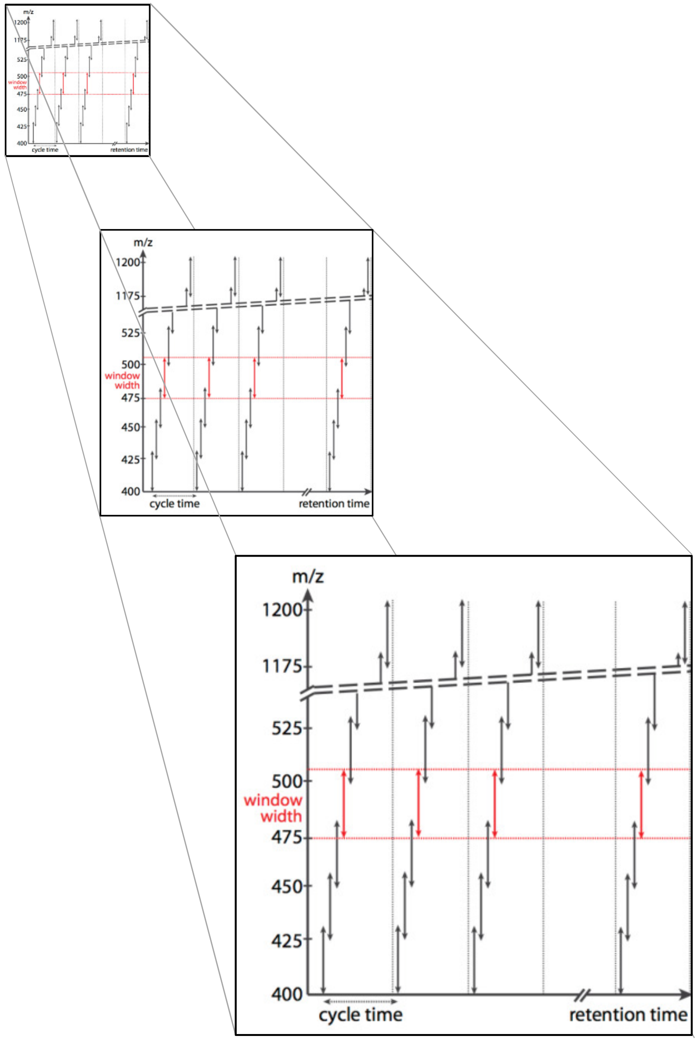
Data Independent Acquisition Strategies for Quantitative Proteomics: The Challenges of Scaling Up to Meet Demand



Data Independent Acquisition Interest Group

Presiding: Ben Collins (ETH Zurich), Isabell Bludau (ETH Zurich)

65th ASMS Conference on Mass Spectrometry and Allied Topics

June 7th, 2017

Room: 235 – 238 -- Indiana Convention Center, Indianapolis, IN

Real time poll

bit.ly/2sEPrpu

The breadth of DIA methodology is increasing

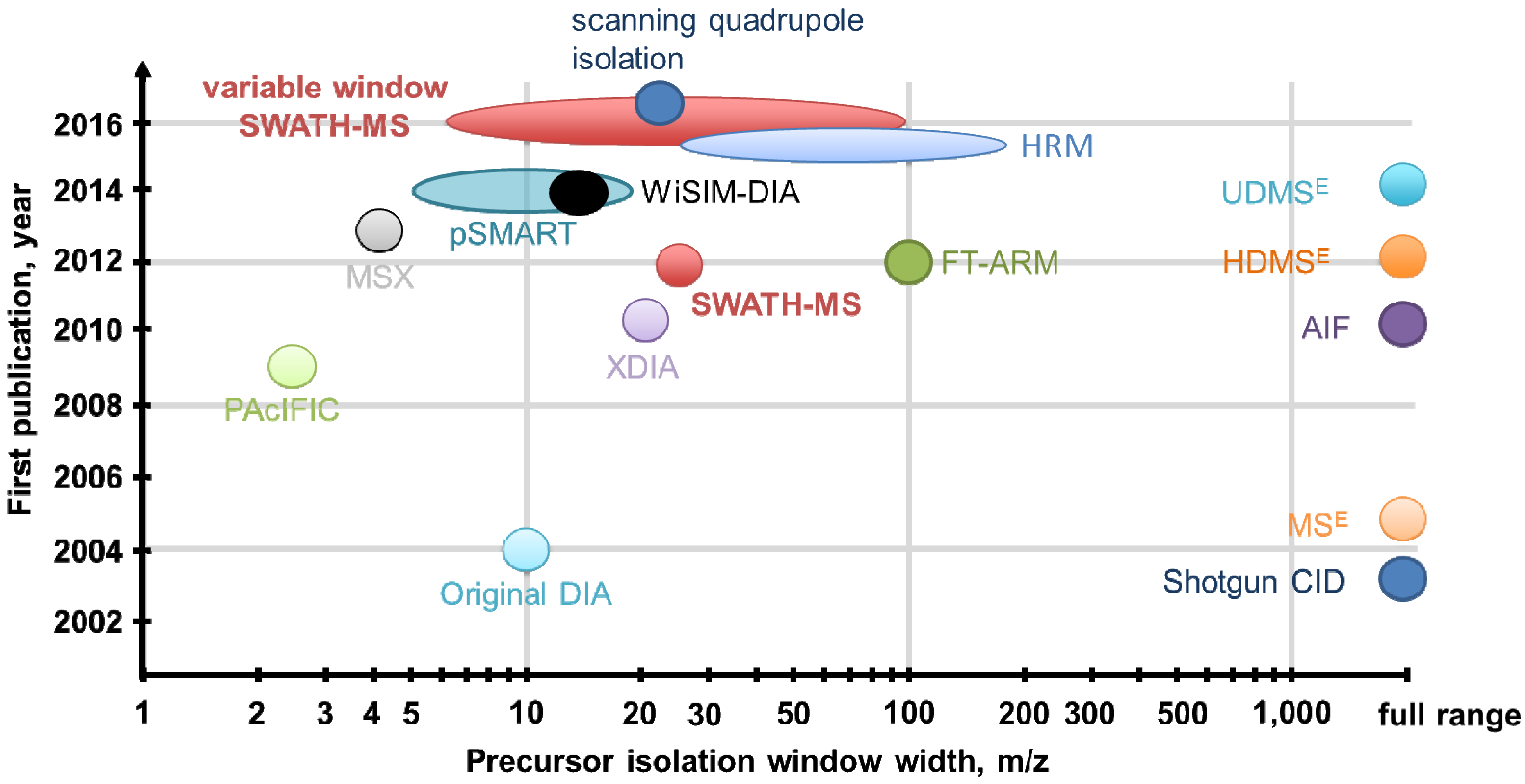
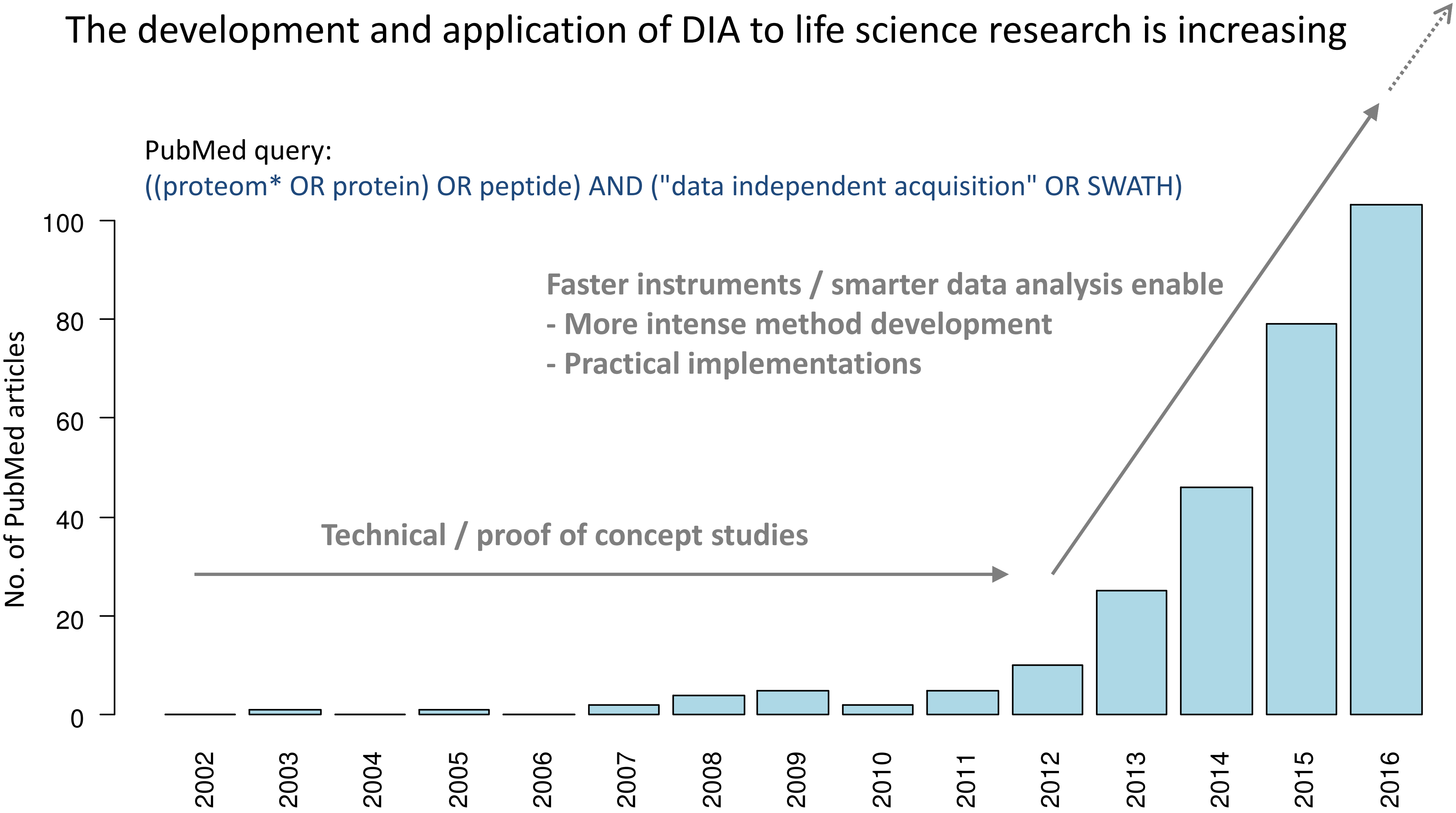


Figure from Tina Ludwig – SWATH-MS tutorial (in prep)

The development and application of DIA to life science research is increasing



PubMed query:

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

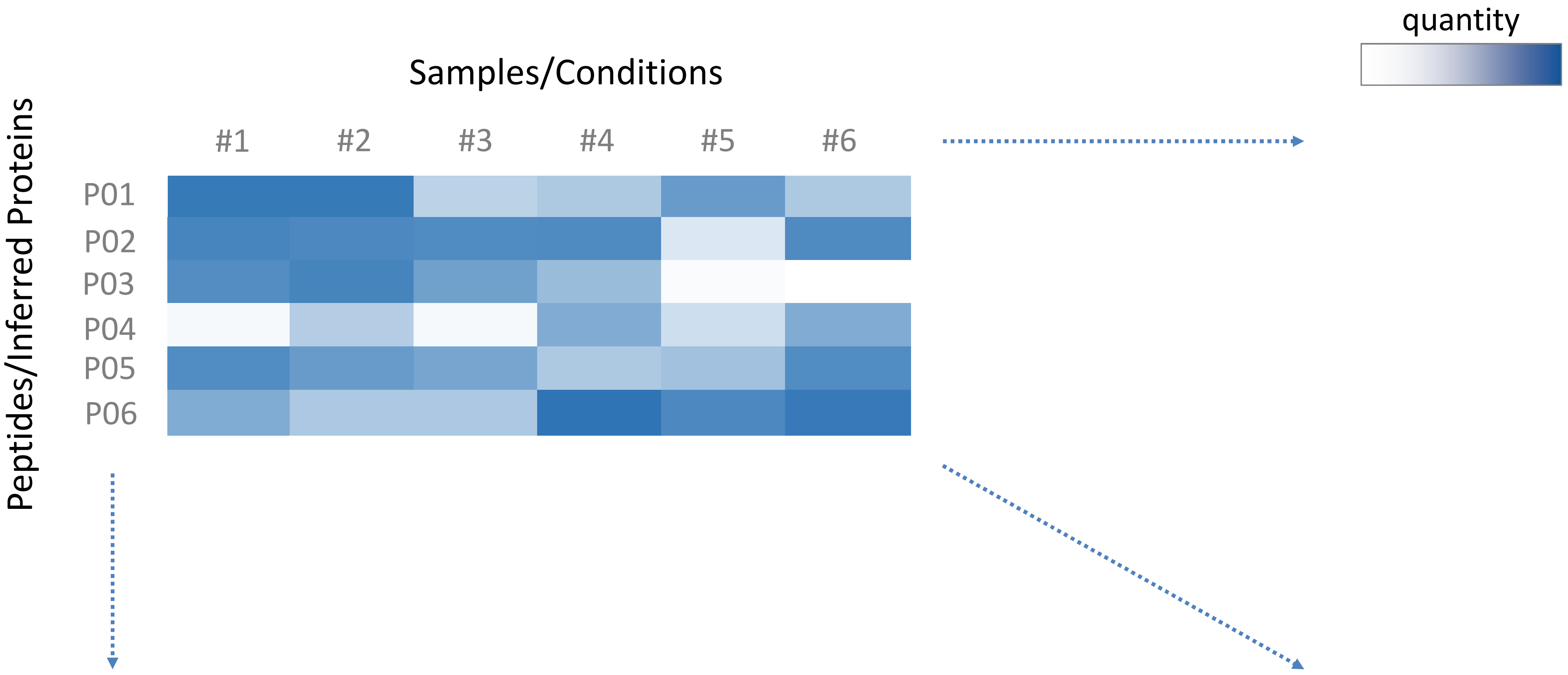
Faster instruments / smarter data analysis enable

- More intense method development

- Practical implementations

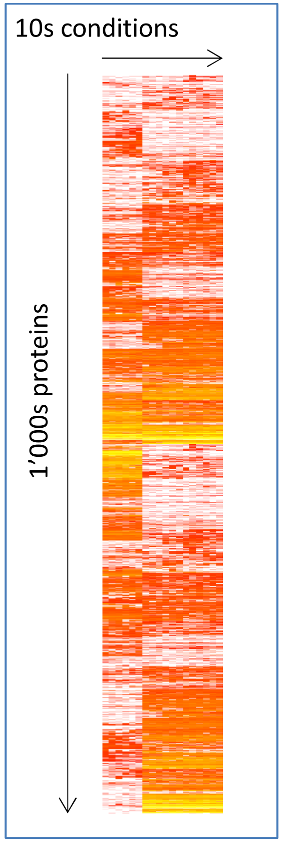
Technical / proof of concept studies

Scaling up – in which dimensions?

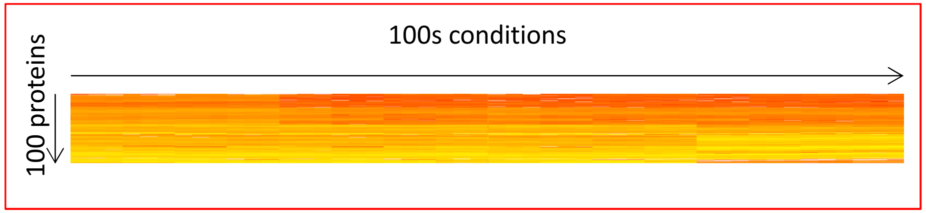


The primary goal of DIA is data completeness

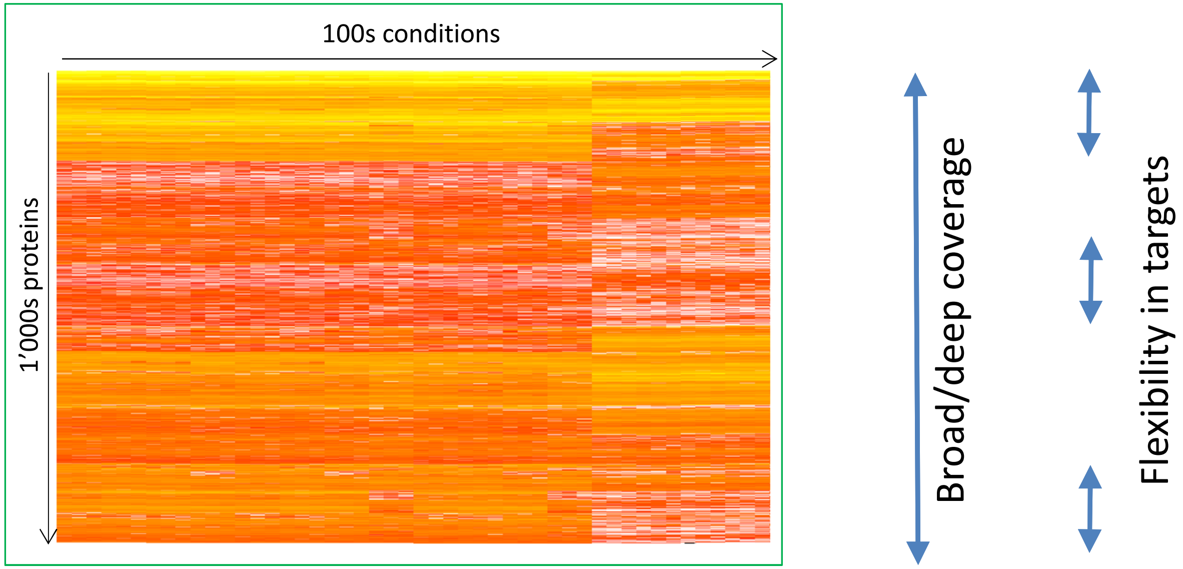
Historical problem of DDA based approaches



Targeted proteomics (SRM/PRM)



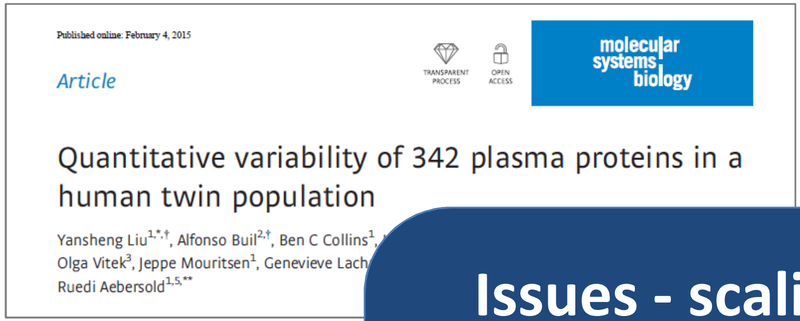
DIA



Figures from Gillet, L. C., et al. *Annual Review of Analytical Chemistry* **9,** (2016).

Scaling up DIA – no. of samples/conditions

Population-based analysis (personalized med., etc)



Published online: February 4, 2015

Article

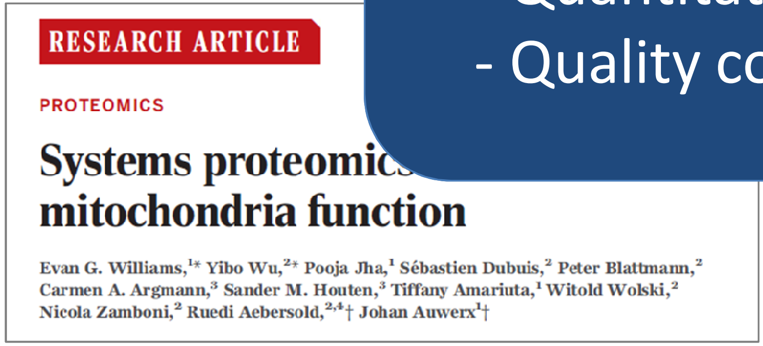
TRANSPARENT PROCESS OPEN ACCESS molecular systems biology

Quantitative variability of 342 plasma proteins in a human twin population

Yansheng Liu[1,*,†], Alfonso Buil[2,†], Ben C Collins[1], ...
Olga Vitek[3], Jeppe Mouritsen[1], Genevieve Lach...
Ruedi Aebersold[1,5,**]

232 human p

Genetic as



RESEARCH ARTICLE

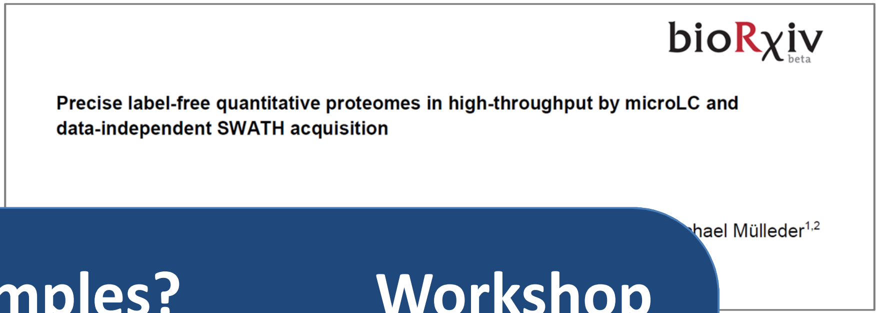
PROTEOMICS

Systems proteomic... mitochondria function

Evan G. Williams,[1*] Yibo Wu,[2*] Pooja Jha,[1] Sébastien Dubuis,[2] Peter Blattmann,[2]
Carmen A. Argmann,[3] Sander M. Houten,[3] Tiffany Amariuta,[1] Witold Wolski,[2]
Nicola Zamboni,[2] Ruedi Aebersold,[2,4]† Johan Auwerx[1]†

386 mouse liver samples

Large-scale knock-out screens



bioRχiv beta

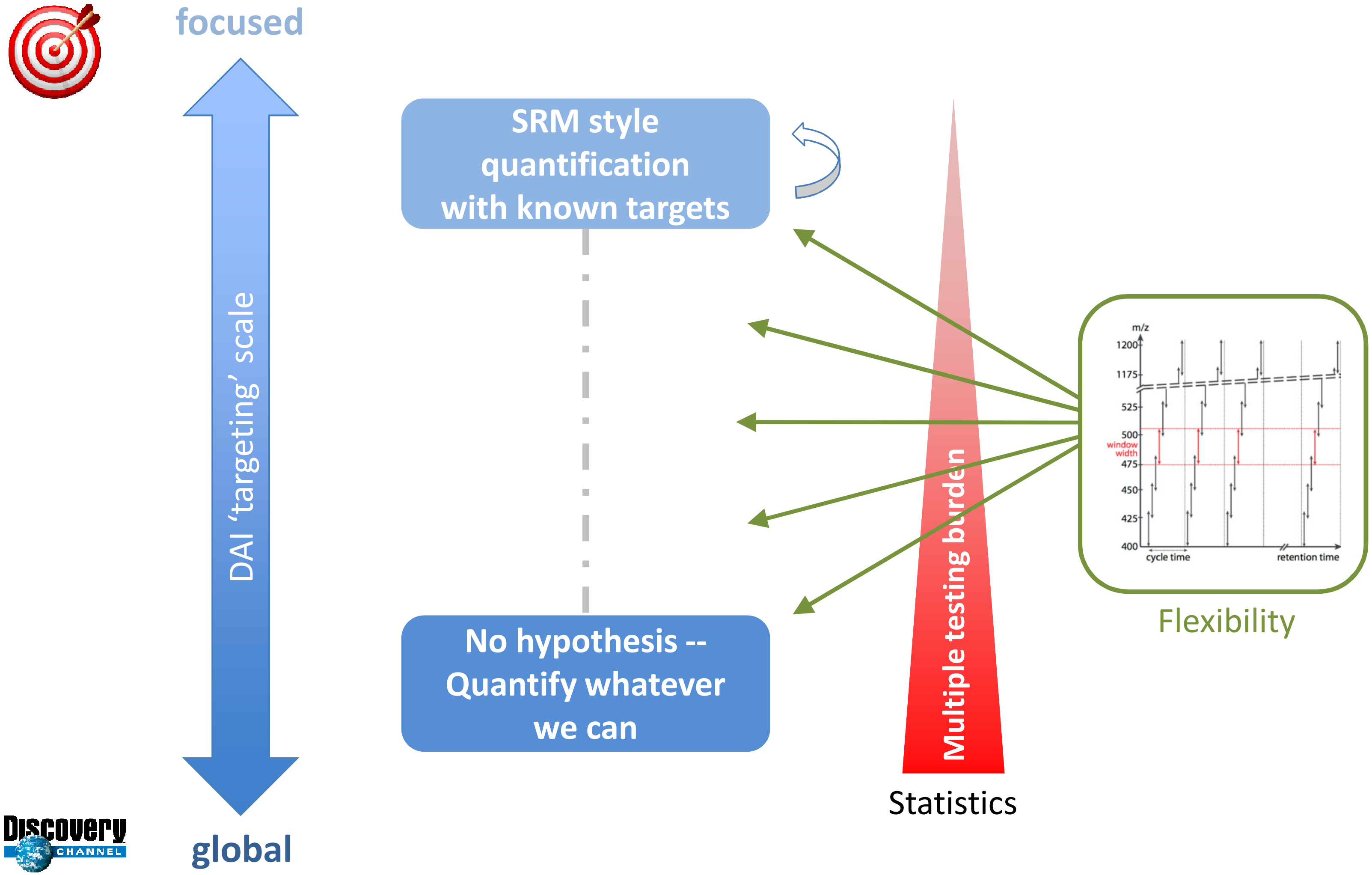
Precise label-free quantitative proteomes in high-throughput by microLC and data-independent SWATH acquisition

...hael Mülleder[1,2]

...ple numbers

Issues - scaling up samples?	Workshop
- Error control (FDR)	✓
- Data completeness	✓
- Quantitative precision/accuracy	✓ ✗
- Quality control	✗

Scaling up DIA – no. of peptides/inferred proteins



focused

DAI 'targeting' scale

global

SRM style quantification with known targets

No hypothesis -- Quantify whatever we can

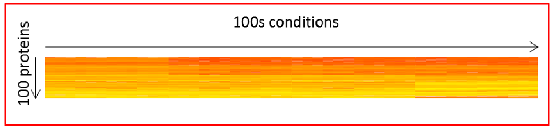
Multiple testing burden

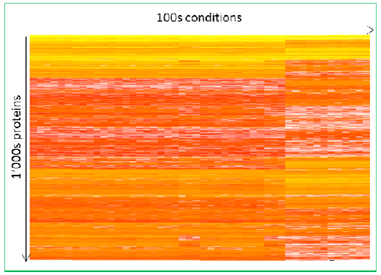
Statistics

Flexibility

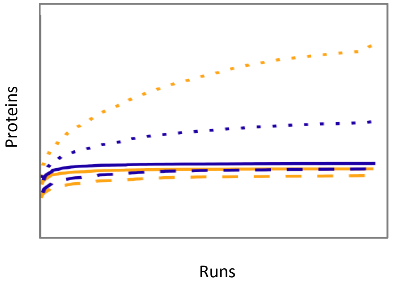
Topics for discussion

1. Mike MacCoss (Univ. of Washington)
   - DIA as targeted proteomics

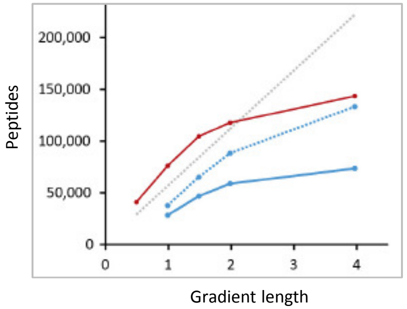




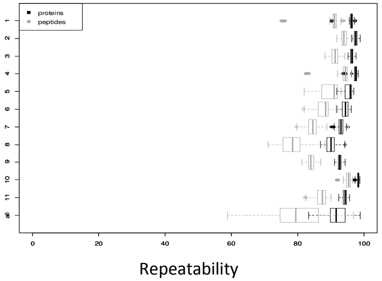
2. Alexey Nesvizhskii (Univ. of Michigan)
   - DIA as discovery proteomics



3. Isabel Bludau (ETH Zurich)
   - Error rate control at various levels



4. Lukas Reiter (Biognosys)
   - Depth of proteome coverage



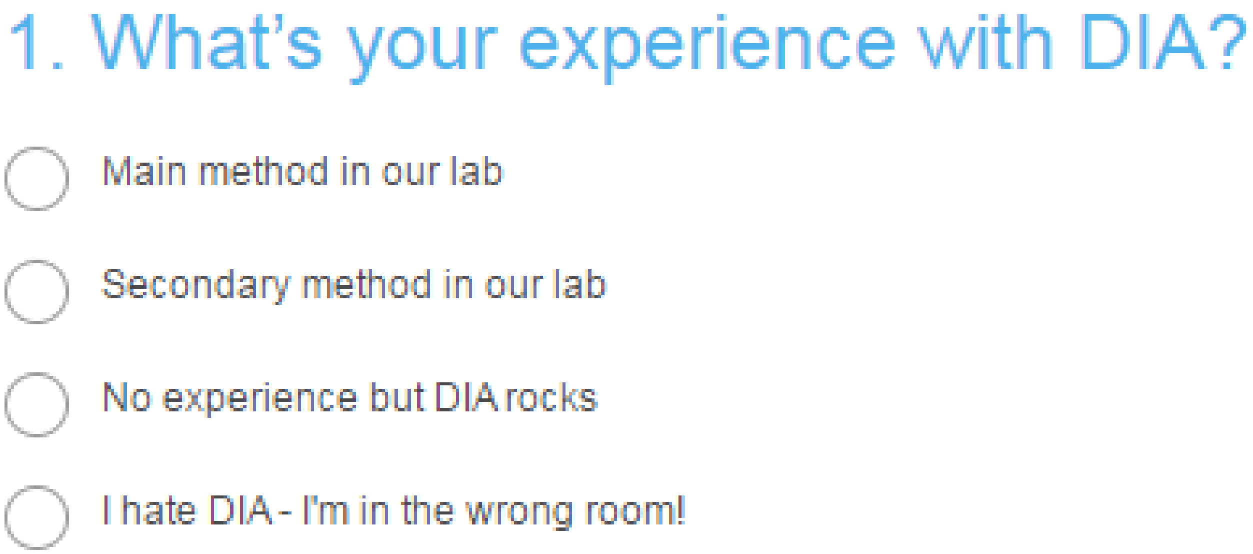
5. Ben Collins (ETH Zurich)
   - Repeatability and data completeness (optional - depending on time)

The goal is a community discussion!

- If you have a question or comment
  1. Raise your hand
  2. Shout
  3. Throw something
  4. Use the ASMS app

- Let’s answer Question 1 in the poll

Survey question 1



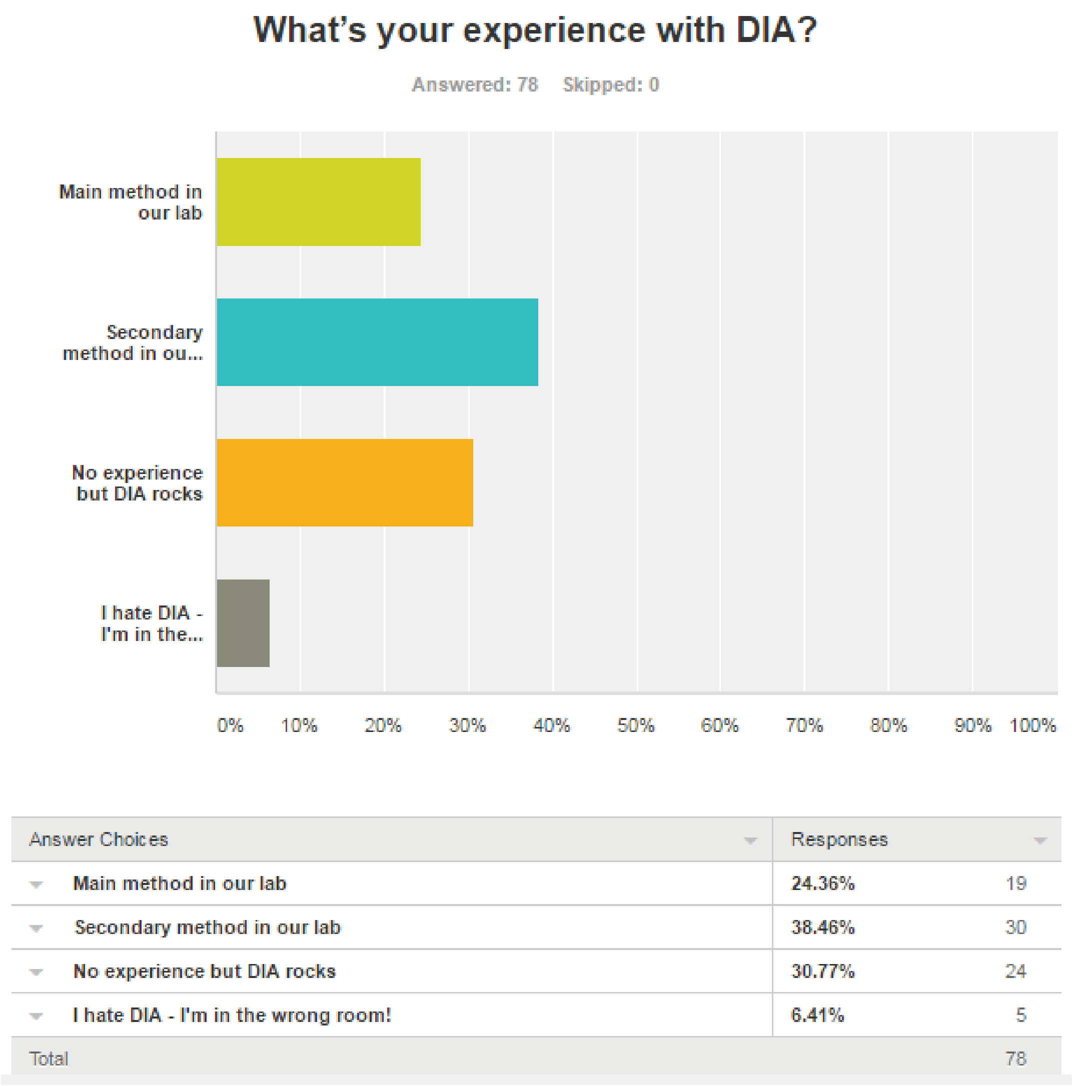
1. What’s your experience with DIA?

○ Main method in our lab

○ Secondary method in our lab

○ No experience but DIA rocks

○ I hate DIA - I'm in the wrong room!



Main method in our lab
Secondary method in ou...
No experience but DIA rocks
I hate DIA - I'm in the...
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%

What’s your experience with DIA?

Answered: 78 Skipped: 0

Answer Choices	Responses	
Main method in our lab	24.36%	19
Secondary method in our lab	38.46%	30
No experience but DIA rocks	30.77%	24
I hate DIA - I'm in the wrong room!	6.41%	5
Total		78

Mike

Improving DIA Assay Design using Lessons Learned from Targeted Assay Development

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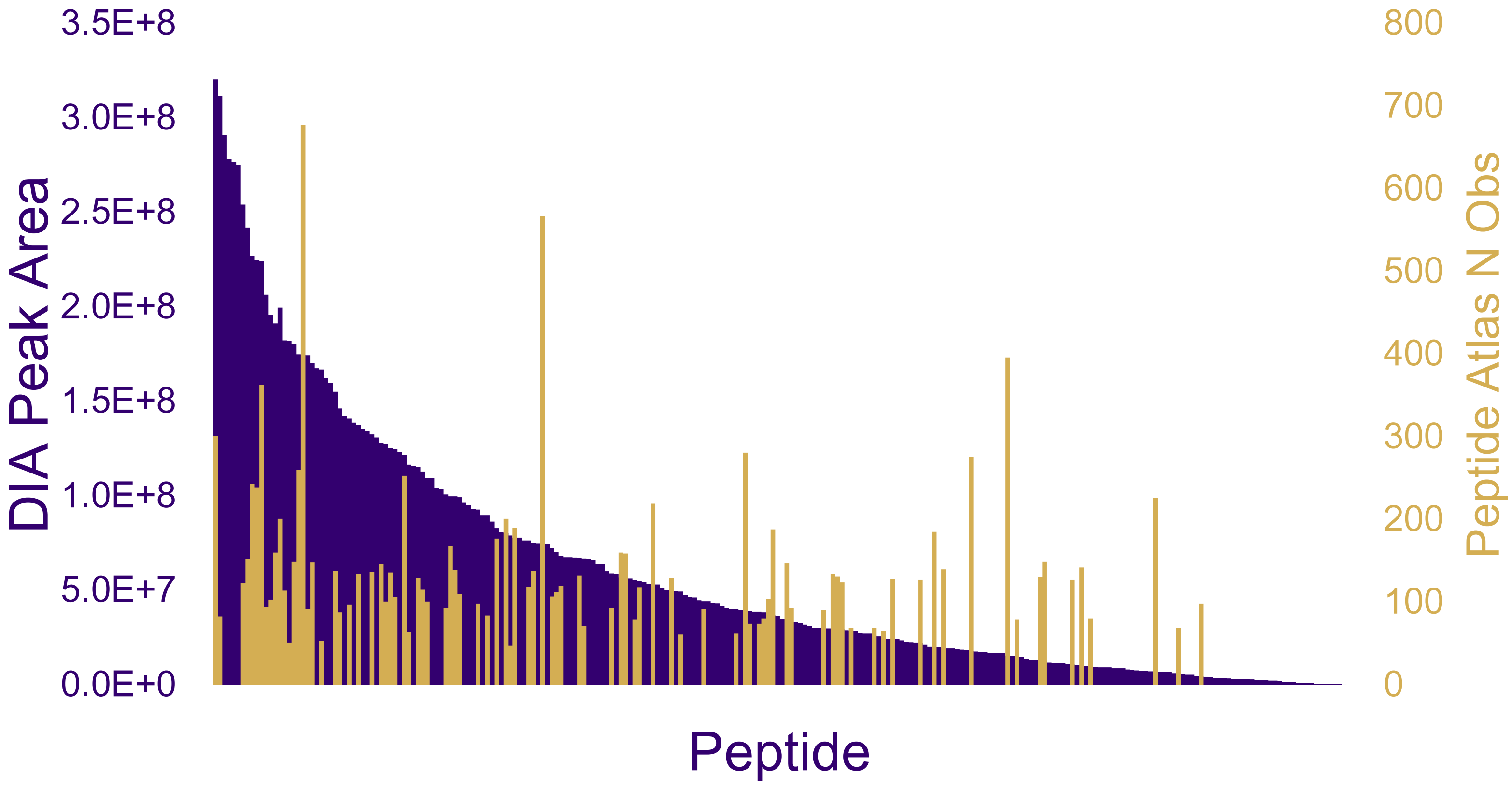
Apolipoprotein B100

DIA vs. PeptideAtlas



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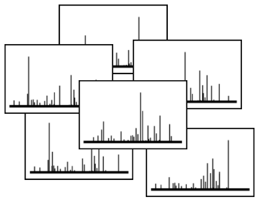
Pecan: Detecting Peptides Directly from DIA Data

Spectrum-centric analysis

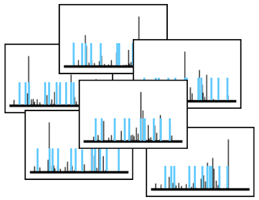
What peptides best explain the data?



MS/MS spectra

example tools:
SEQUEST, MASCOT … etc.

Protein sequence DB



Peptide spectrum matches (PSMs)

p-value
q-value

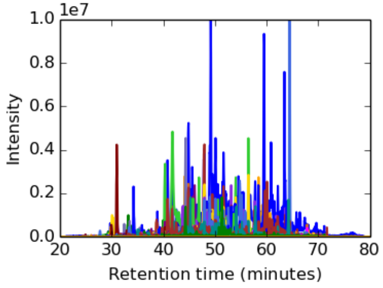
Peptide-centric analysis

Which peptides are detected in our data?

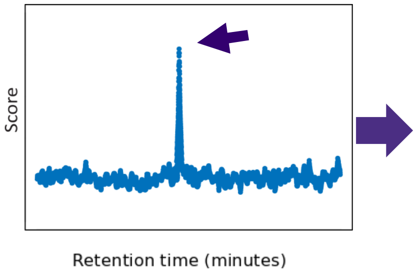
Peptides of interest

APTNVTCILK

example tools:
OpenSWATH, Skyline



Extracted MS/MS data



Evidence for detection

p-value
q-value

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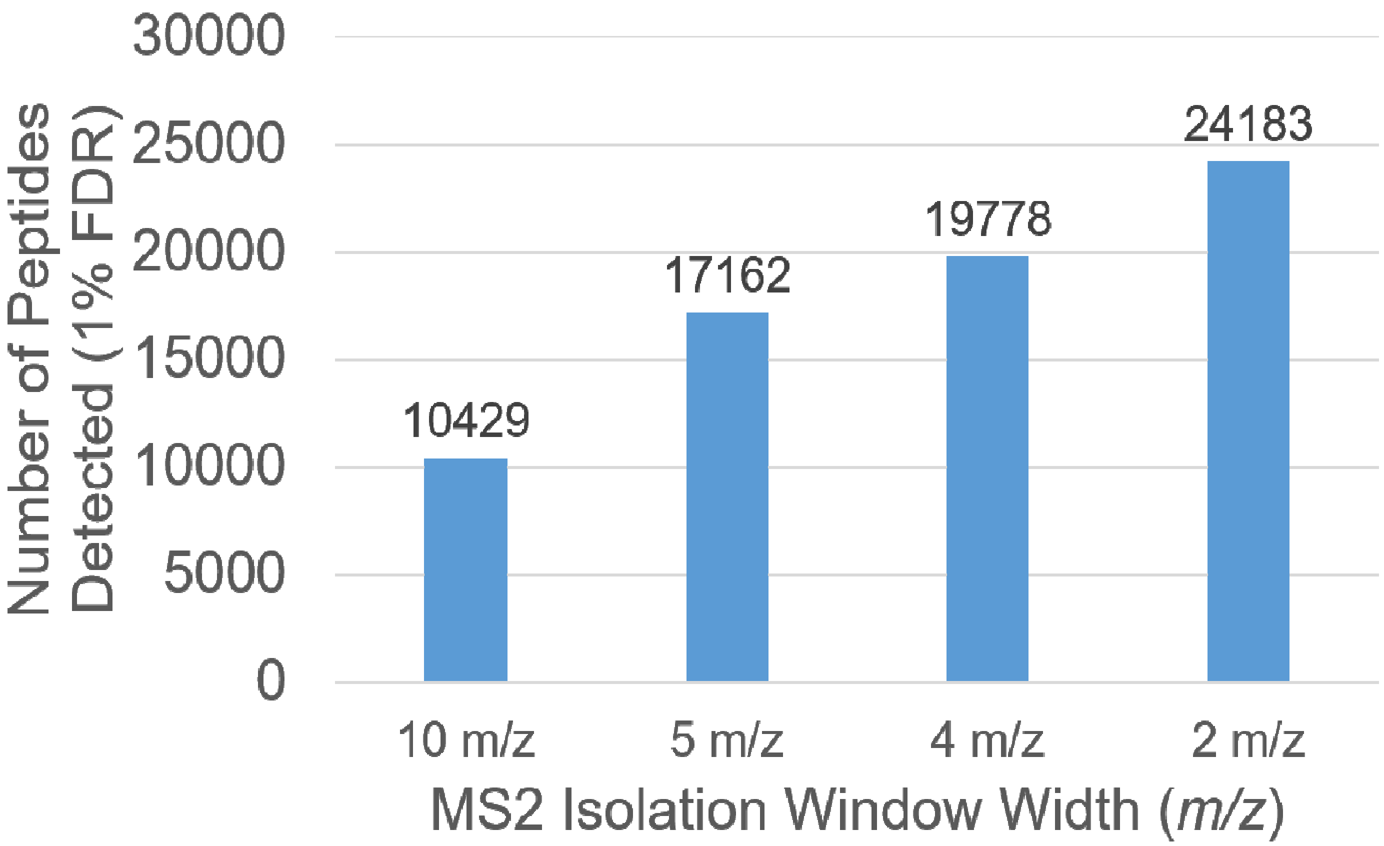
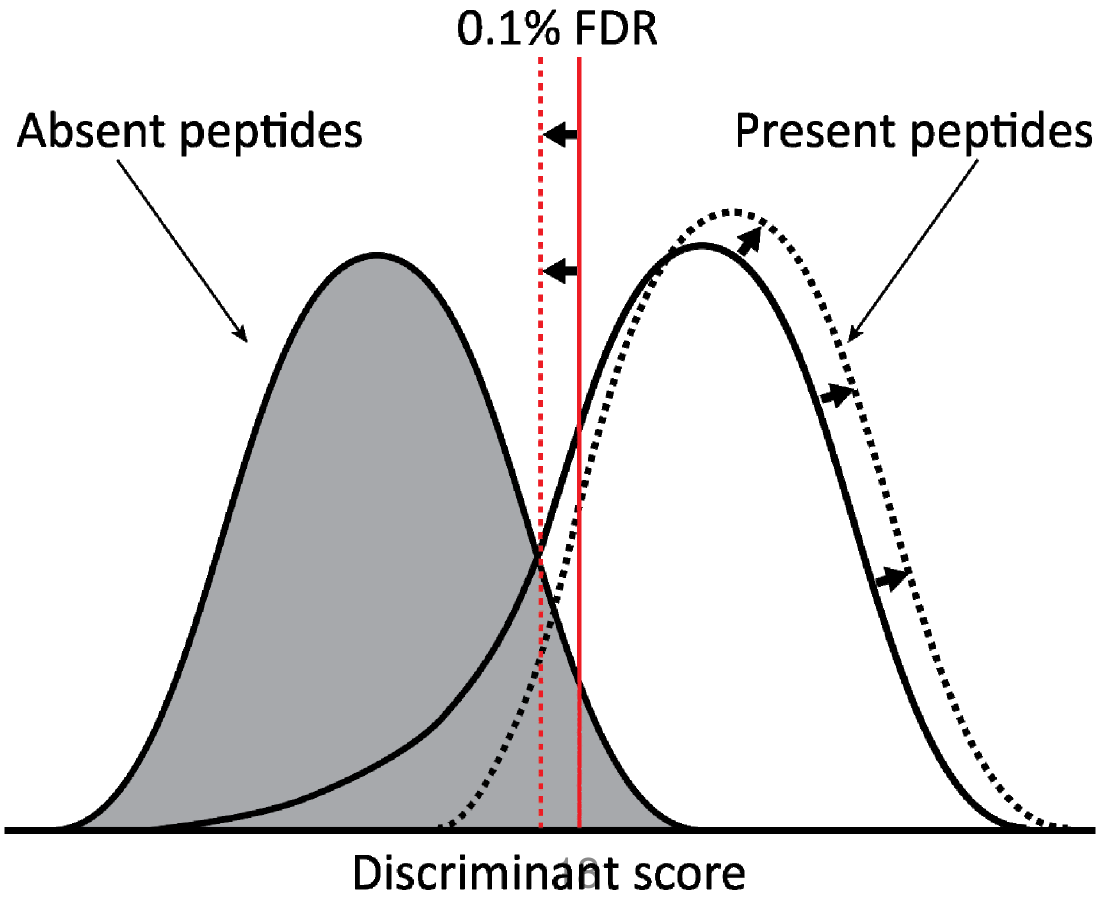
Ying Sonia Ting, MCP 2015

Problem #1: Detection of Peptides in DIA Data is Inversely Proportional to Isolation Width

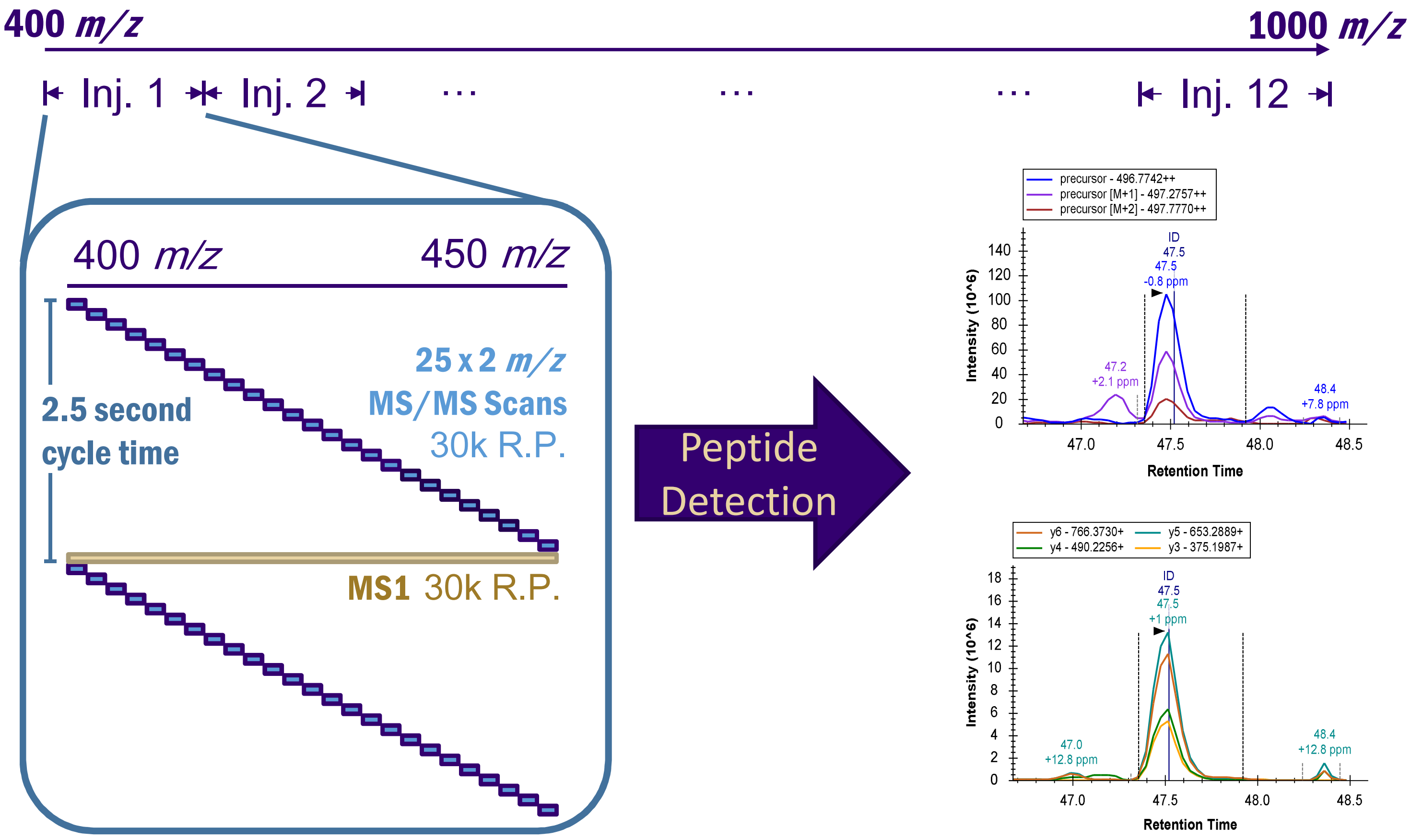


Narrow Isolation Windows Improves the Score Discrimination Between Absent and Present Peptides



Picking Peptides Directly from DIA Data

12 LC–MS/MS Runs: ~1 µL plasma



400 *m/z* — 1000 *m/z*

Inj. 1 · Inj. 2 · … · … · … · Inj. 12

400 *m/z* — 450 *m/z*

2.5 second cycle time

25 x 2 *m/z* MS/MS Scans 30k R.P.

MS1 30k R.P.

Peptide Detection

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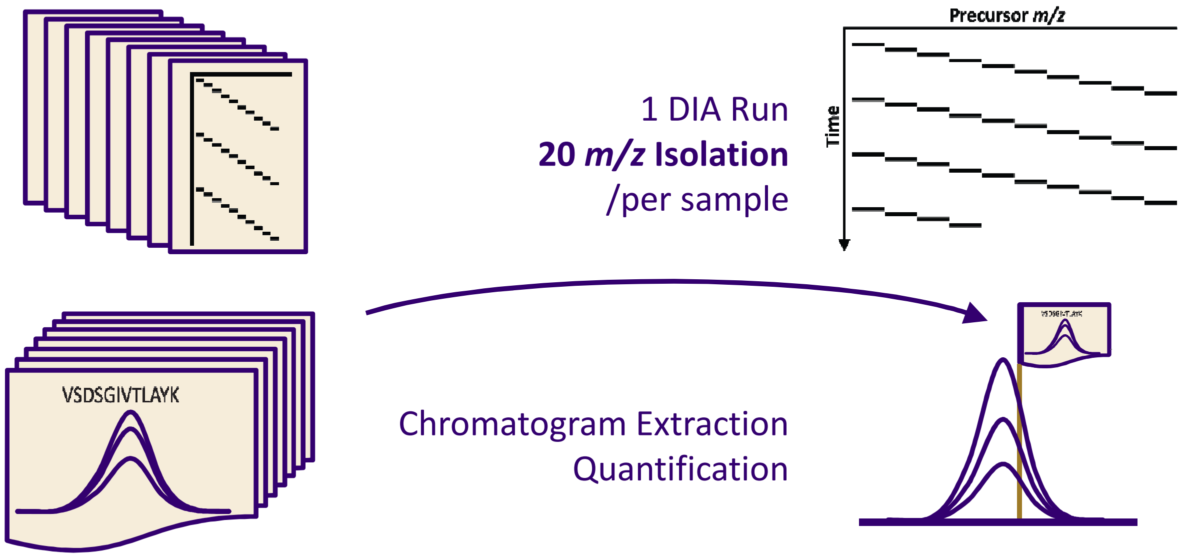
Chromatogram

Spectral ~~Library~~ Workflow



Build a Chromatogram Library

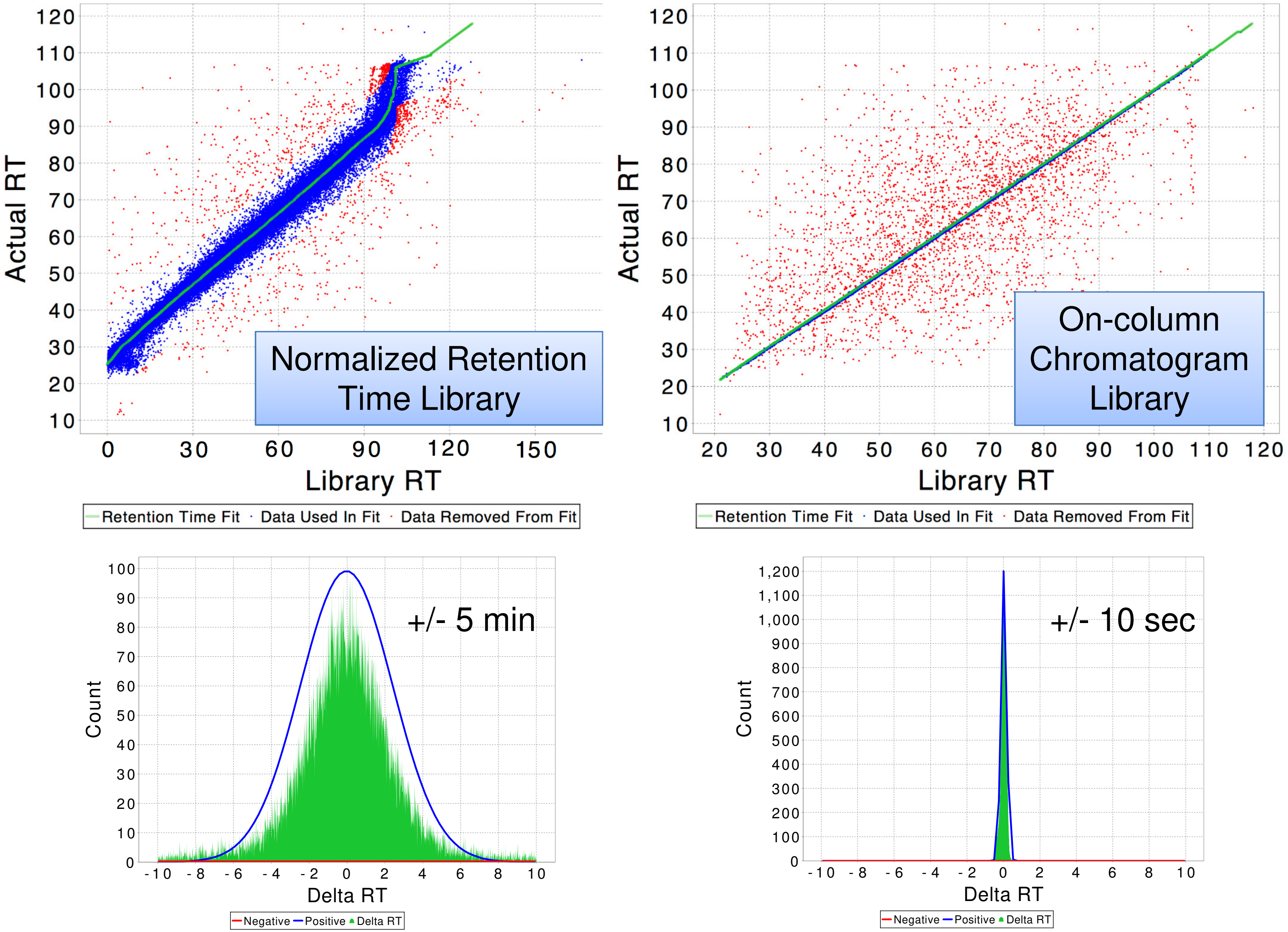
12 DIA Runs
2 *m/z* Isolation
Pooled Sample

Pecan Peptide Detection

Acquire DIA Data

1 DIA Run
20 *m/z* Isolation
/per sample

Chromatogram Extraction
Quantification



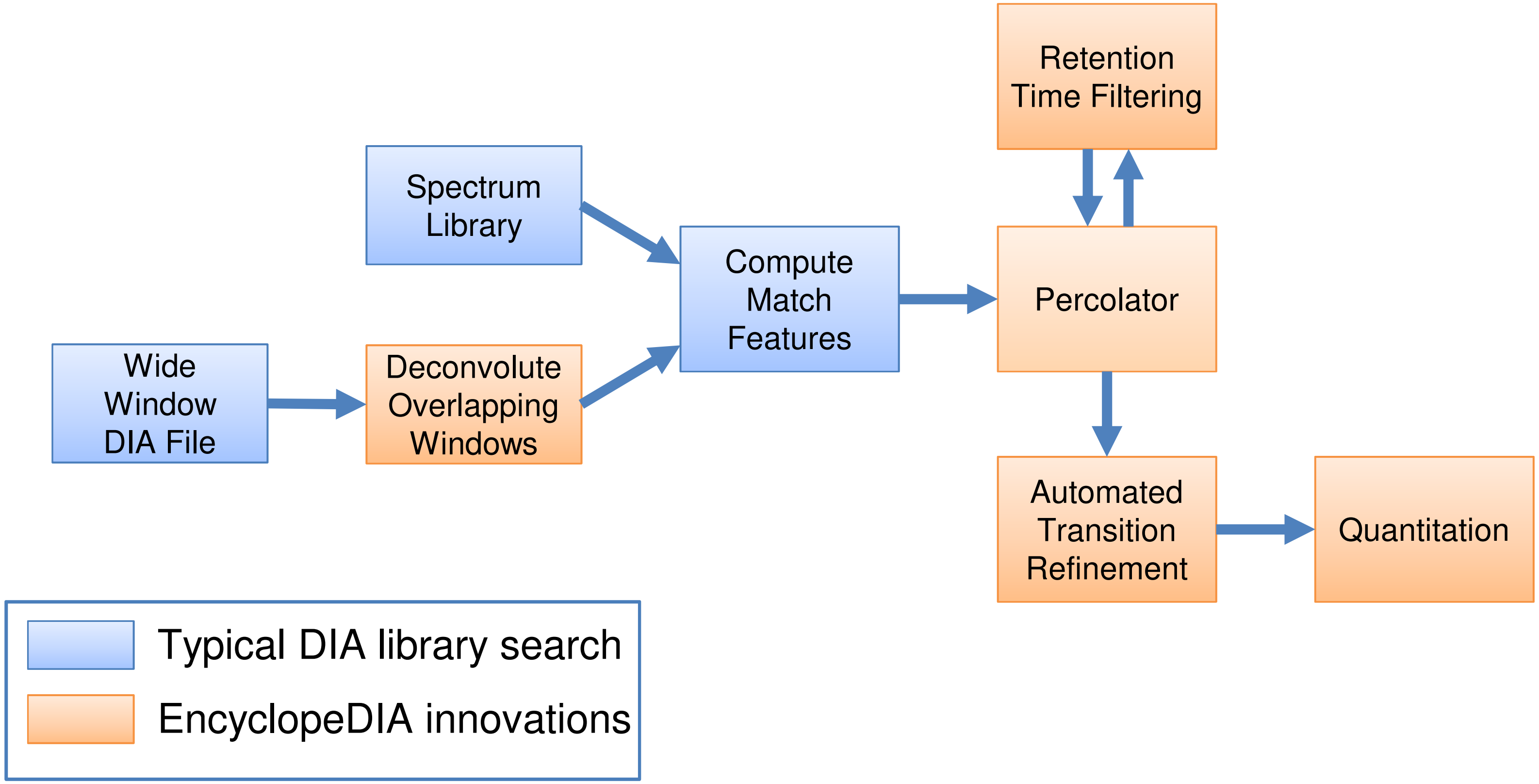
Normalized Retention Time Library

On-column Chromatogram Library

+/- 5 min

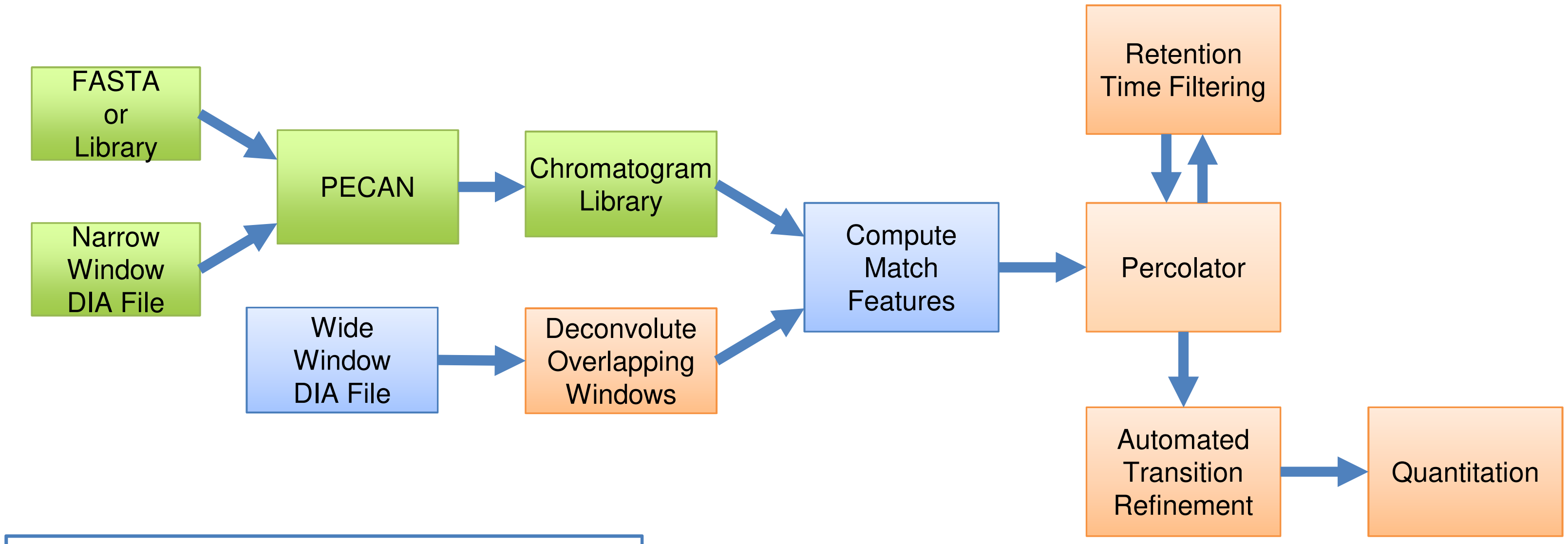
+/- 10 sec

EncyclopeDIA workflow


Spectrum Library
Wide Window DIA File
Deconvolute Overlapping Windows
Compute Match Features
Retention Time Filtering
Percolator
Automated Transition Refinement
Quantitation
🟦 Typical DIA library search
🟧 EncyclopeDIA innovations

Percolator from Käll L et al, Nat Methods. 2007 Nov;4(11):923-5.

EncyclopeDIA workflow



FASTA or Library

Narrow Window DIA File

PECAN

Chromatogram Library

Wide Window DIA File

Deconvolute Overlapping Windows

Compute Match Features

Retention Time Filtering

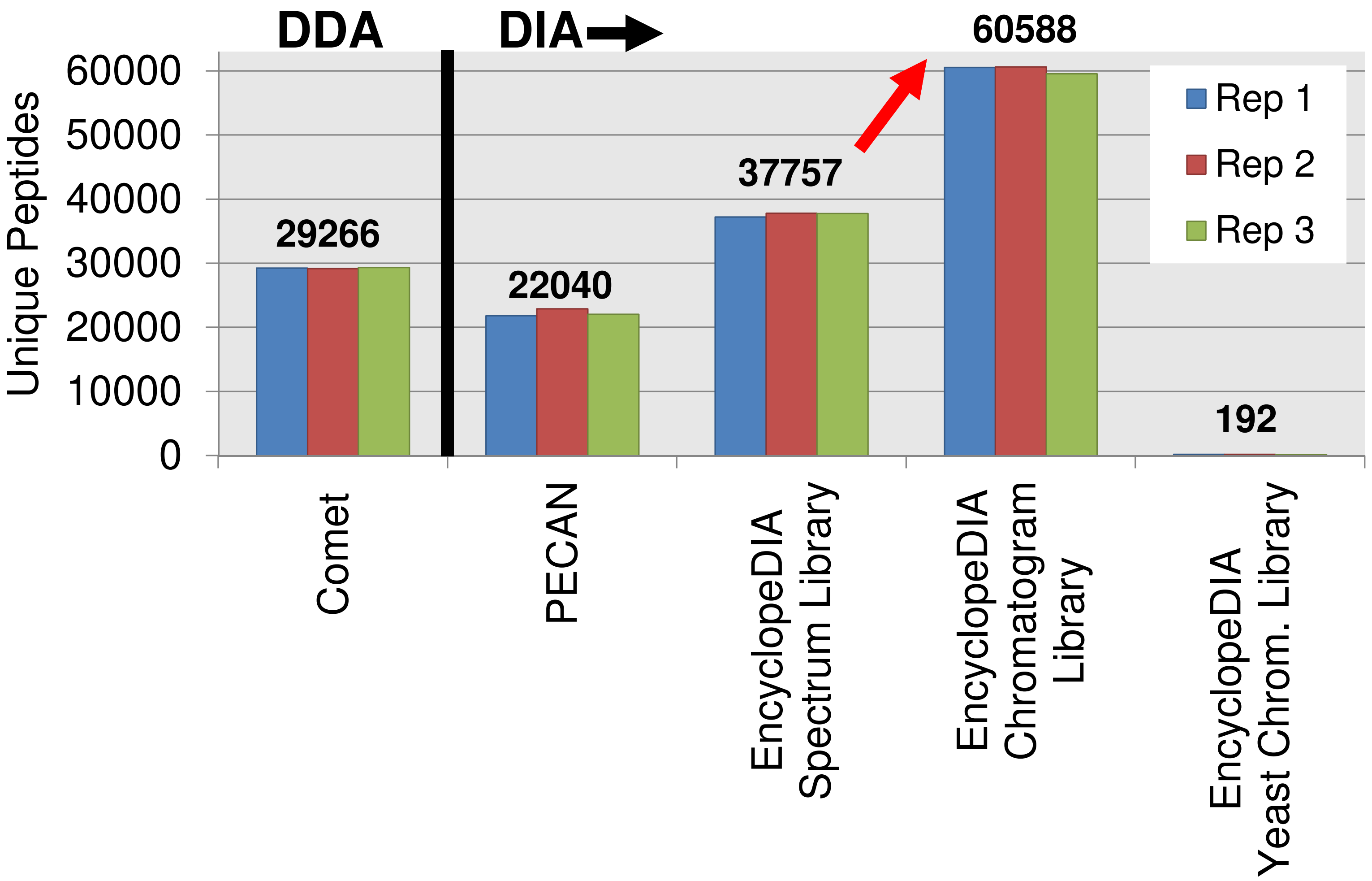
Percolator

Automated Transition Refinement

Quantitation

- 🟦 Typical DIA library search
- 🟧 EncyclopeDIA innovations
- 🟩 Chromatogram libraries

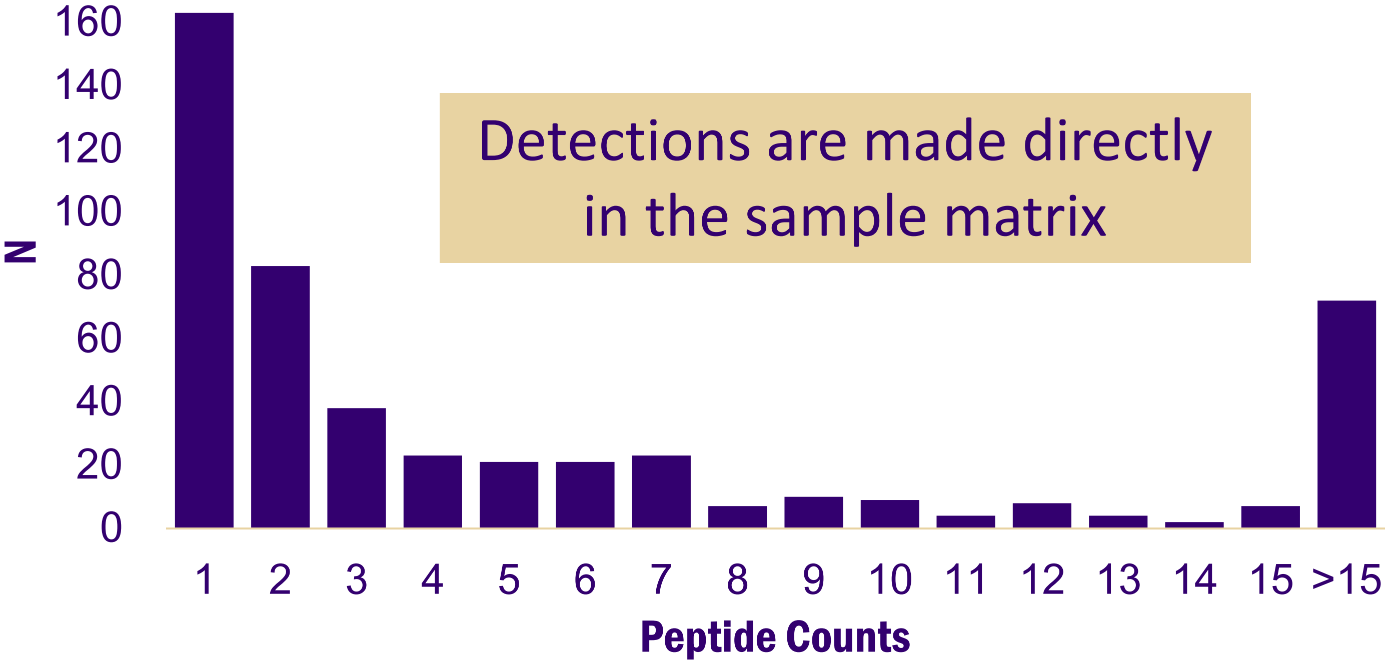
Chromatogram libraries are significantly more powerful than spectrum libraries



Human Plasma Chromatogram Library

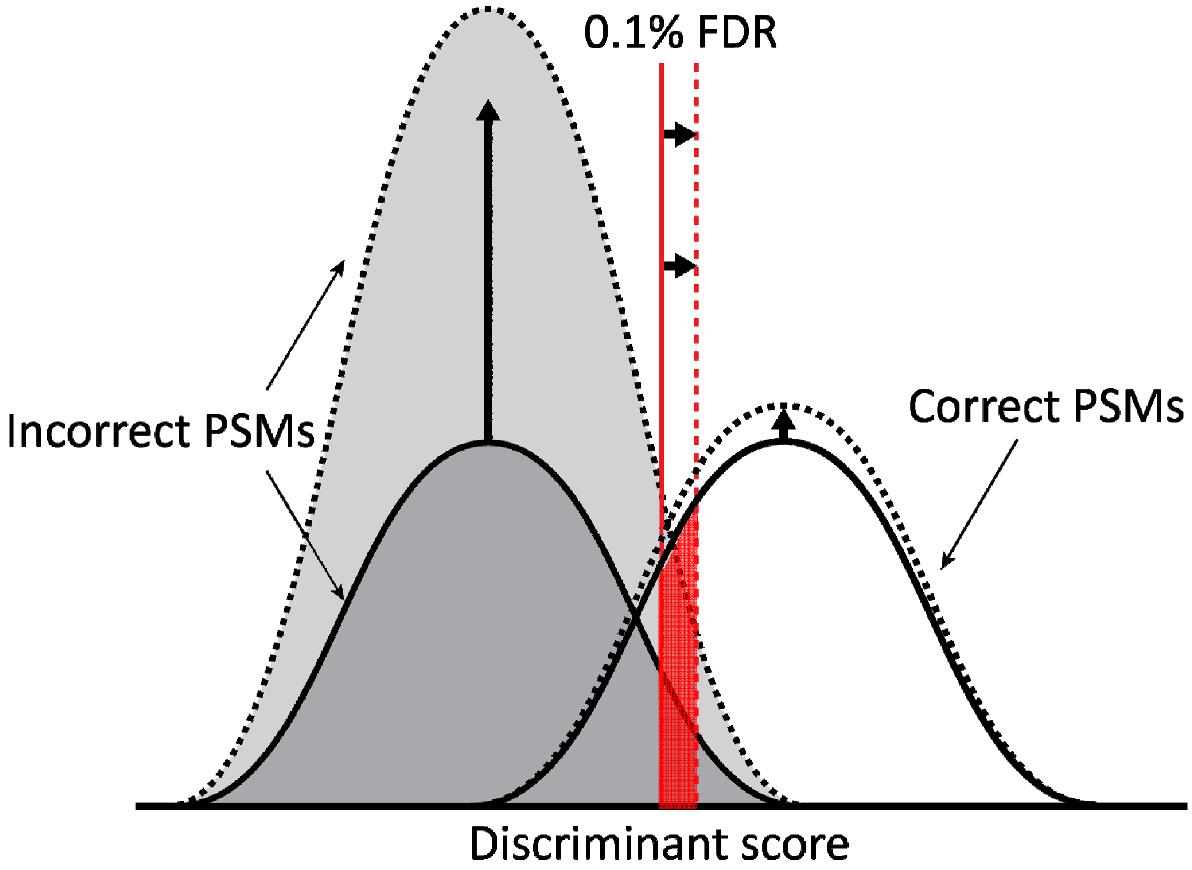
4,244 unique peptides | 495 proteins

3,880 peptides mapping to one protein

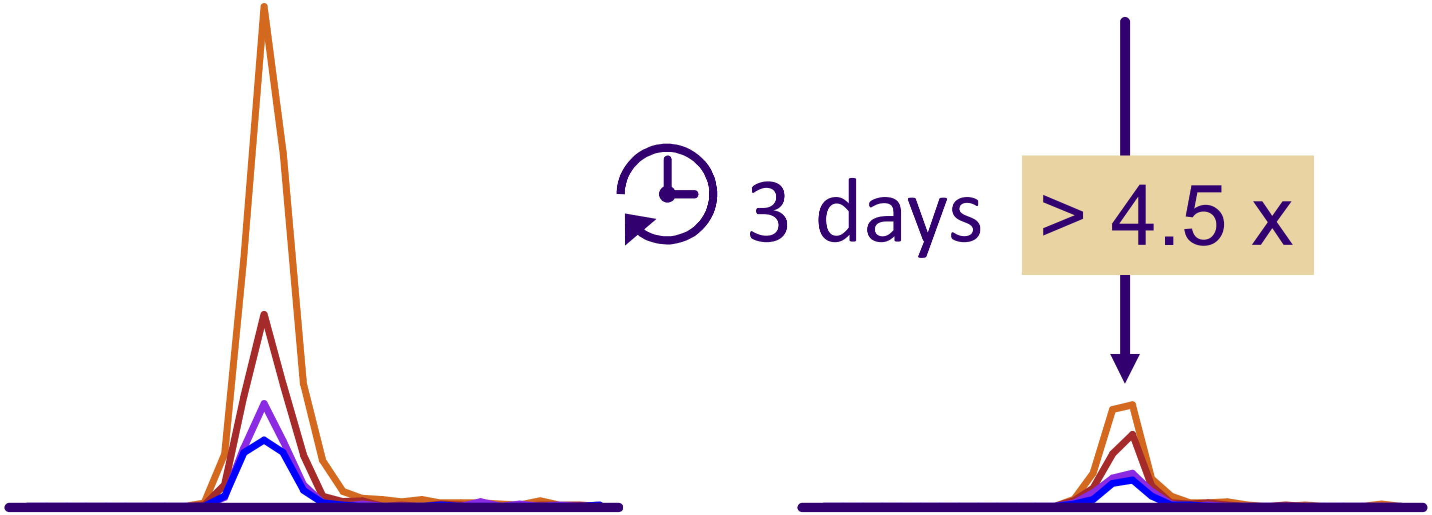


Detections are made directly in the sample matrix

Lesson 2: Don’t Use a Large Library Just Because You Can



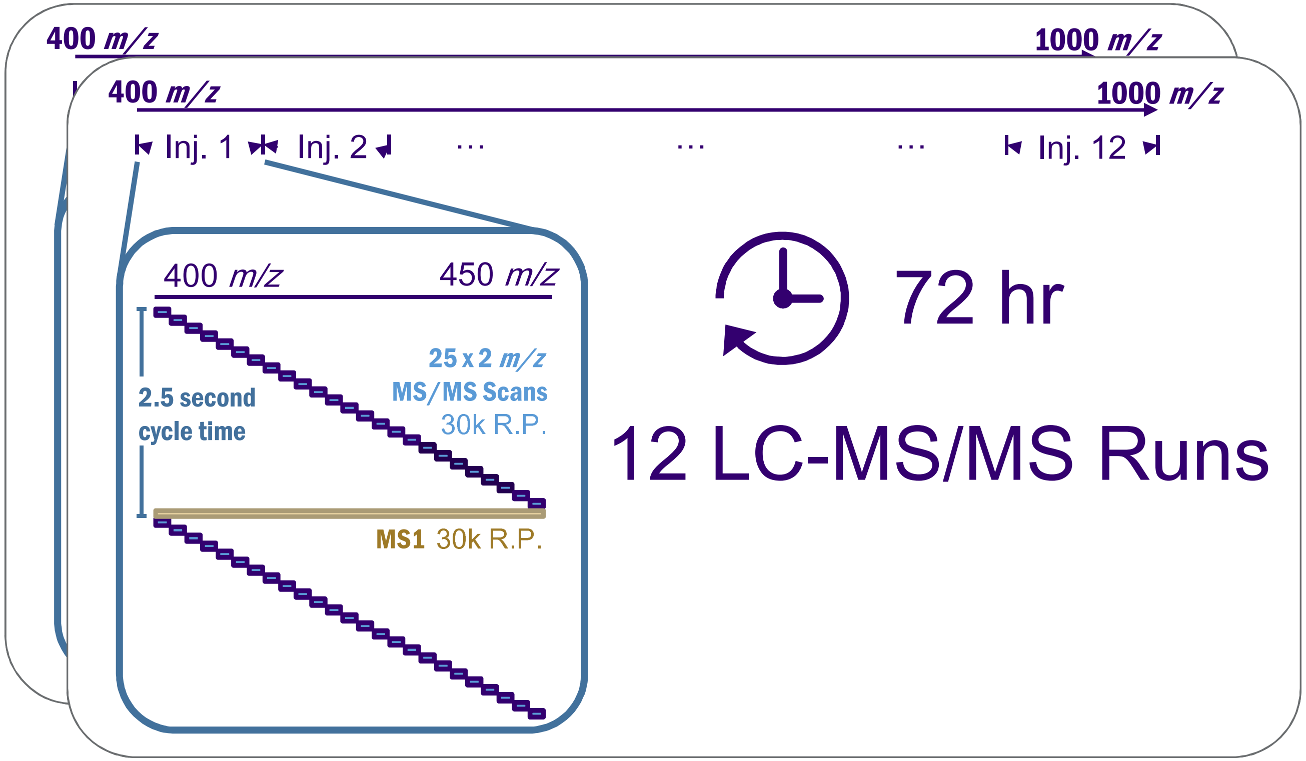
Lesson 3: Not all Peptides are Stable



Apolipoprotein B100 | TTLTAFGFASADLIEIGLEGK @ 4°C

Jim Bollinger | ASMS 2014

Assessing Peptide Stability with DIA

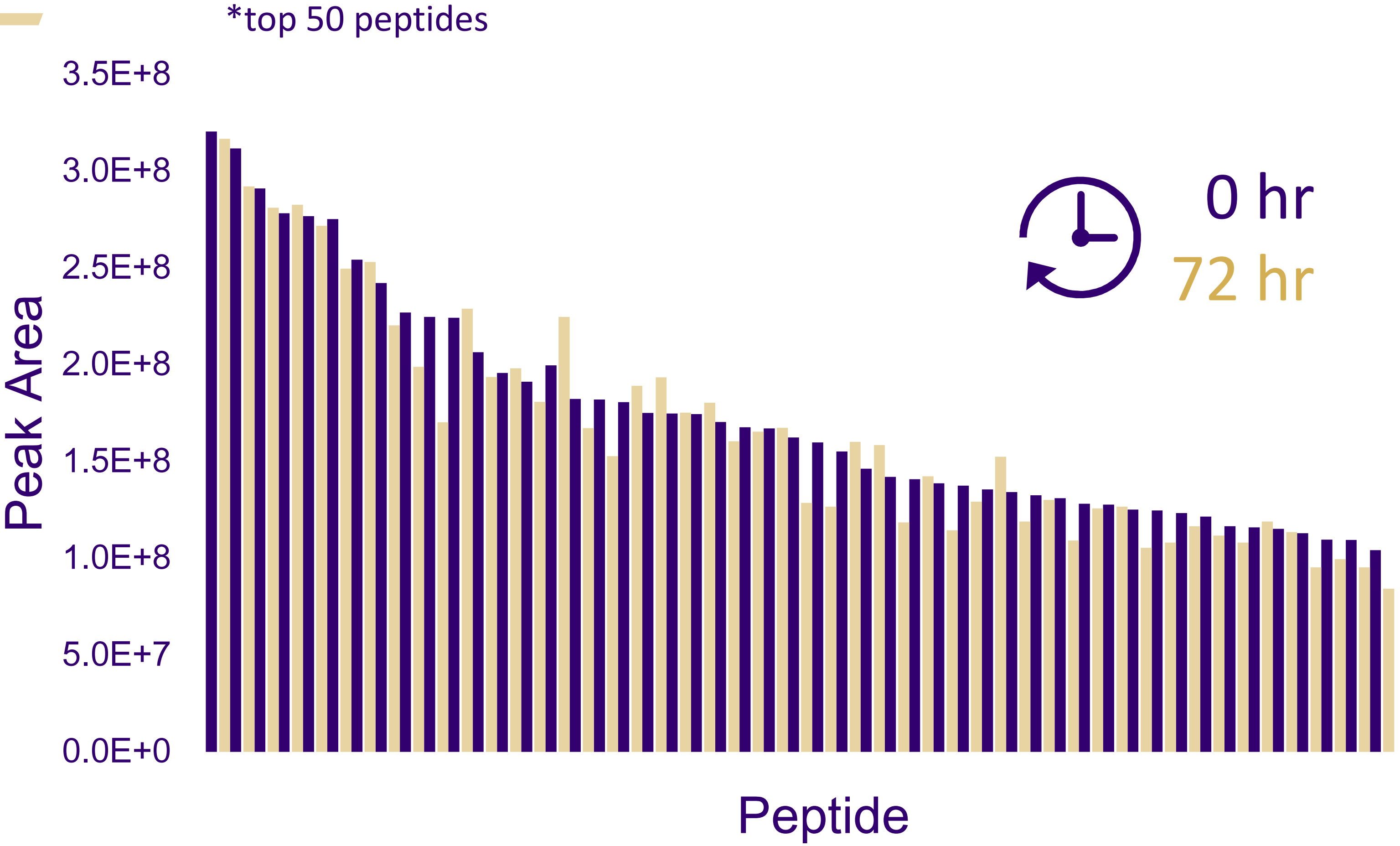


72 hr

12 LC-MS/MS Runs

Peptide Stability

Apolipoprotein B100



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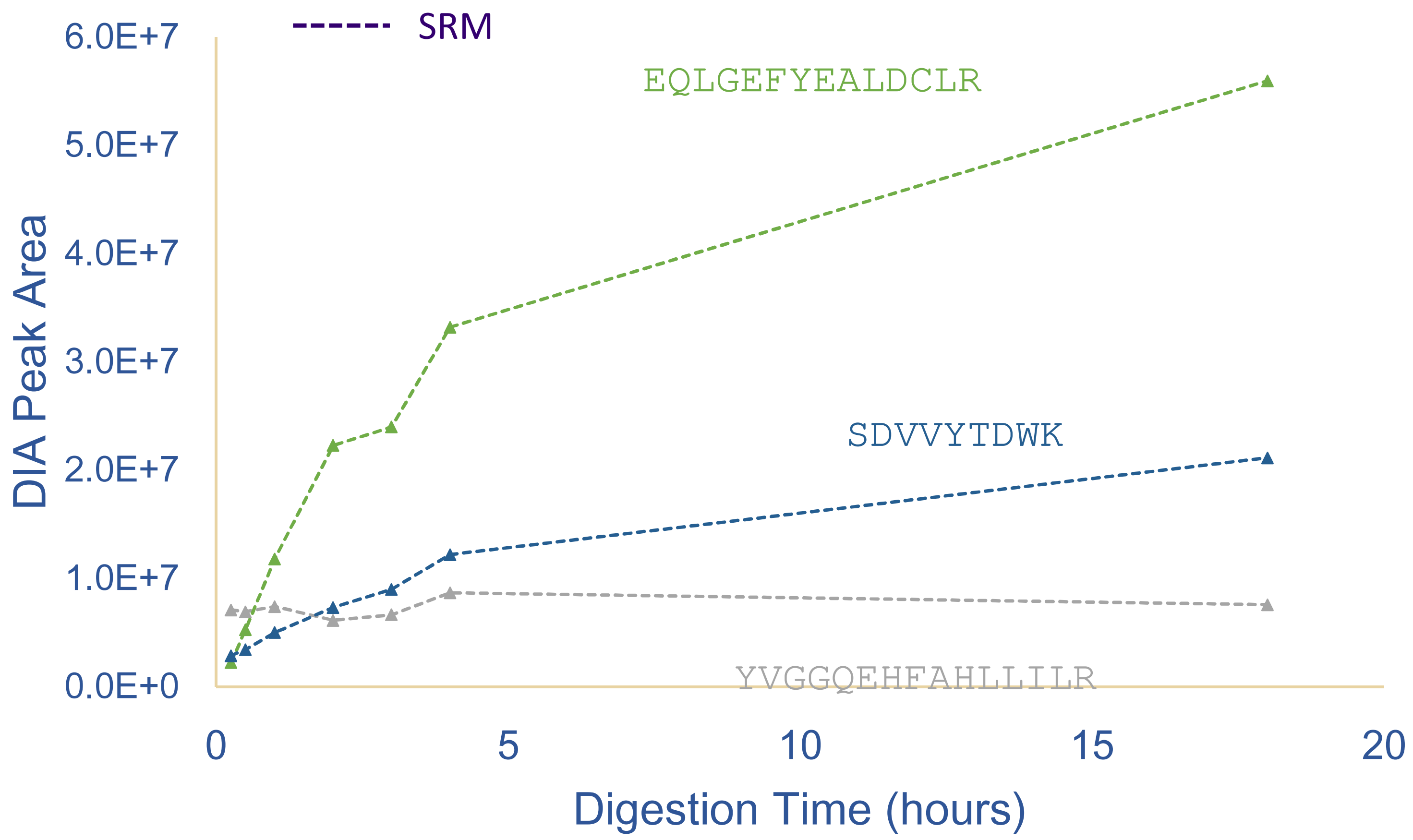
Lesson 4: Know your Digestion

Alpha-1-acid glycoprotein



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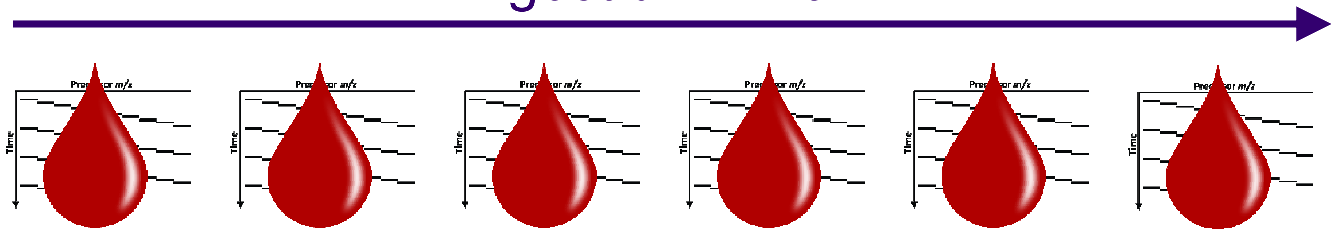
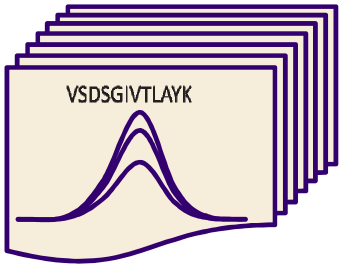
Digestion Time Course by DIA

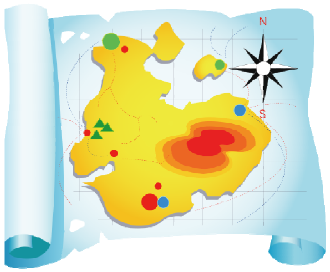
Digestion Time



15 min 1 hr 2 hr 4 hr 10 hr 18 hr

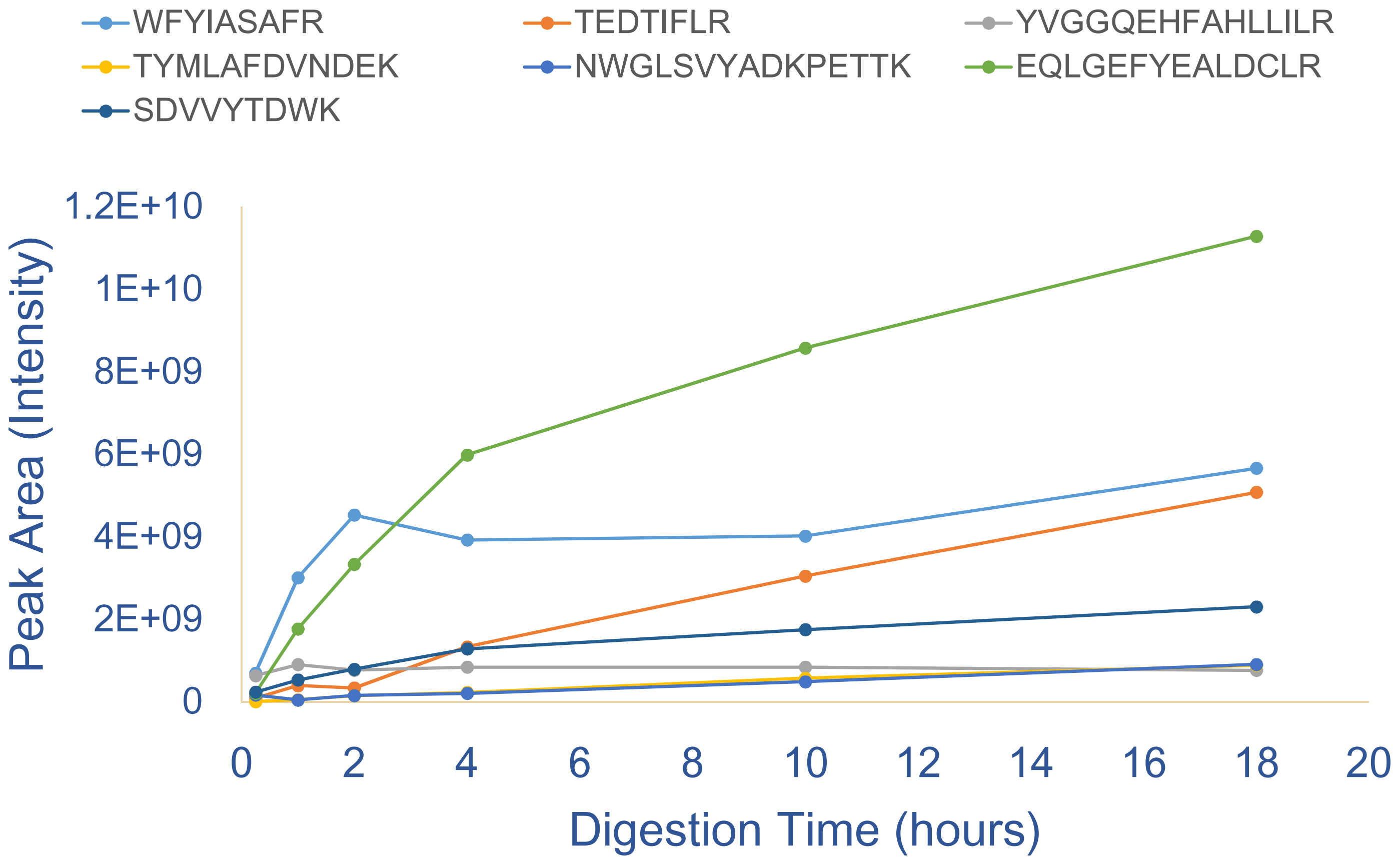
1 DIA Run
20 *m/z* Isolation
/per sample





Digestion Time Course (DIA)

Alpha-1-acid glycoprotein



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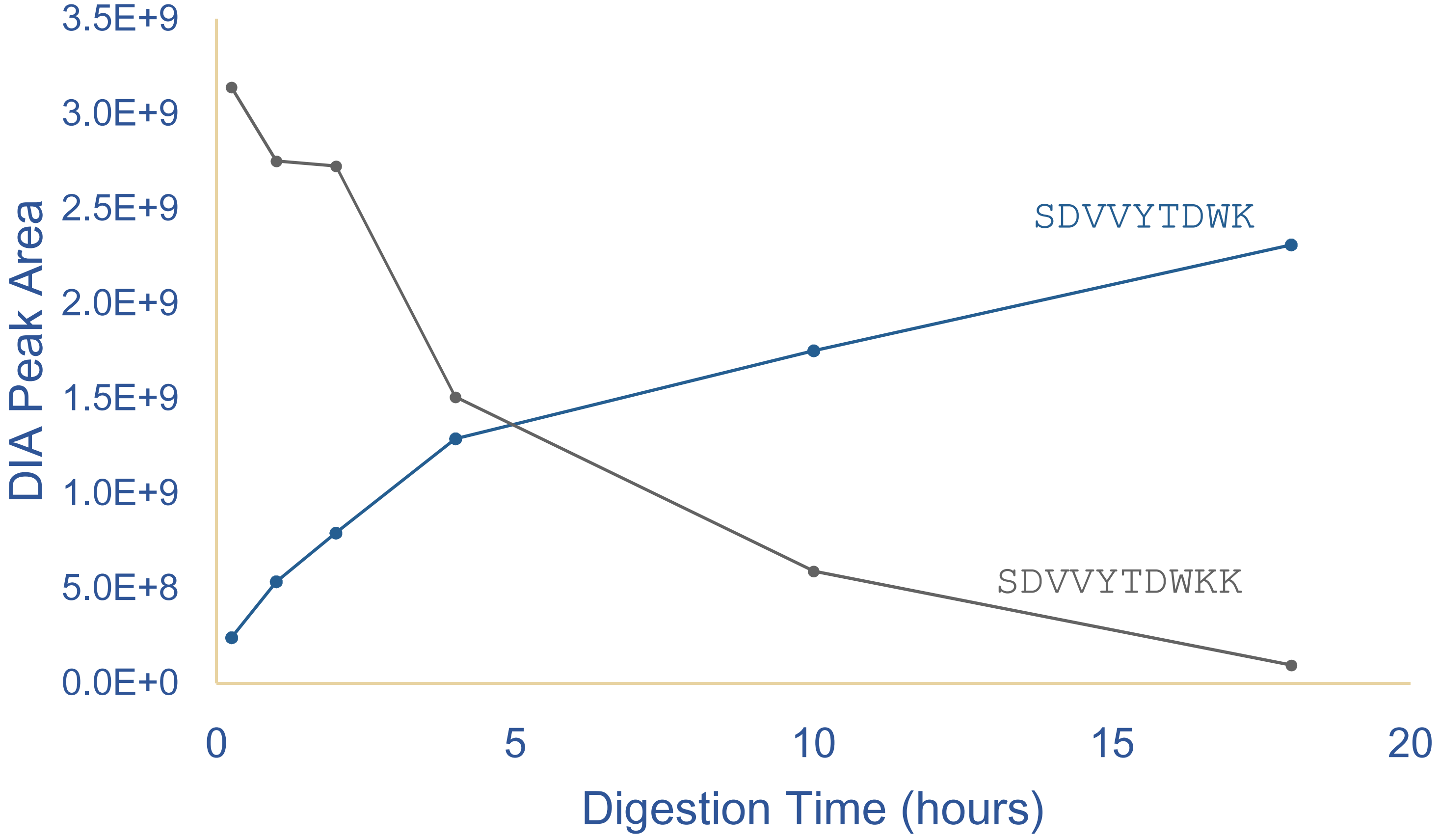
Investigating Digestion Kinetics

Alpha-1-acid glycoprotein



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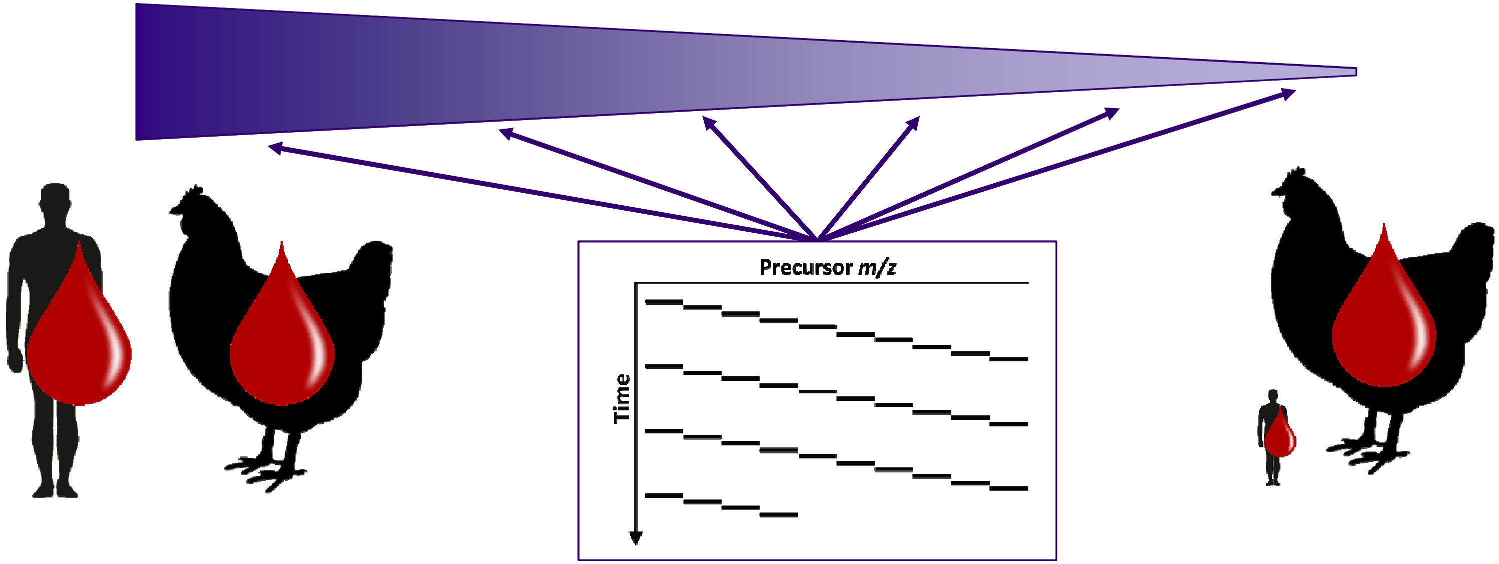
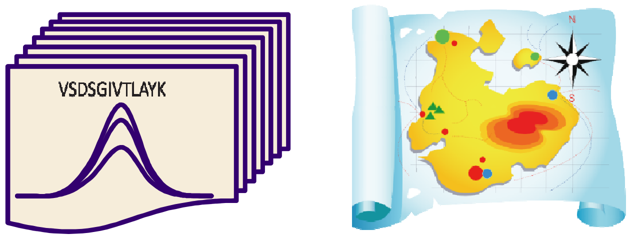
Lesson 5: Evaluate Linearity and LoQ

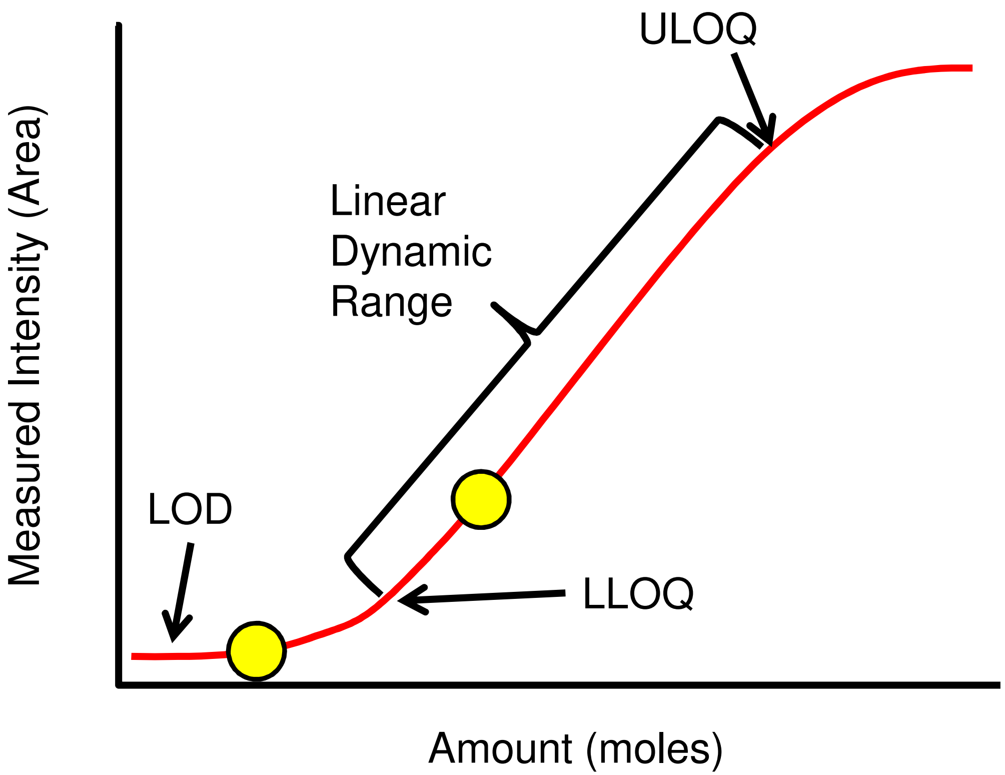




6 LC-MS/MS Runs
(not including replicates)

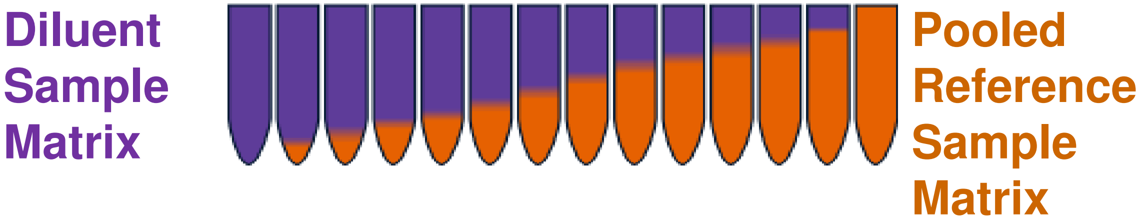
Grant RP, Hoofnagle AN, Clinical Chemistry 2014

Are the measurements quantitative or just differential?



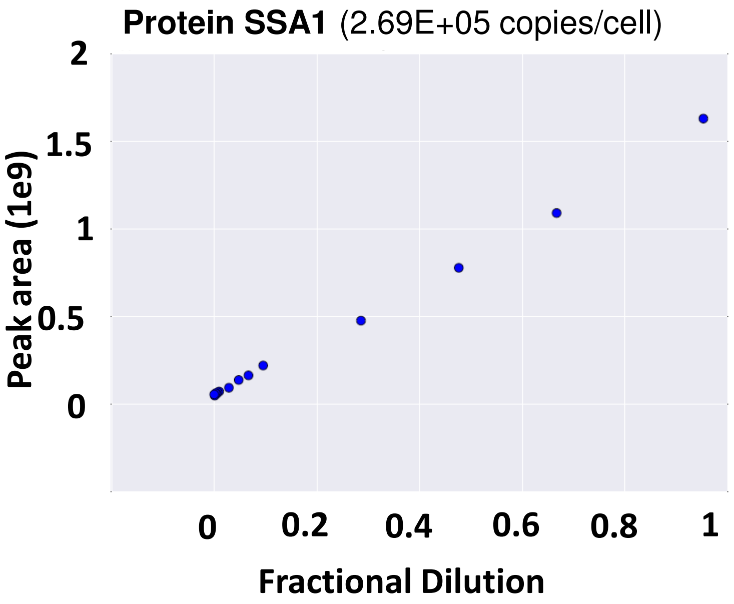
LOD: Limit of Detection
LLOQ: Lower Limit of Quantitation
ULOQ: Upper Limit of Quantitation

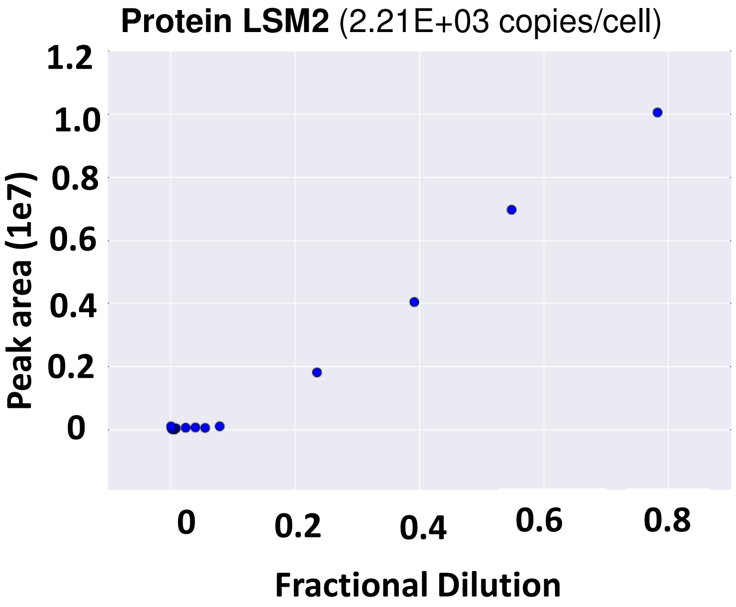
Method to measure both LOQ and Linearity



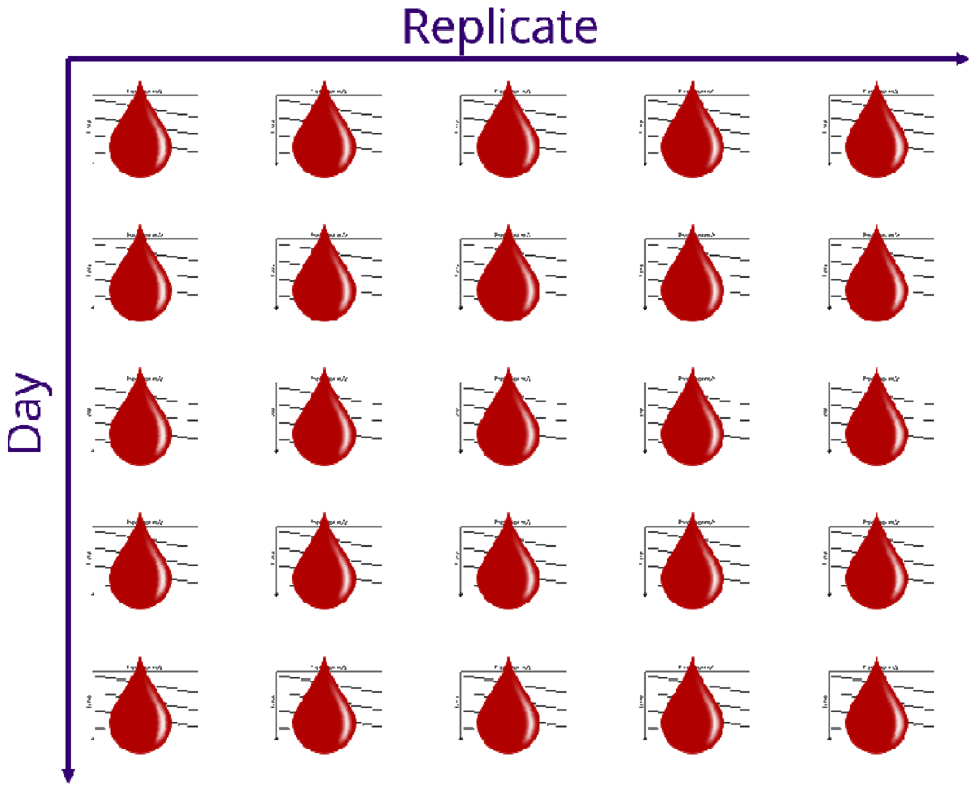
- Possible Samples to Use as a Diluent Matrix
  - Stable isotope labeled version of the matrix.
    - 15N or SILAC labeled cells
  - A diverged species
    - For human plasma we use chicken plasma.

Reference Yeast BY4742 Diluted in 15N Yeast (S288c)





Assess Reproducibility (5x5)



Grant RP, Hoofnagle AN, Clinical Chemistry 2014

DIA Assay Workflow

Peptide Detection and Stability
24 x 2 hr LC-MS/MS

Digestion Time Course
8 x 2 hr LC-MS/MS

Linearity / LoQ Assessment
10 x 2 hr LC-MS/MS

Reproducibility
25 x 2 hr LC-MS/MS

67 LC-MS/MS Runs

Alexey

YEAH, WELL, THAT'S JUST, LIKE, YOU KNOW, YOUR OPINION, MAN.

DIA

Is it a shotgun proteomics method?

Yes, DIA as not "less shotgun" than DDA

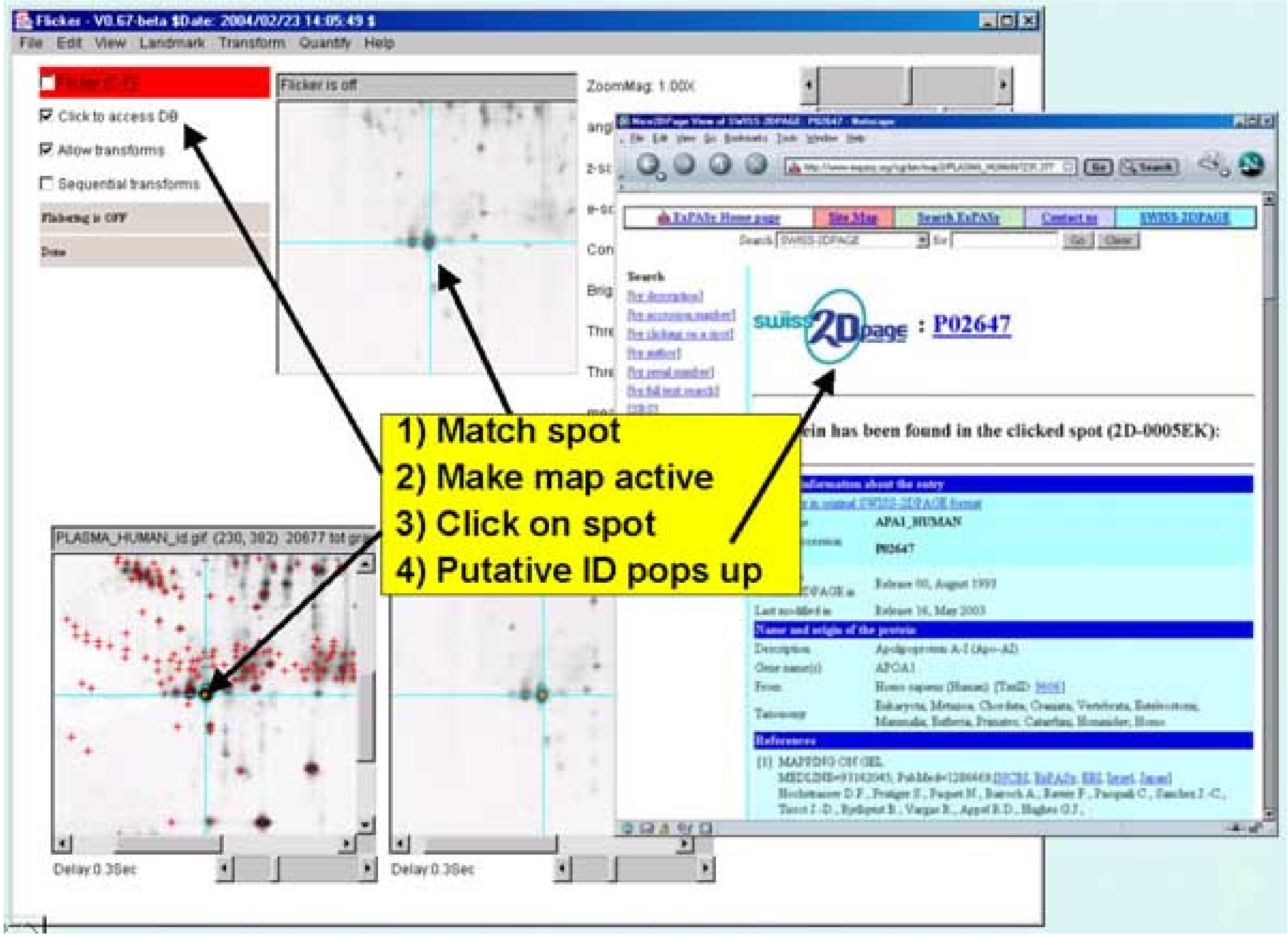
Is it a discovery proteomics method?

Yes, DIA is a untargeted data acquisition method. It is even "less targeted" than DDA

Lessons from History

High throughput proteomics methods that rely on the previously generated (proteomics) data have not been very successful

- Reference databases of 1D and 2D SDS Page gels (SWISS-2DPAGE database)
- AMT (accurate mass and time) approach
- Spectral library searching as replacement for database search



1) Match spot
2) Make map active
3) Click on spot
4) Putative ID pops up

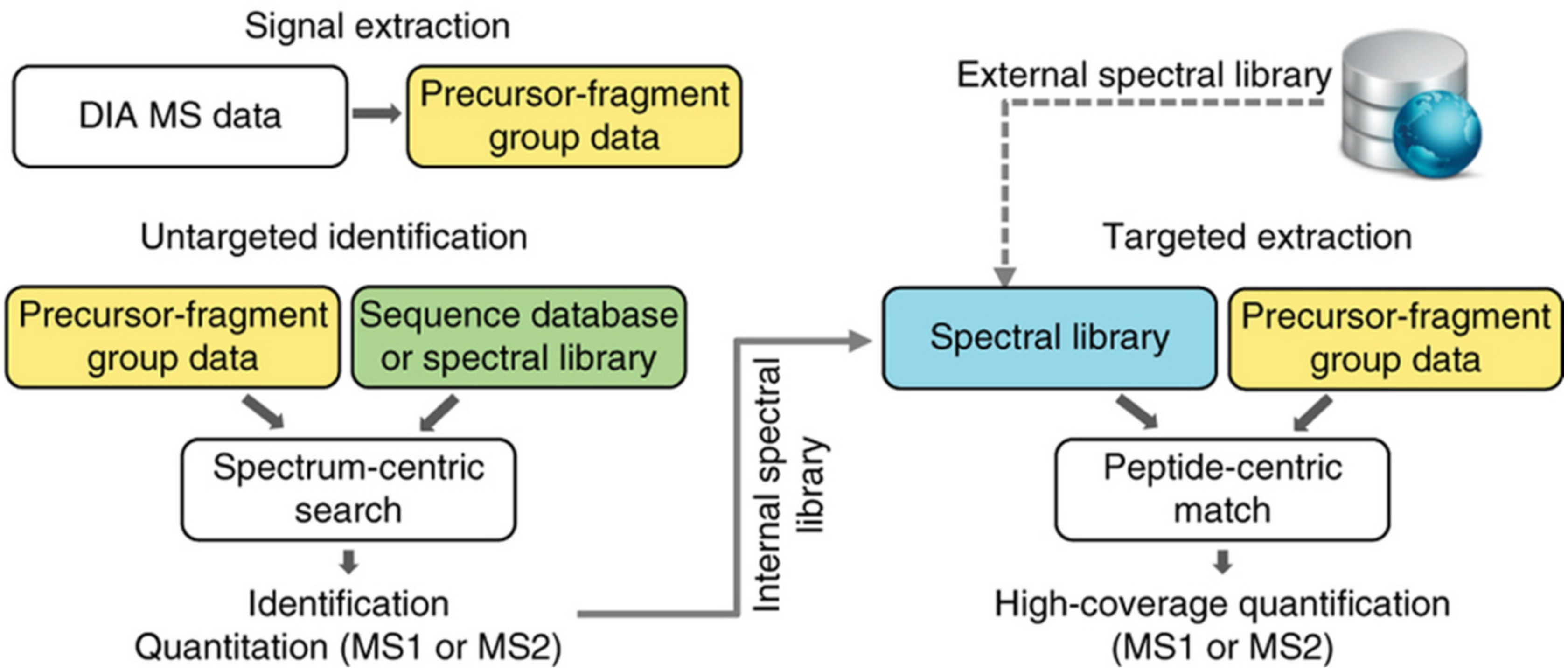
swiss2Dpage : P02647

…ein has been found in the clicked spot (2D-0005EK):

…nformation about the entry	
…y in original SWISS-2DPAGE format	
…e	APA1_HUMAN
…ccession	P02647
…2DPAGE in	Release 00, August 1993
Last modified in	Release 16, May 2003
Name and origin of the protein	
Description	Apolipoprotein A-I (Apo-AI)
Gene name(s)	APOA1
From	Homo sapiens (Human). (TaxID: 9606)
Taxonomy	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo
References	

[1] MAPPING ON GEL.
MEDLINE=93142045; PubMed=1286669 [NCBI, ExPASy, EBI, Israel, Japan]
Hochstrasser D.F., Frutiger S., Paquet N., Bairoch A., Ravier F., Pasquali C., Sanchez J.-C., Tissot J.-D., Bjellqvist B., Vargas R., Appel R.D., Hughes G.J.;

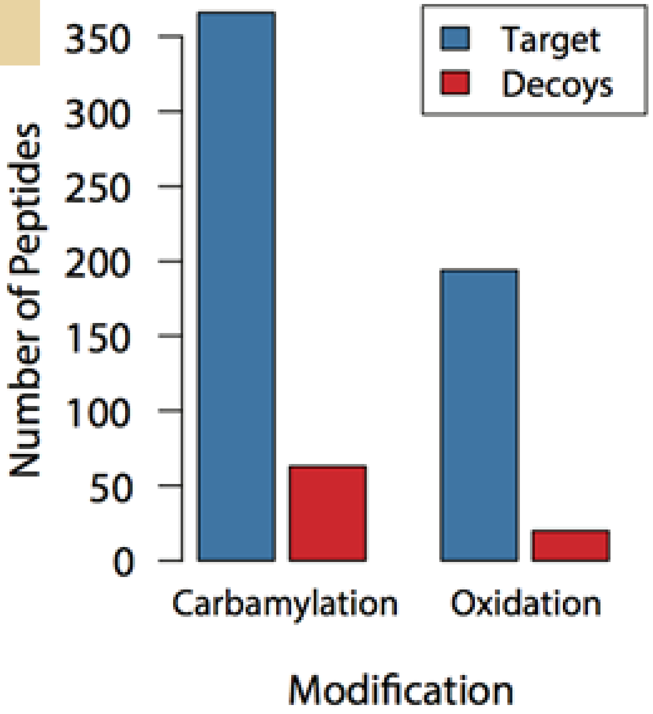
Hybrid (Direct+ Targeted) Strategy


Signal extraction
DIA MS data
Precursor-fragment group data
External spectral library
Untargeted identification
Targeted extraction
Precursor-fragment group data
Sequence database or spectral library
Spectral library
Precursor-fragment group data
Spectrum-centric search
Internal spectral library
Peptide-centric match
Identification
Quantitation (MS1 or MS2)
High-coverage quantification (MS1 or MS2)

C.C. Tsou *et al.* DIA-Umpire: comprehensive computational framework for data independent acquisition proteomics ***Nature Methods***, 2015

Oh this is bullshit...
8
7
6
5
4
3
2

Violation of the Target-Decoy Assumption in Closed Searches



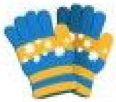
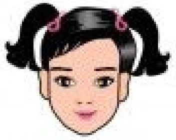
- Selected spectra corresponding to common modifications identified in open search and examined their identifications in closed search

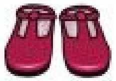
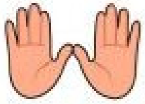
- Under target-decoy assumptions, these spectra should all be incorrect and match equally to target and decoy sequences

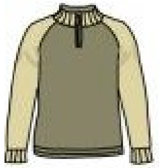
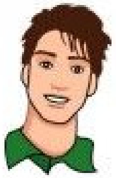
- Target-decoy assumption is violated: 6X difference for carbamylation, 9X for oxidation

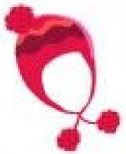
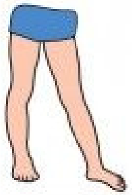
Clothes And Body Parts

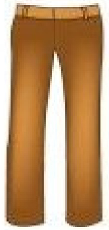
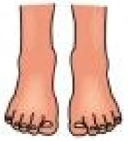
Match the images on the left to their corresponding images on the right.

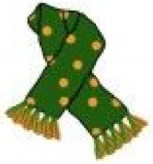
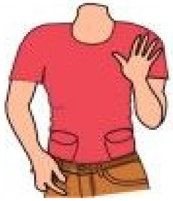
 ● ● 

 ● ● 

 ● ● 

 ● ● 

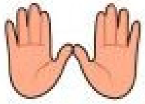
 ● ● 

 ● ● 

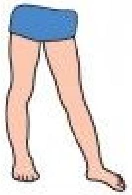
Clothes And Body Parts

Match the images on the left to their corresponding images on the right.

●

● ● 

●

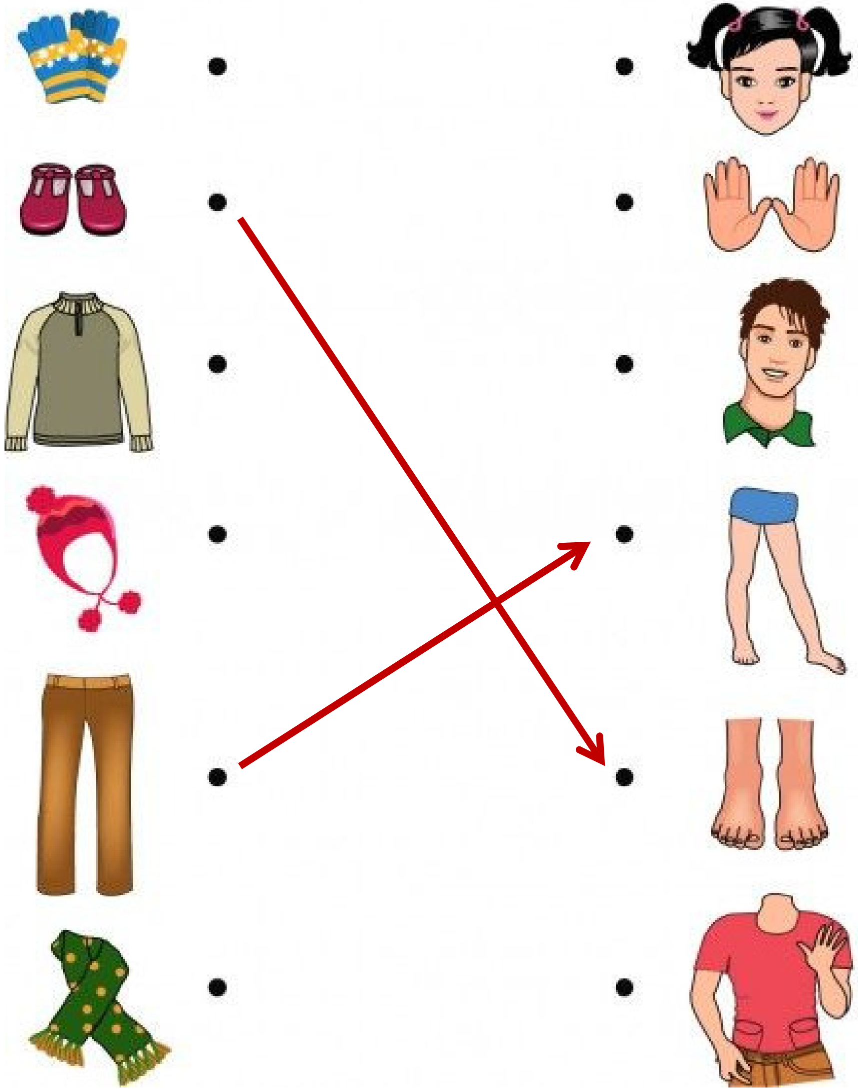
● 

●

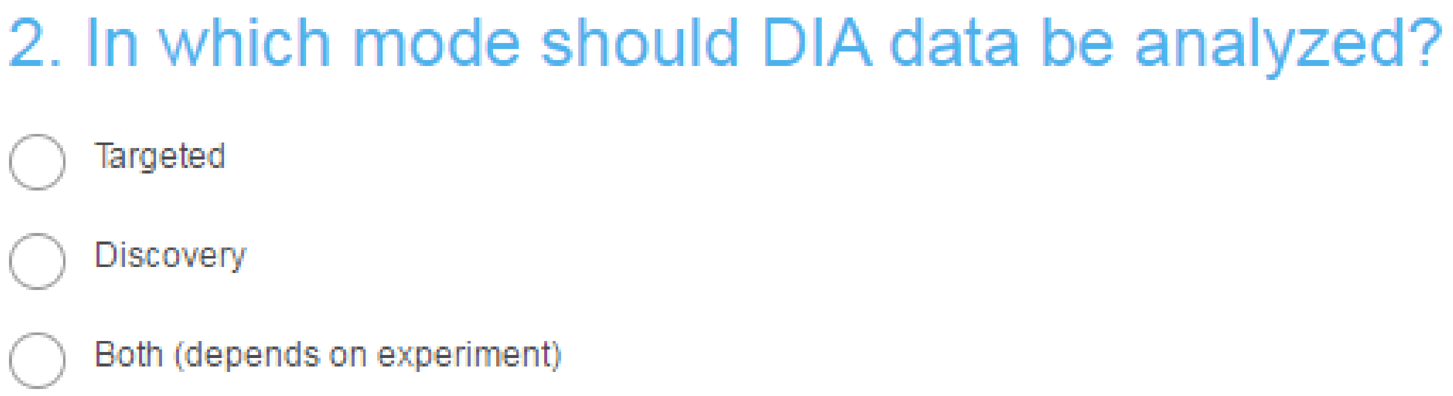
●

Clothes And Body Parts

Match the images on the left to their corresponding images on the right.



Survey question 2



2. In which mode should DIA data be analyzed?

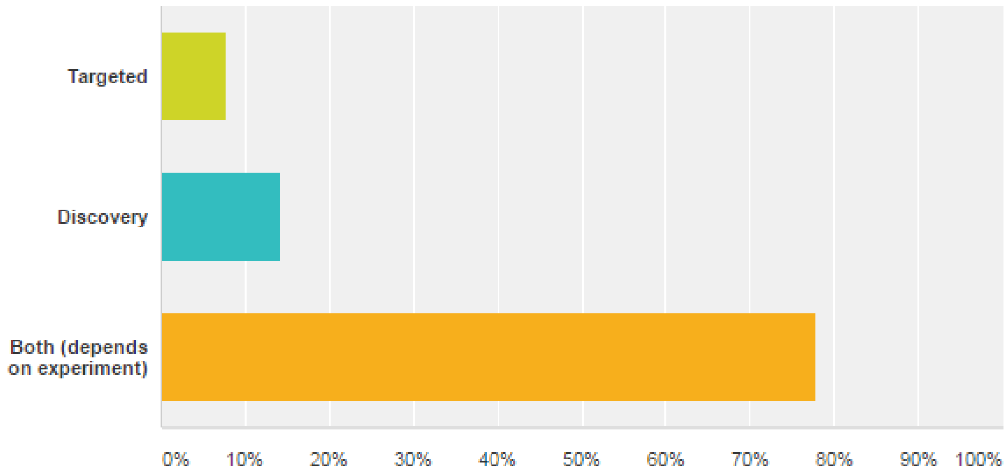
○ Targeted

○ Discovery

○ Both (depends on experiment)

In which mode should DIA data be analyzed?

Answered: 77 Skipped: 1



Answer Choices	Responses	
Targeted	7.79%	6
Discovery	14.29%	11
Both (depends on experiment)	77.92%	60
Total		77

Isabell

Scaling up DIA – Error rate control

focused

DIA ‘targeting’ scale

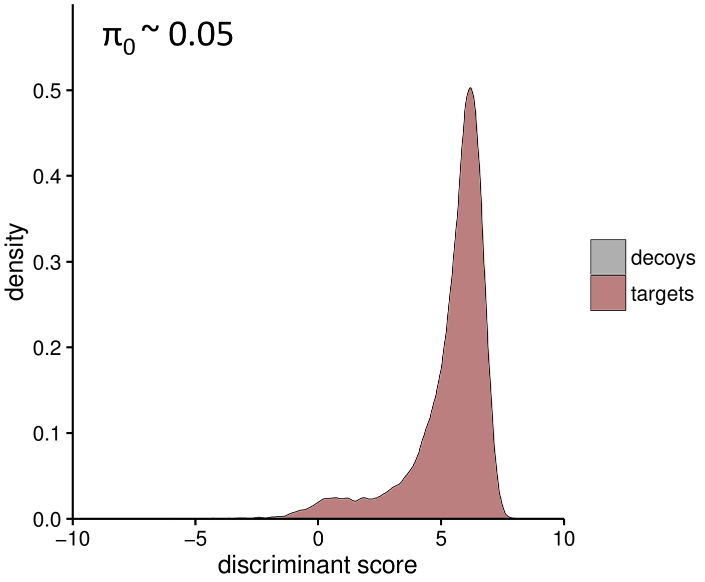
global

Sample-specific spectral library

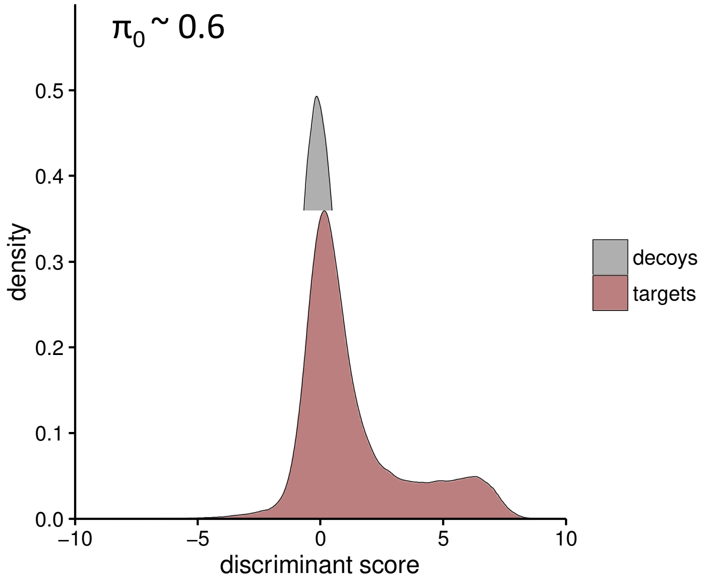
- Built from same samples as DIA data
- Majority of peptides/proteins detectable

Comprehensive deep spectral library

- Built from multiple different samples
- Majority of peptides/proteins NOT detectable



$\pi_0 \sim 0.05$



$\pi_0 \sim 0.6$

Scaling up DIA – Error rate control

focused

DIA ‘targeting’ scale

global

Sample-specific spectral library

- Built from same samples as DIA data
- Majority of peptides/proteins detectable

Comprehensive deep spectral library

- Built from multiple different samples
- Majority of peptides/proteins NOT detectable

Protein-level error can b handled on library generation level

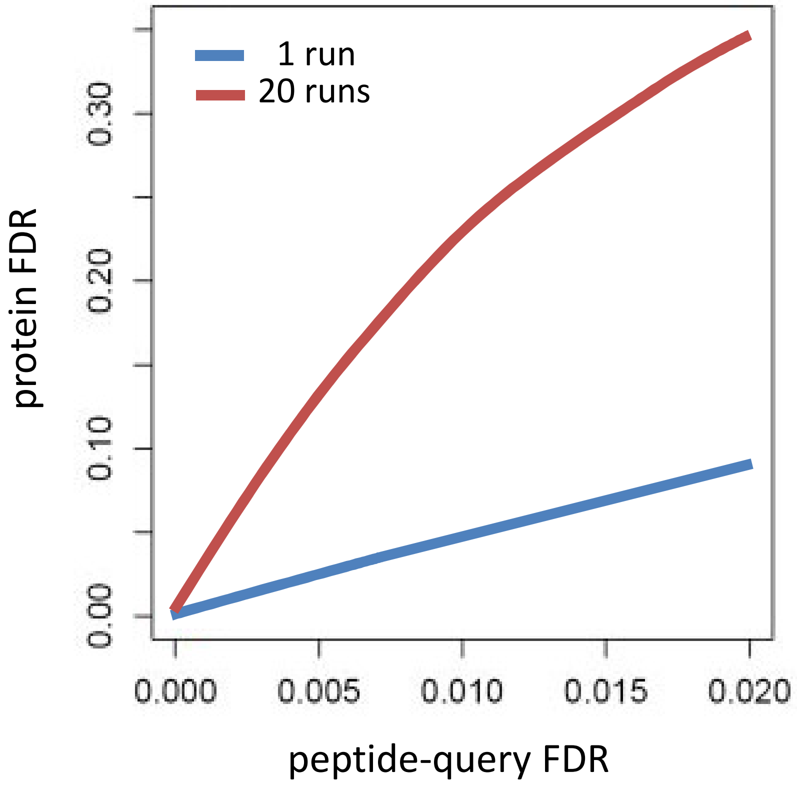
Protein-level error accumulates if not carefully controlled

Scaling up DIA – Error rate control

focused

DIA ‘targeting’ scale

global



Comprehensive deep spectral library

- Built from multiple different samples
- Majority of peptides/proteins NOT detectable

Protein-level error accumulates if not carefully controlled

Scaling up DIA – Error rate control

1. **Control error rate on protein level**:
   Take best peptide peak group per protein for FDR / q-value estimation on protein level

2. **Control error rate globally across all samples within a study: Protein master list**
   Take best peptide peak group per protein across all samples in a study to generate a protein master list at 1% FDR

Extended version of PyProphet: https://github.com/PyProphet

Rosenberger & Bludau *et al.* (submitted)

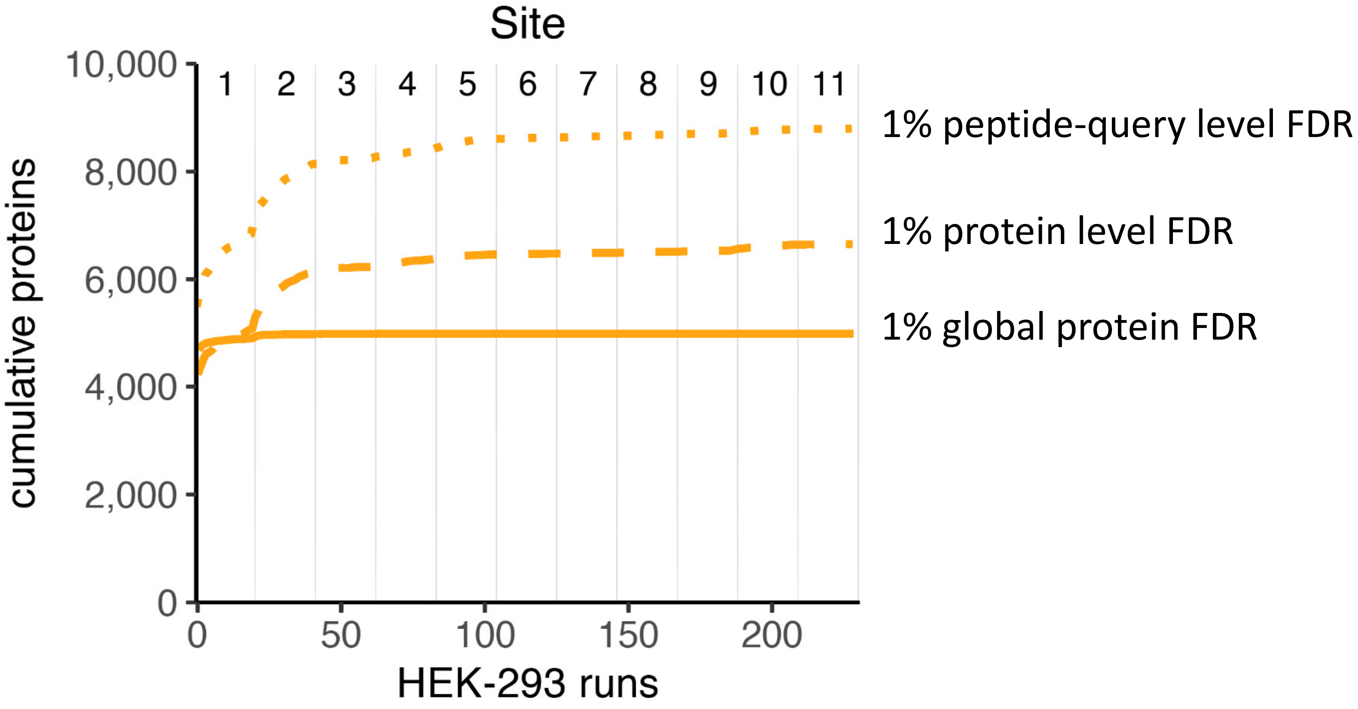
Scaling up DIA – Error rate control

DIA data:

- Inter-laboratory study:
  229 DIA measurements of same HEK-293 cell lysate
  Collins et al. (2017)

Spectral library:

- Combined assay library (CAL):
  331 DDA injections of different human tissues and cell types including HEK293
  Rosenberger, G. *et al.* (2014)



Scaling up DIA – Error rate control

DIA data:

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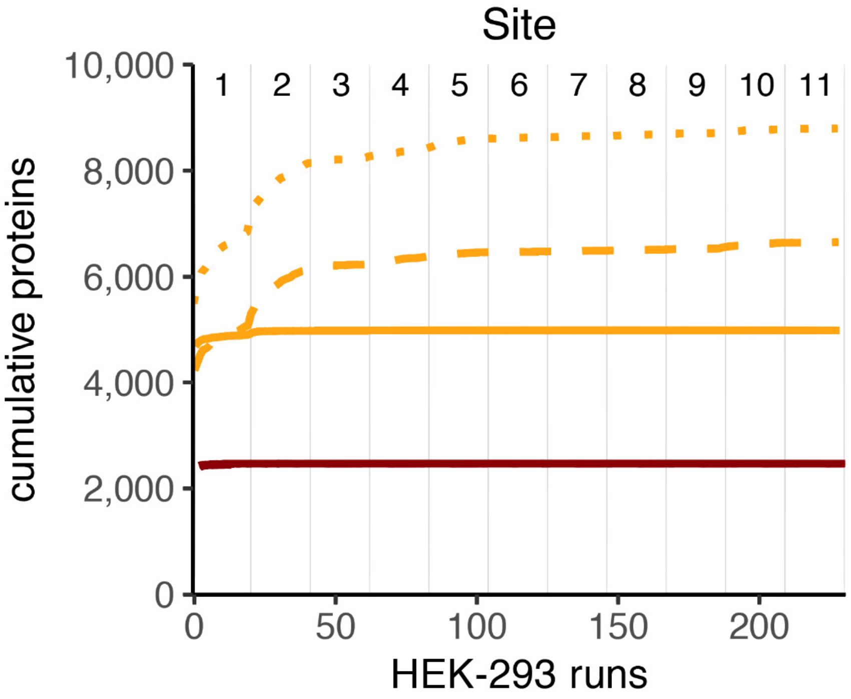


1% peptide-query level FDR

1% protein level FDR

1% global protein FDR

Sample specific spectral library

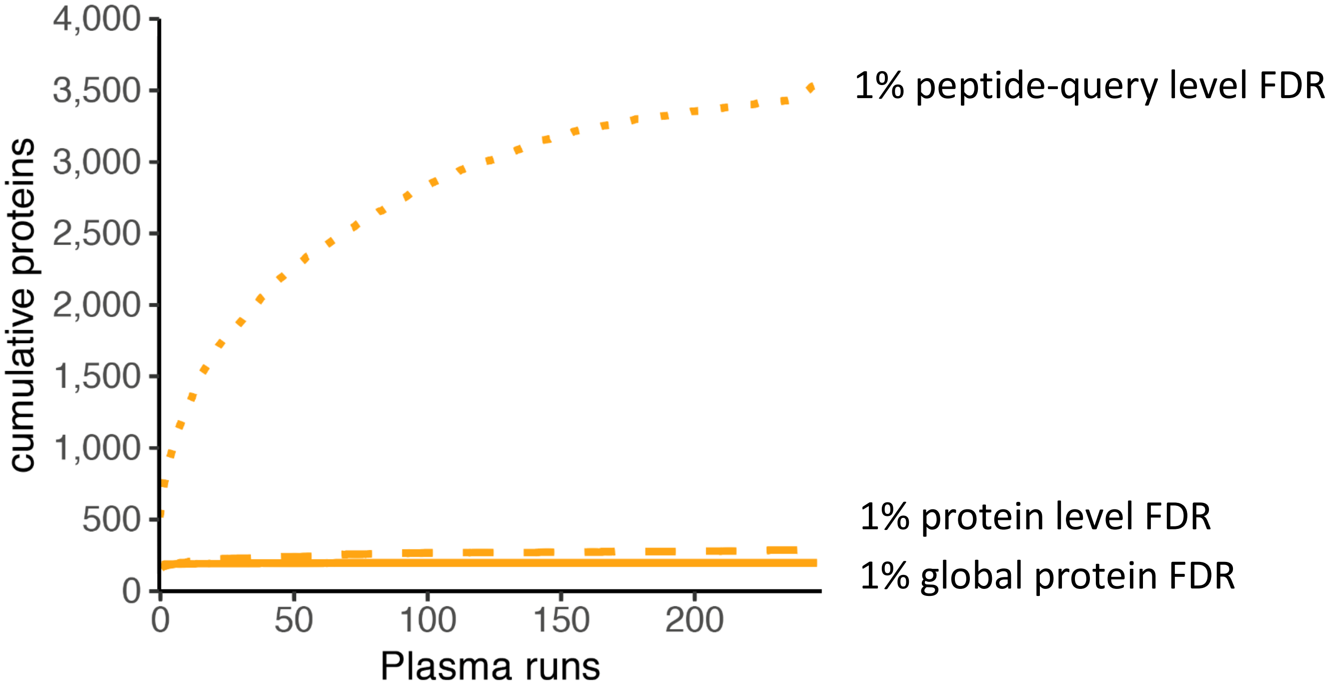
Scaling up DIA – Error rate control

DIA data:

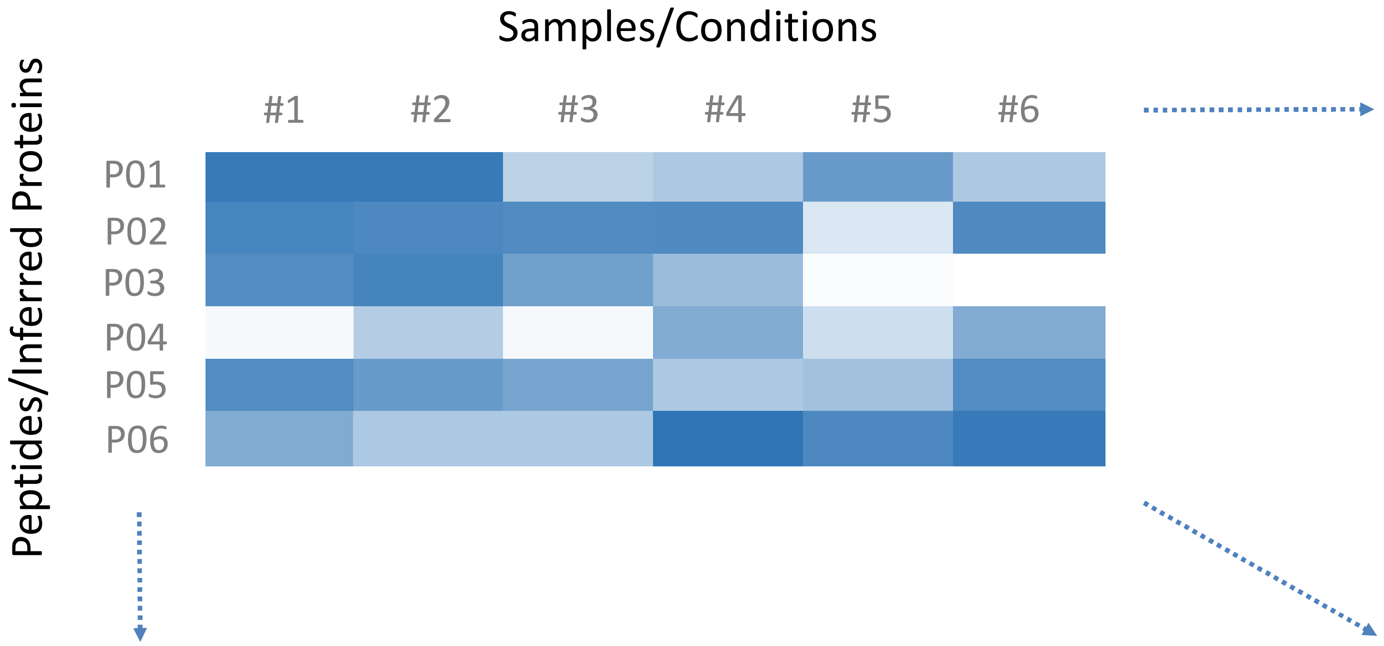
- Blood plasma dataset with 246 samples
  Liu et al. (2015)

Spectral library:

- Combined assay library (CAL):
  331 DDA injections of different human tissues and cell types including HEK293
  Rosenberger, G. *et al.* (2014)



Scaling up DIA – Error rate control

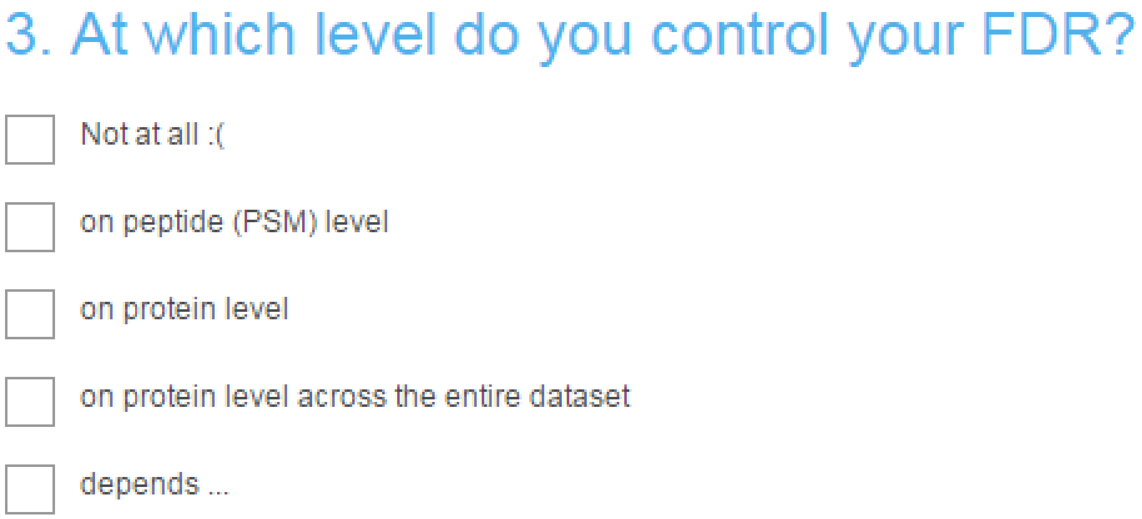


For large comprehensive spectral libraries:
Use protein-level FDR

&

Context matters!
Use global protein master list constraint to avoid error accumulation across samples!

Survey question 3



3. At which level do you control your FDR?

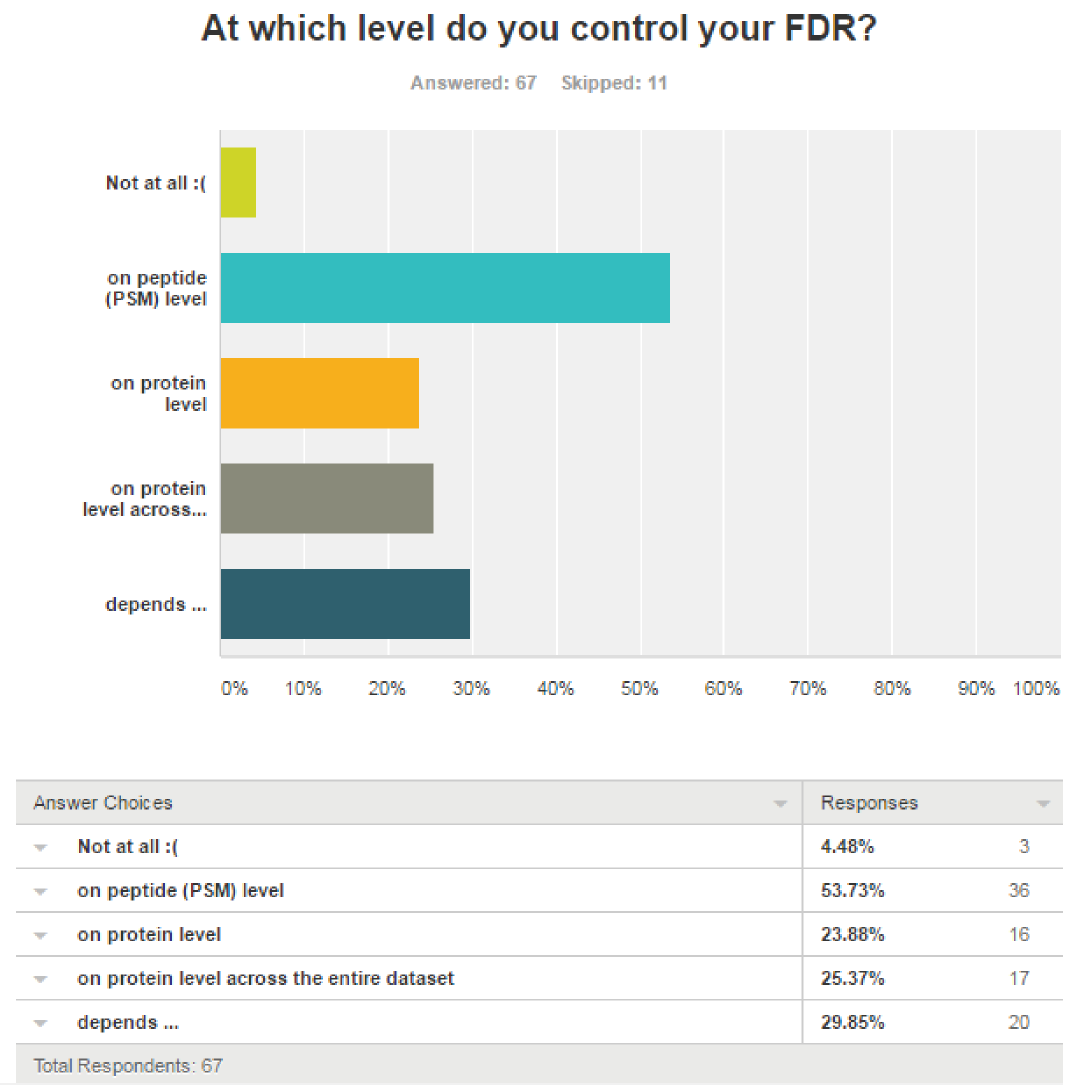
□ Not at all :(

□ on peptide (PSM) level

□ on protein level

□ on protein level across the entire dataset

□ depends ...



At which level do you control your FDR?

Answered: 67 Skipped: 11

Answer Choices	Responses	
Not at all :(	4.48%	3
on peptide (PSM) level	53.73%	36
on protein level	23.88%	16
on protein level across the entire dataset	25.37%	17
depends ...	29.85%	20
Total Respondents: 67		

Lukas

ASMS 2017

DIA Workshop – “Depth of Proteome Coverage”

Lukas Reiter, Biognosys

Depth of Proteome Coverage

Why having a large proteome coverage?

- Discovery
  - E.g. drug target deconvolution with Limited proteolysis (LiP) *
- Low abundant wish list proteins combined with discovery
- Multi OMICS -> increase overlap with other data sets

How can the proteome coverage be increased?

- Sample (prep)
- **Chromatography**
- Instrumentation
- **DIA Method**
- **Spectral library**
- **Precision iRT**

Leuenberger et al. Cell-wide analysis… Science (2017)

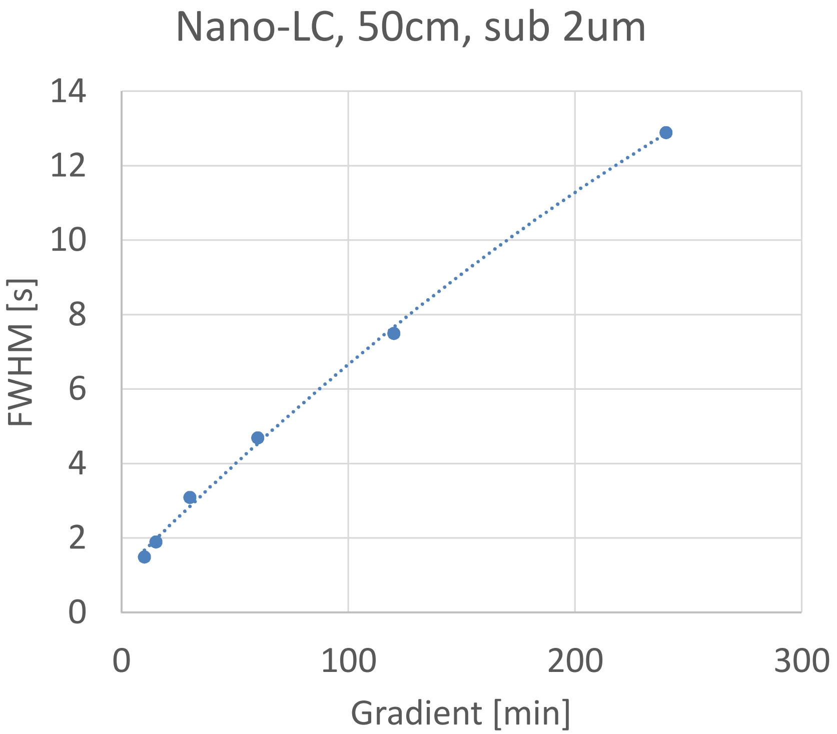
Chromatography

How to get a high peak capacity?

- UHPLC
- Nano-LC
- 75um ID long columns
- Sub 2um beads
- Long gradients
- Low dead volumes

1m column, 4h gradient

- Peak capacity > 700



FWHM:	measured as median for all peptides identified in a HeLa digest
Peak capacity:	1 + g / (FWHM*1.7)

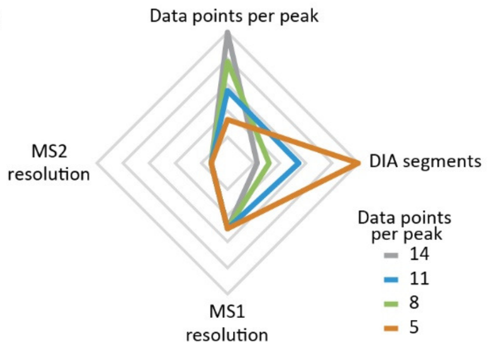
DIA Method

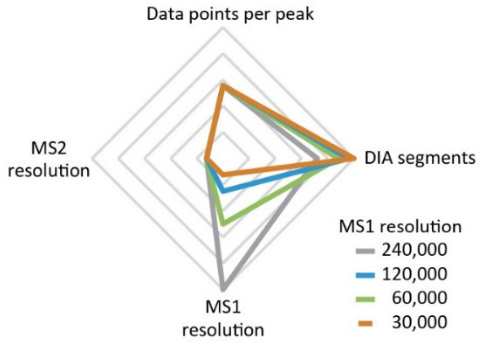
Step by step optimization

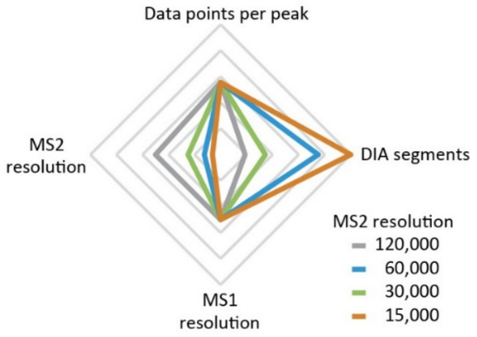
1) Data points per peak
   - Generate a scouting method which over samples the peaks
   - Scale the number of MS2 segments from the scouting method to result in 5, 8, 11 and 14 data points per peak
   - Pick the best method

2) MS1 resolution
   - Vary the MS1 resolution from 30’000 to 240’000 (balance the MS2 segments to keep data points per peak constant)
   - Pick the best method

3) MS2 resolution
   - Vary the MS2 resolution from 15’000 to 120’000 (balance the MS2 segments to keep data points per peak constant)
   - Pick the best method







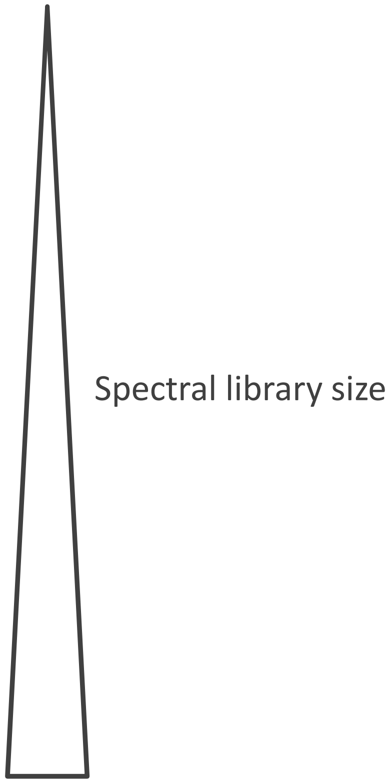
Spectral Library

Evolution over time for Biognosys

- Replicate injections and DDA on project samples
- Mild fractionation
  - 6 high pH reversed phased fractions
- Deep fractionation
  - Two condition pools
  - Pooled micro fractions from UHPLC
  - 10 fractions each

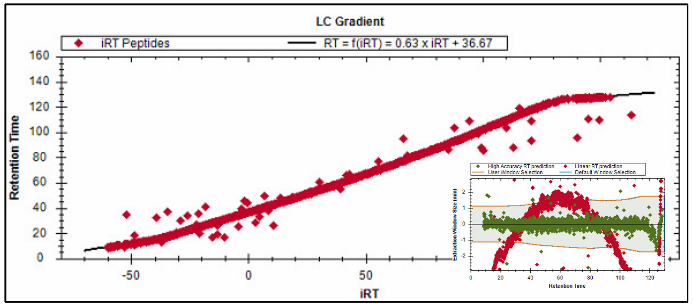
…

- Resource spectral libraries or from large sets of synthetic peptides



Precision iRT

- Extends the indexed retention time (iRT) concept
- Allows very precise targeting in retention time dimension
- Dynamically adapts
- XIC windows of 1-3% of gradient length can be achieved
    - Especially when using spectral libraries acquired on exactly the same setup



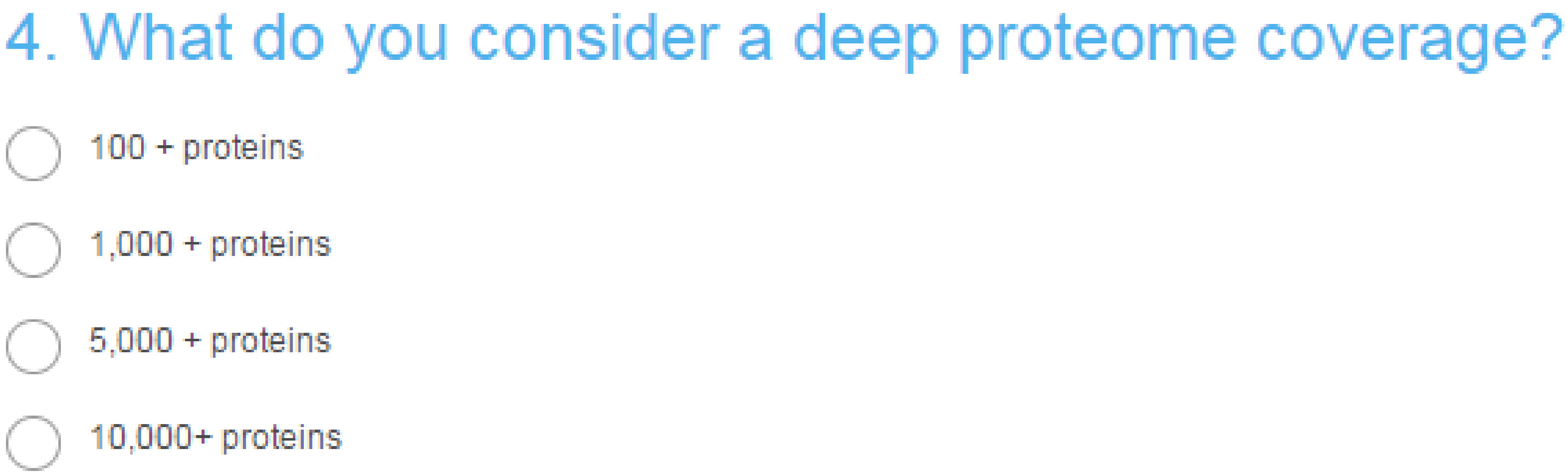
Bruderer et al. High-precision iRT prediction… Proteomics (2016)

Depth of Proteome Coverage

Some example data

- Setup
  - Deep project-specific spectral library (MaxQuant & Spectronaut)
  - Analyzed with Spectronaut
  - Peptide and protein FDR 1%
  - 4h gradients, 1m column
  - HEK-293 sample
- Single run results
  - 7’060 protein groups, 154’643 precursors
  - Median XIC width 8.5 min
  - Peak capacity 710, median FWHM 13 s
- Technical triplicates
  - 6’534 proteins with CVs < 20%
  - 123’700 precursors with CVs < 20%
  - Data completeness for precursors: 91%

Survey Question 4



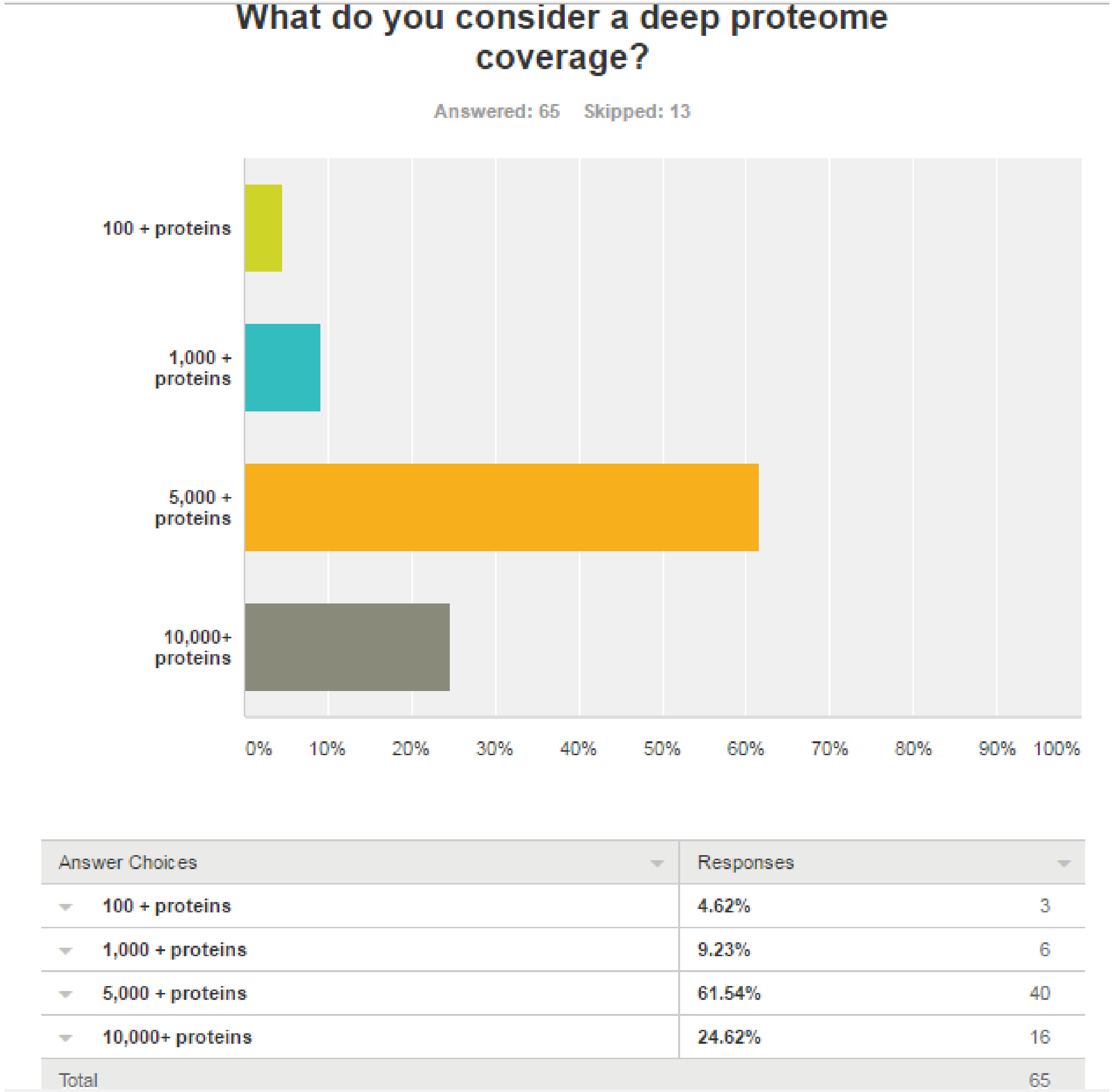
4. What do you consider a deep proteome coverage?

○ 100 + proteins

○ 1,000 + proteins

○ 5,000 + proteins

○ 10,000+ proteins



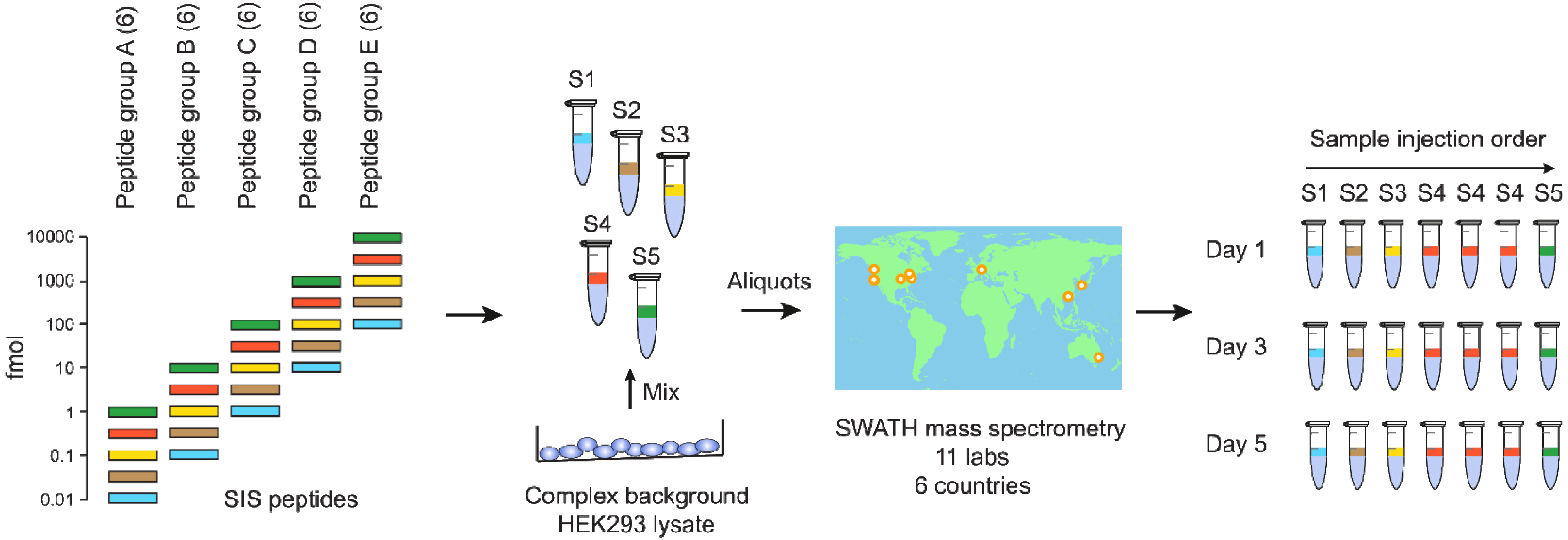
What do you consider a deep proteome coverage?

Answered: 65 Skipped: 13

Answer Choices	Responses	
100 + proteins	4.62%	3
1,000 + proteins	9.23%	6
5,000 + proteins	61.54%	40
10,000+ proteins	24.62%	16
Total		65

Ben

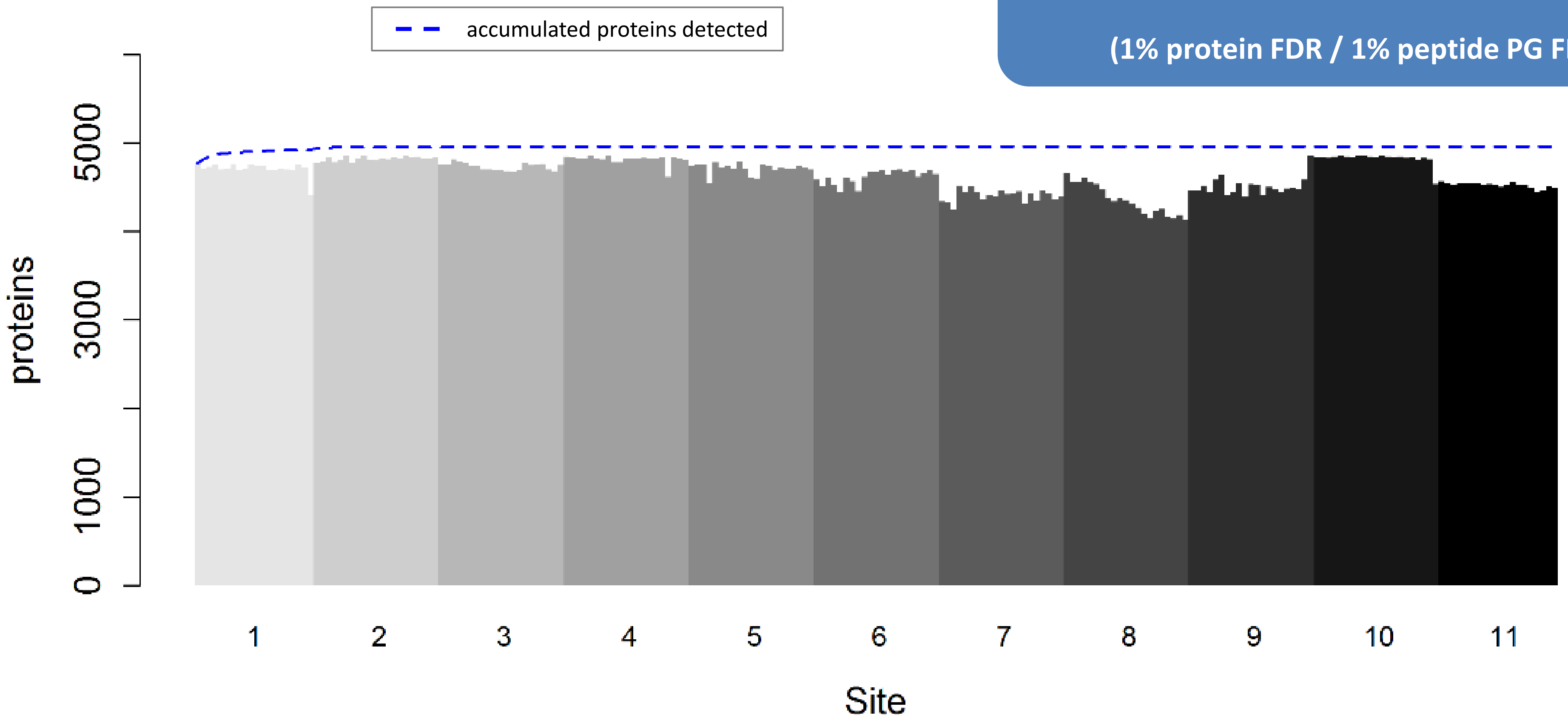
Study design -- Inter-lab SWATH-MS



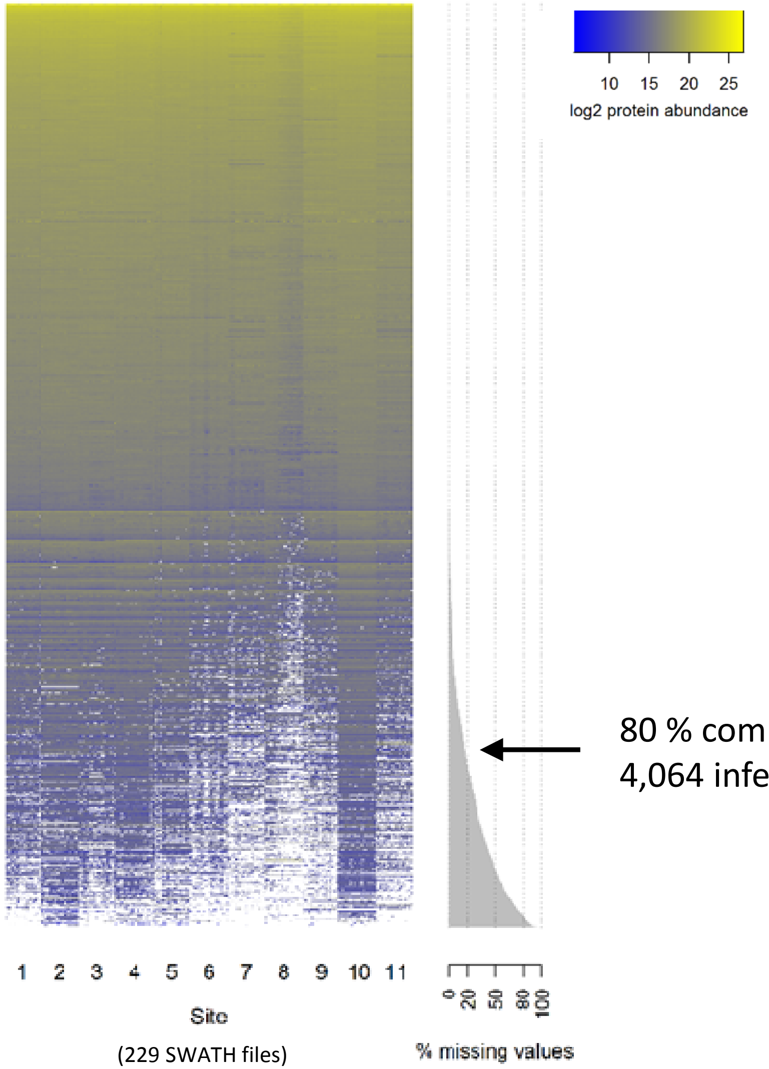
Peptide/protein detection rates (HEK293 lysate)

Total -- 4,960 proteins / 39,928 peptide PGs
Site median – 4,691 / 34,286 PGs

(1% protein FDR / 1% peptide PG FDR)



Data completeness



4,960 inferred proteins

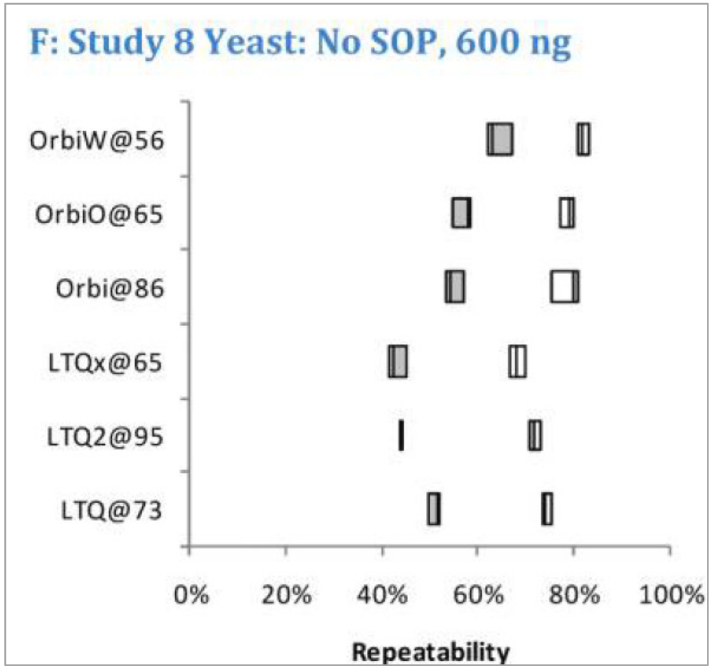
80 % complete
4,064 inferred proteins

No alignment

No ID propagation between runs

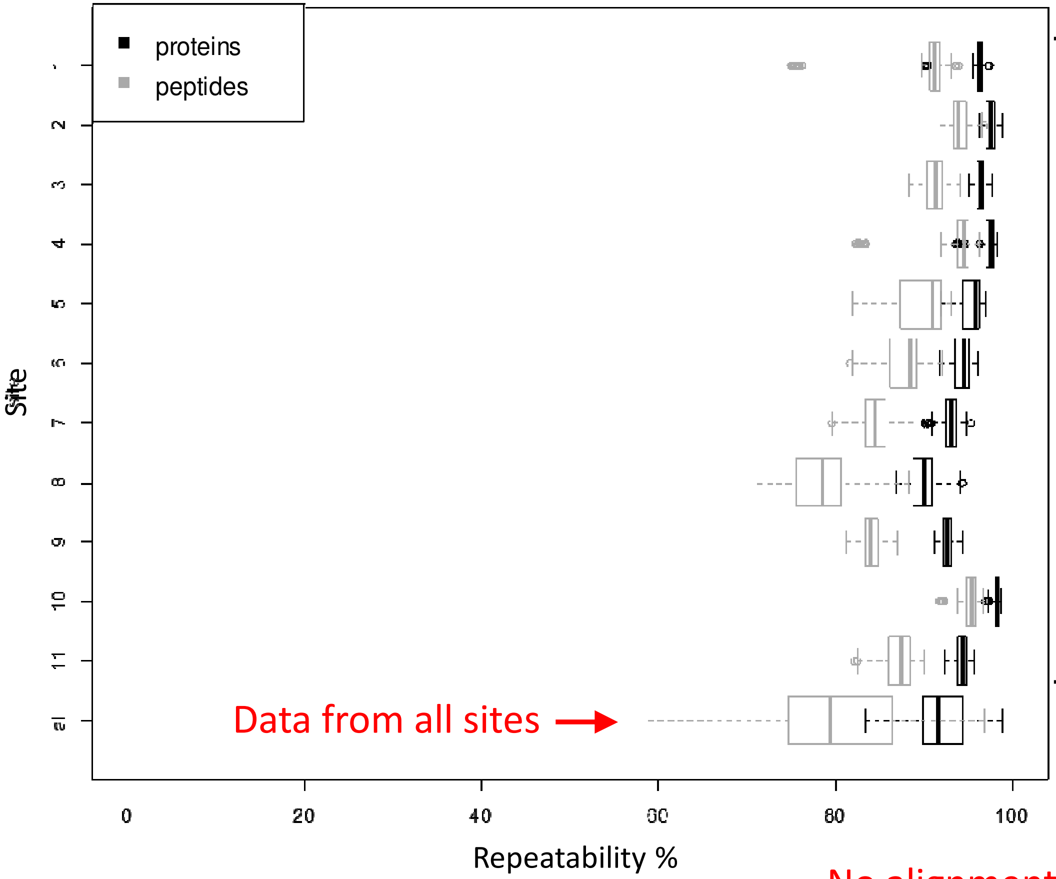
Repeatability of identification

Tabb et al. – DDA (2010)



3 reps

Inter-lab SWATH-MS



21 reps

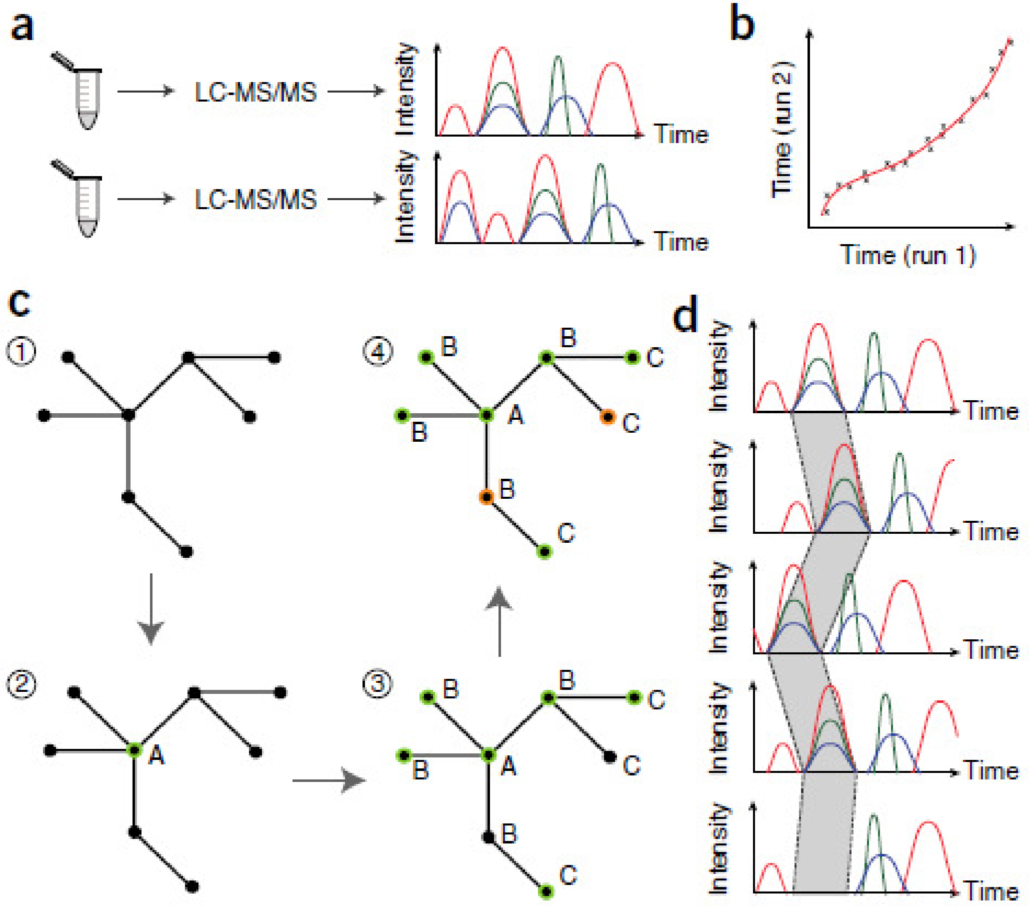
Data from all sites →

229 reps

No alignment!

Tabb, D. L. *et al.* Repeatability and Reproducibility in Proteomic Identifications by Liquid Chromatography–Tandem Mass Spectrometry. *J. Proteome Res.* **9,** 761–776 (2010).

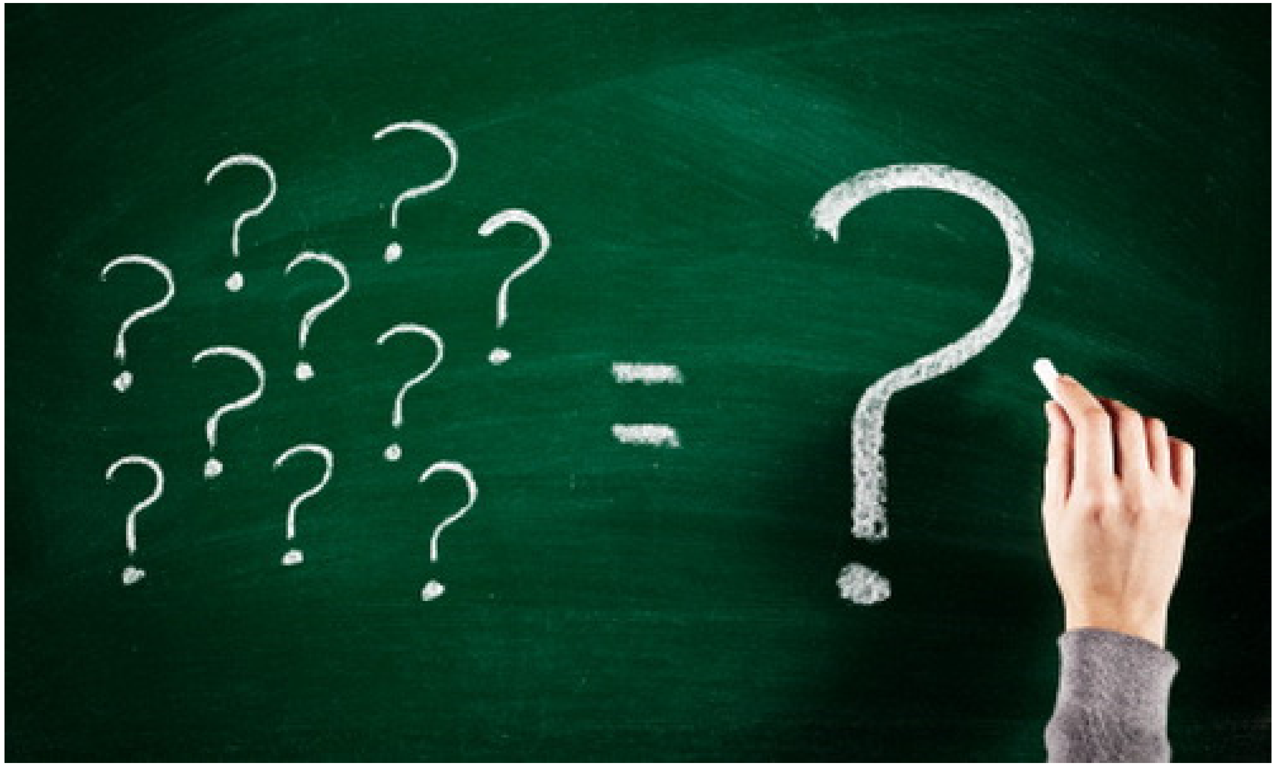
Alignment can only improve completeness…



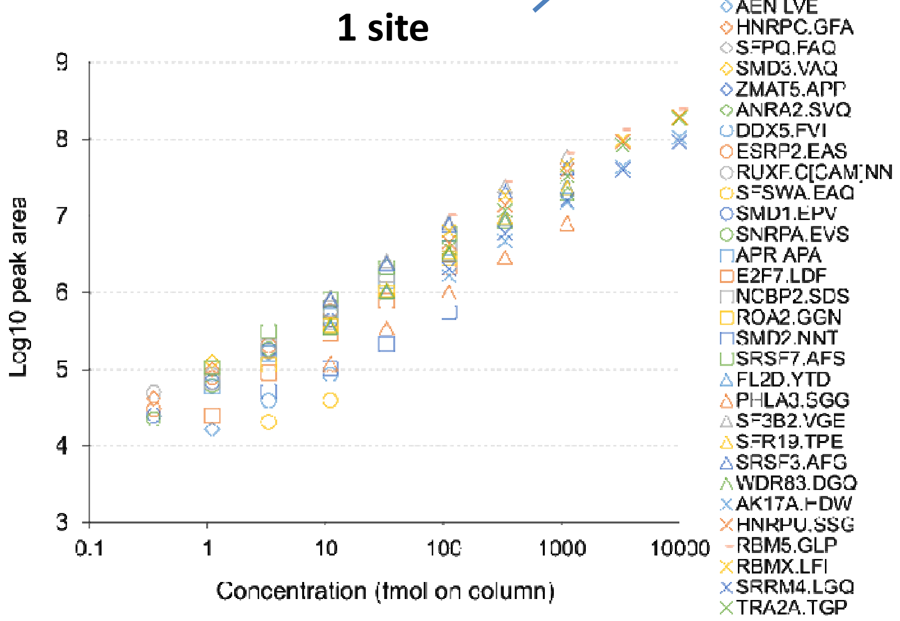
TRIC
TRansfer of
Identification
Confidence

Röst, H. L. *et al. Nat Meth* **13,** 777–783 (2016)

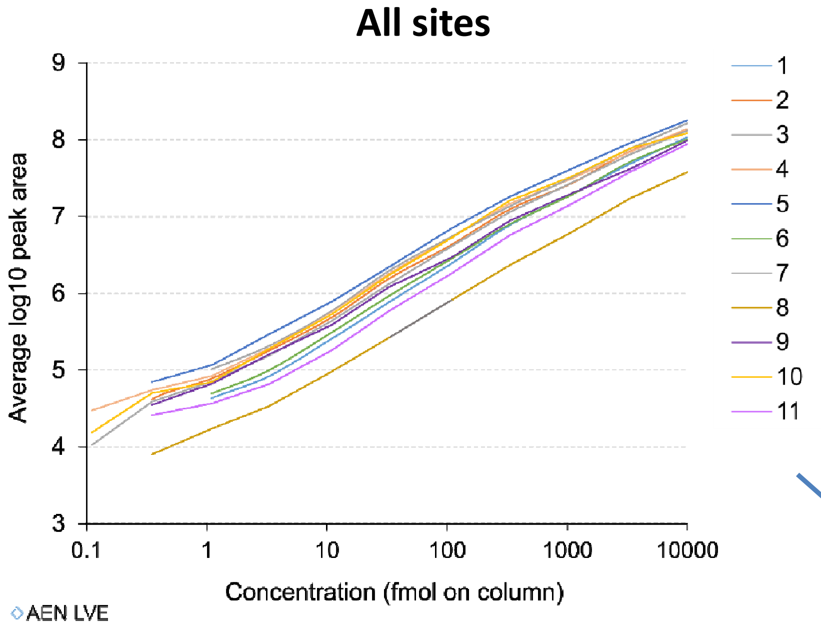
Comments? Questions?



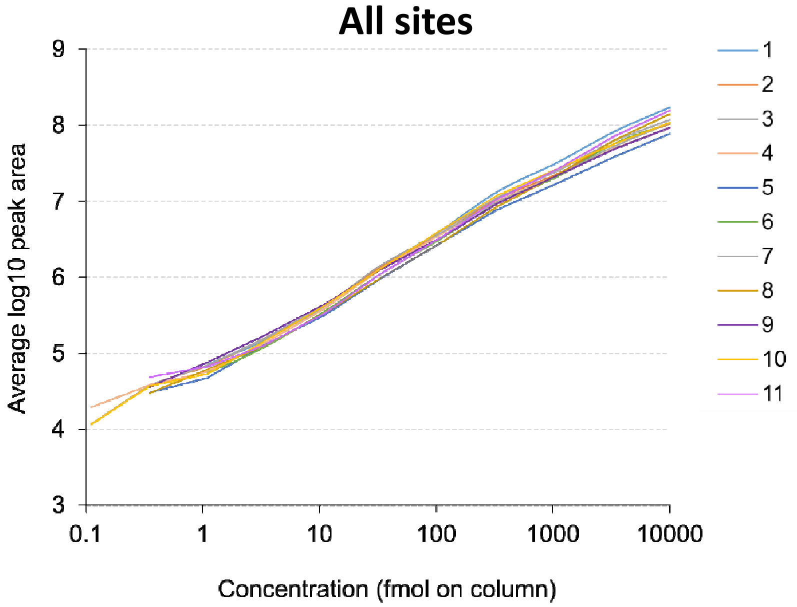
Linearity, dynamic range, and response differences


1 site

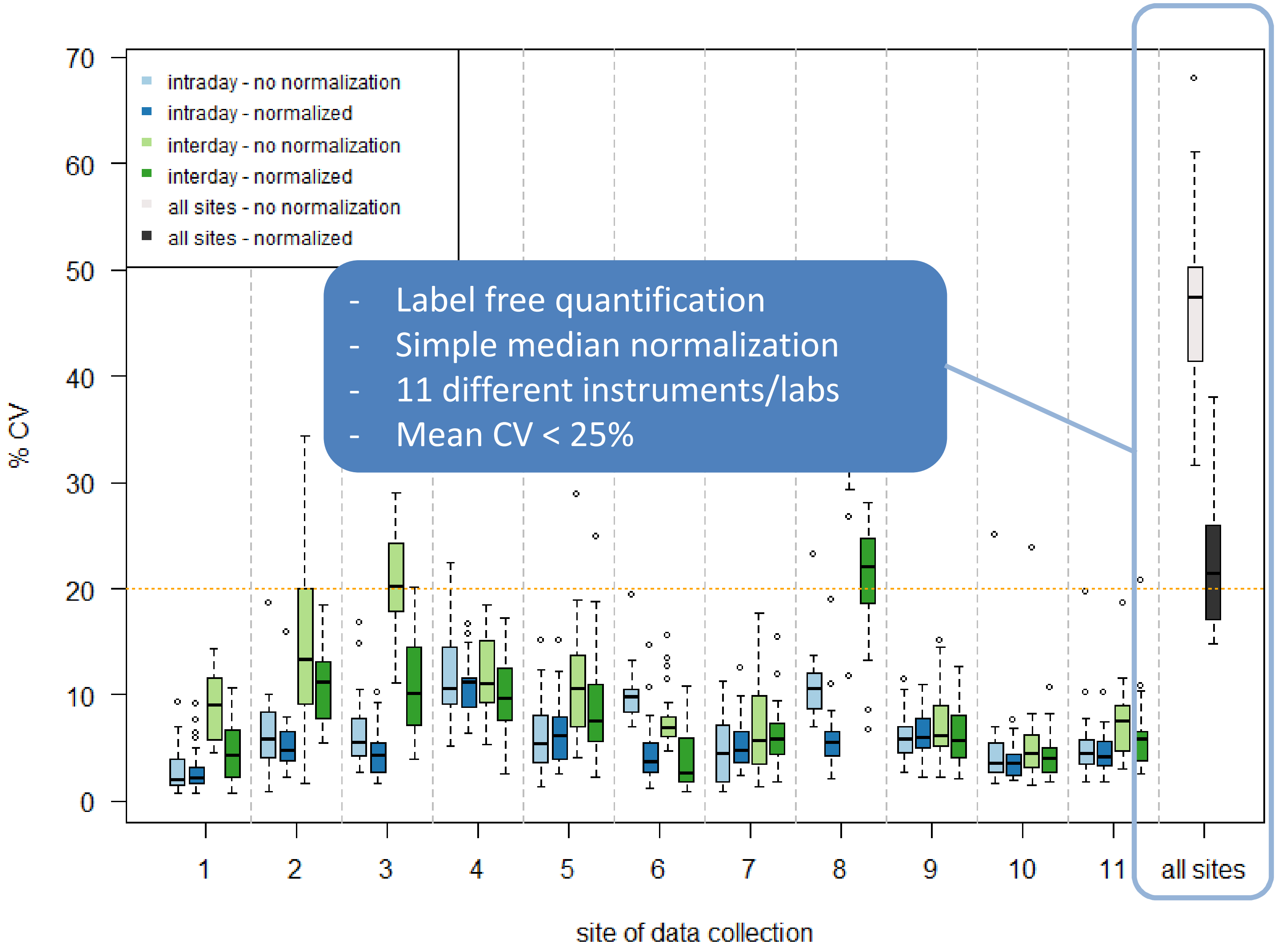
average


All sites

Normalize (based on HEK293 medians)

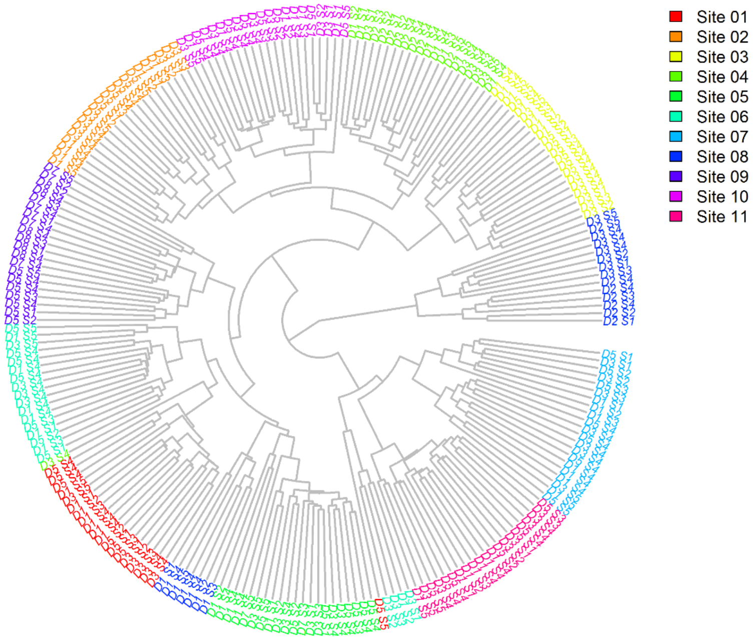

All sites

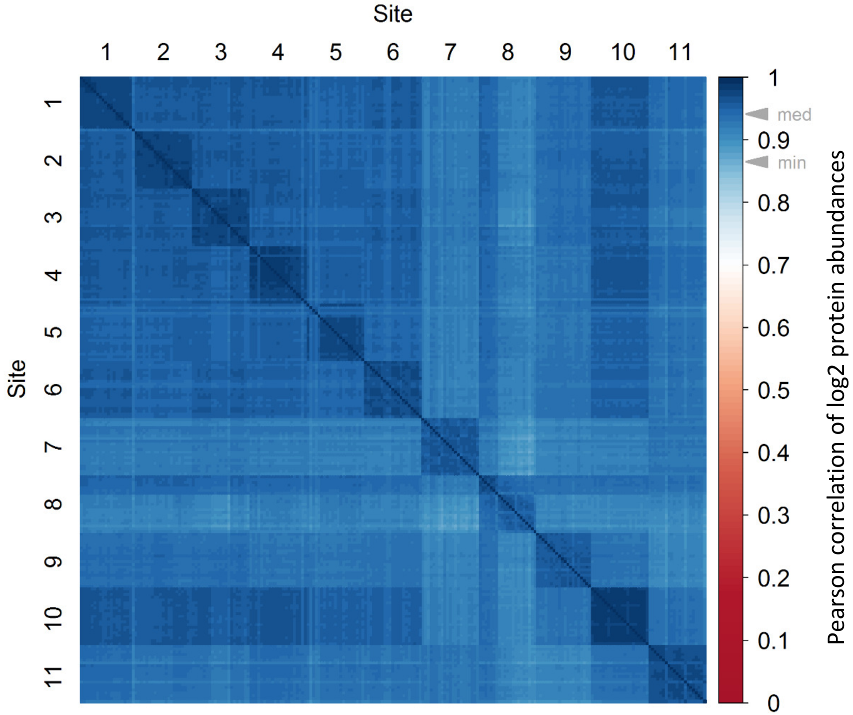
Reproducibility (30 x SIL peptides)



- Label free quantification
- Simple median normalization
- 11 different instruments/labs
- Mean CV < 25%

Global similarity of quantitative protein abundance profiles (HEK293 lysate)

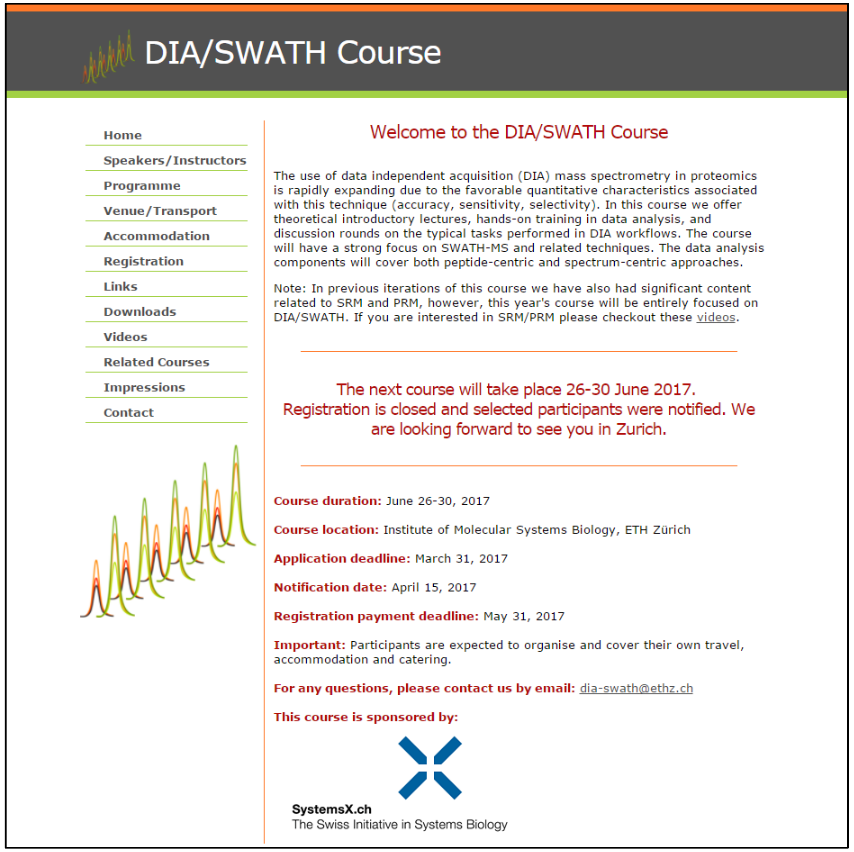




Median Pearson correlation (overall) = 0.940
Median Pearson correlation (within sites) = 0.971
Minimum Pearson correlation = 0.868

Collins BC*, Hunter C*, Liu Y* et al, Nature Communications (in press)

If you want learn more about DIA/SWATH



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 2017**

Thanks for participating!!

Ideas for discussion topics for next year to:

collins@imsb.biol.ethz.ch

bludau@imsb.biol.ethz.ch