New Proteomics Strategies for Studying Alzheimer's and Related Dementias

MacCoss, Hoofnagle, and Montine Labs

Projects Related to Signatures of AD and Related Dementias

- **Neuropathological assessment of resilience in post-mortem brain**
  - **Can we have a molecular assay that can distinguish between:**
    - Different AD genetic risk
    - Associated co-morbities
    - Resilience to AD neuropathologic change
  - **Can we develop a proteomics assay to use as a replacement of traditional histopathological assessment.**
- **Cerebral spinal fluid assay that can reflect brain pathophysiology**
  - **Can we expand beyond the use of CSF $A\beta_{42}$, tau, and P181-tau, and PET imaging for amyloid and pathologic tau protein**

So what do we need?

- We need methods that sample the same peptides in all samples
- We need methods where the change in signal reflects the change in quantity
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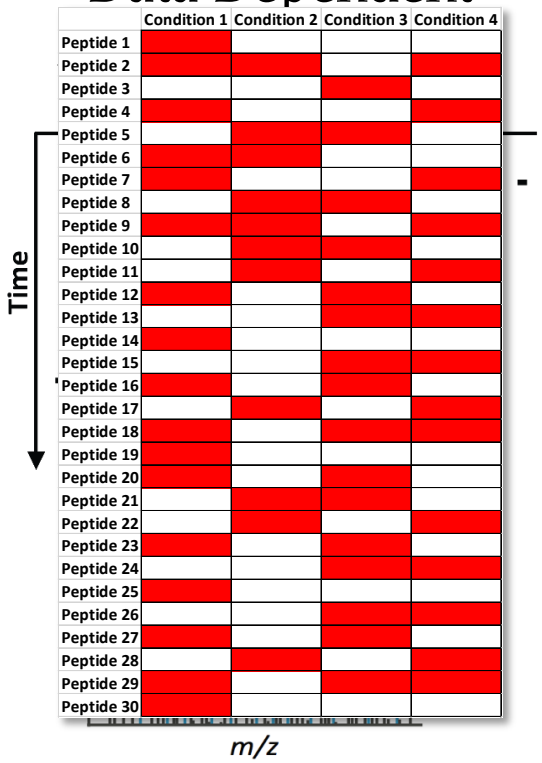
Mass Spectrometry Data Acquisition Strategies Used in Proteomics

Data Dependent



Database Searching

Peptide Identification

Mass Spectrometry Data Acquisition Strategies Used in Proteomics

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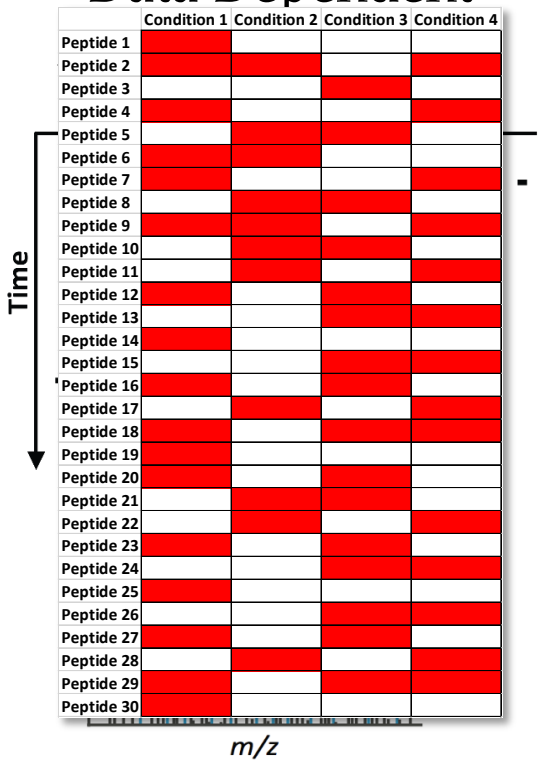
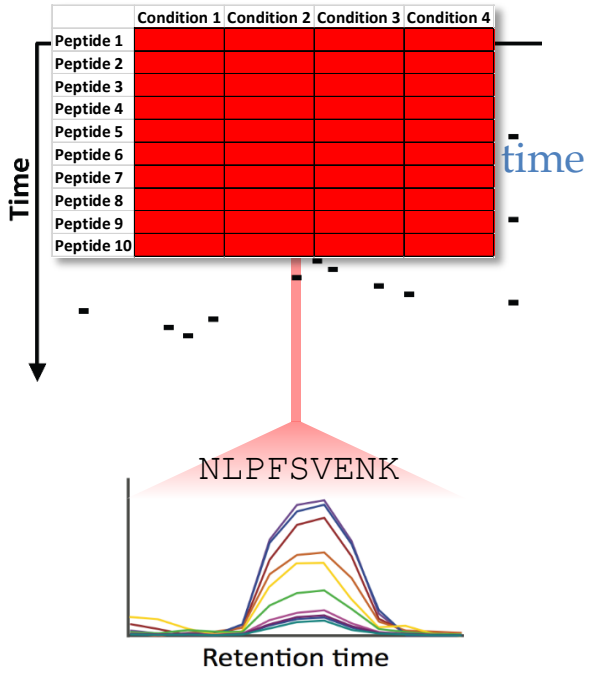


Database Searching

Peptide Identification

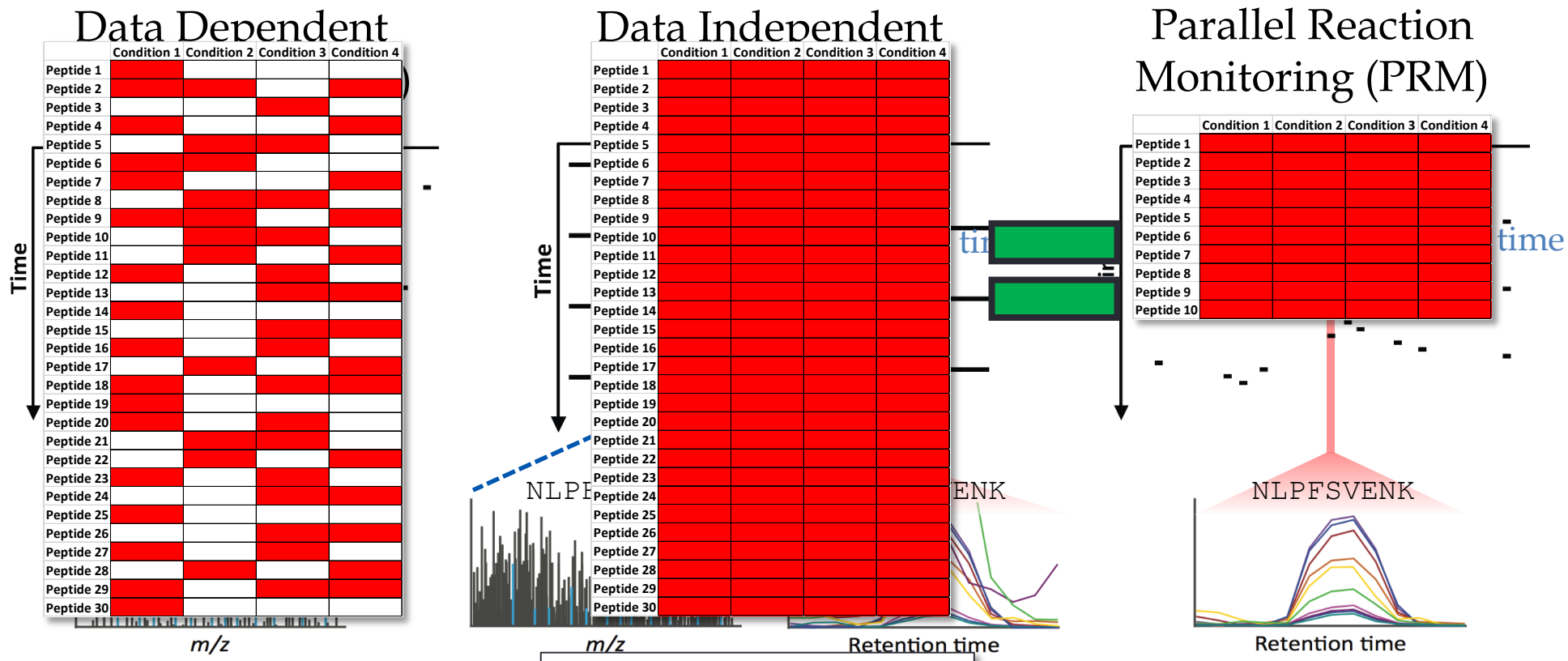
Parallel Reaction Monitoring (PRM)



Targeted Analysis

Peptide Quantification

Mass Spectrometry Data Acquisition Strategies Used in Proteomics



Database Searching

Peptide Identification

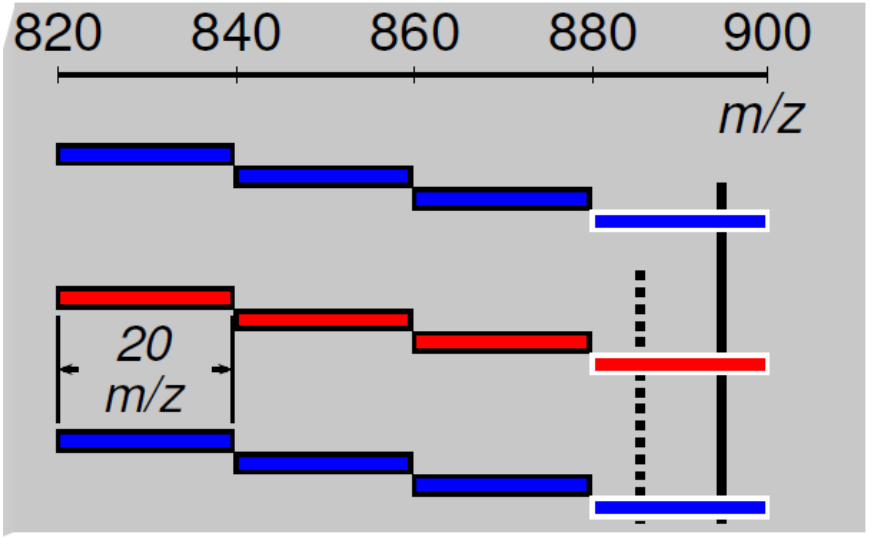
~50 Hz

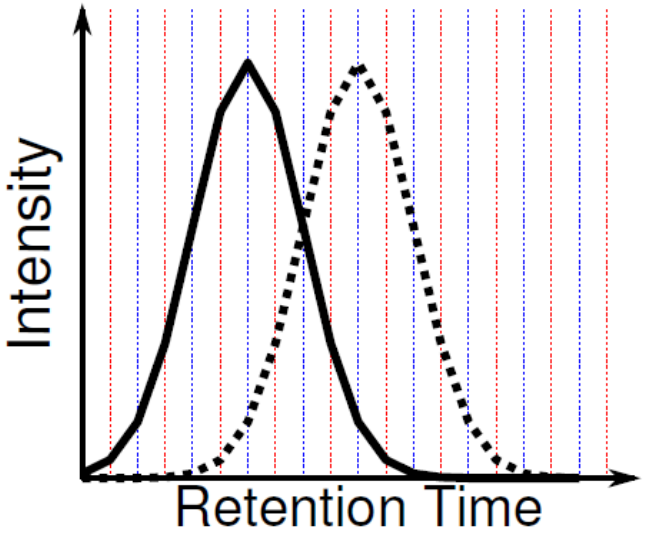
- 4 m/z isolation (2 m/z effective)
- 500-900 m/z
- 2 s Cycle Time

Targeted Analysis

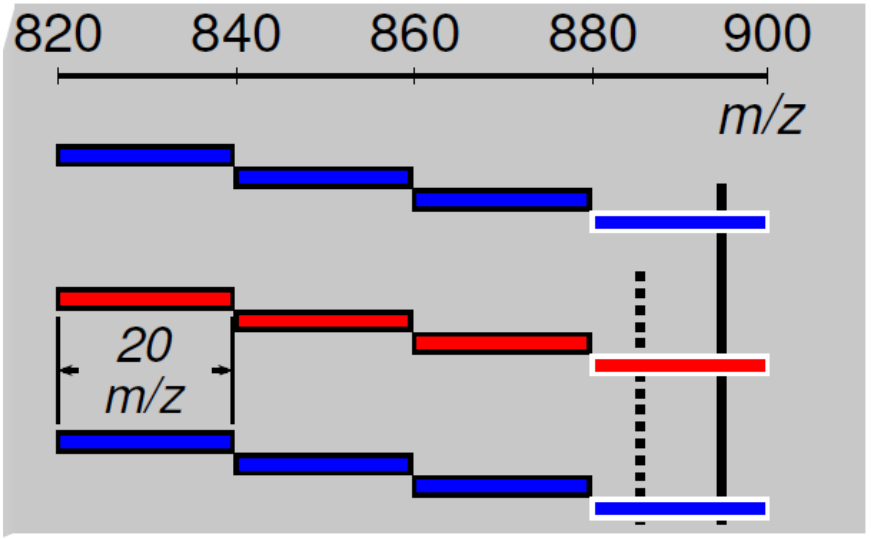
Peptide Quantification

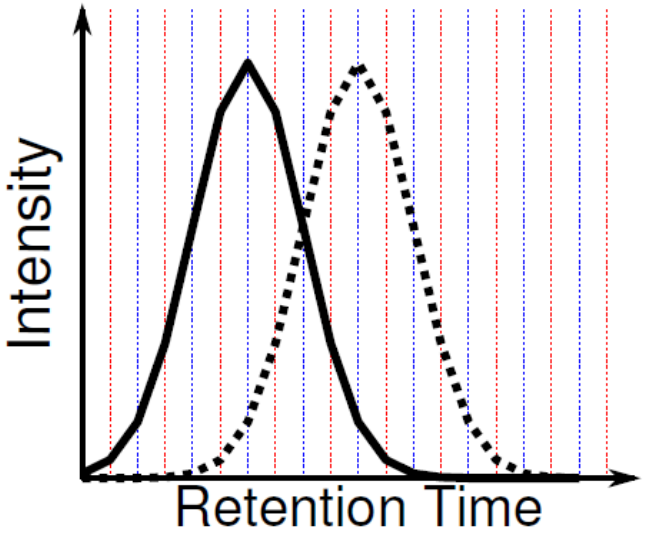
Improving Precursor Selectivity

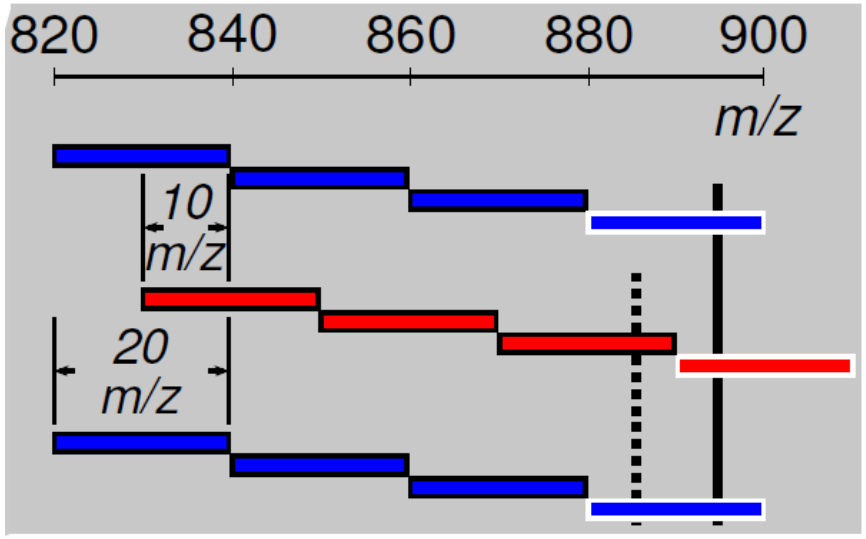


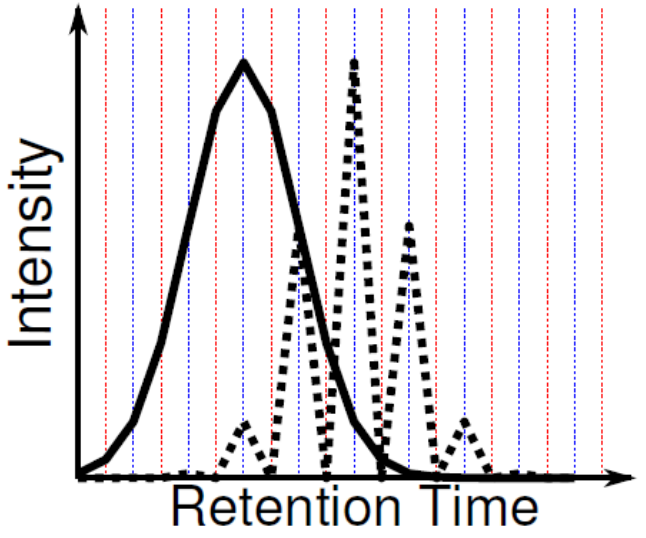


Improving Precursor Selectivity





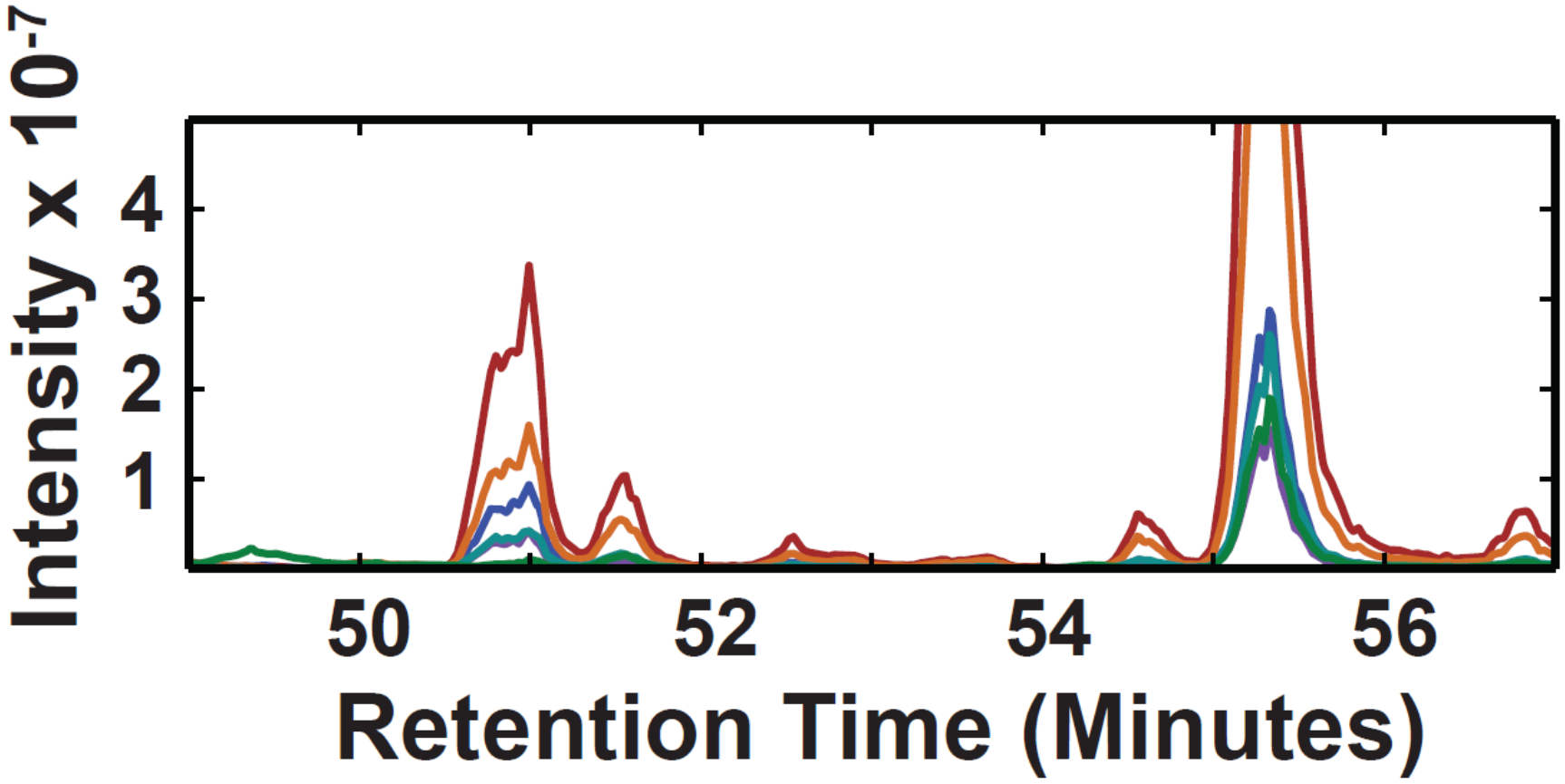




Amodei et al JASMS 2019

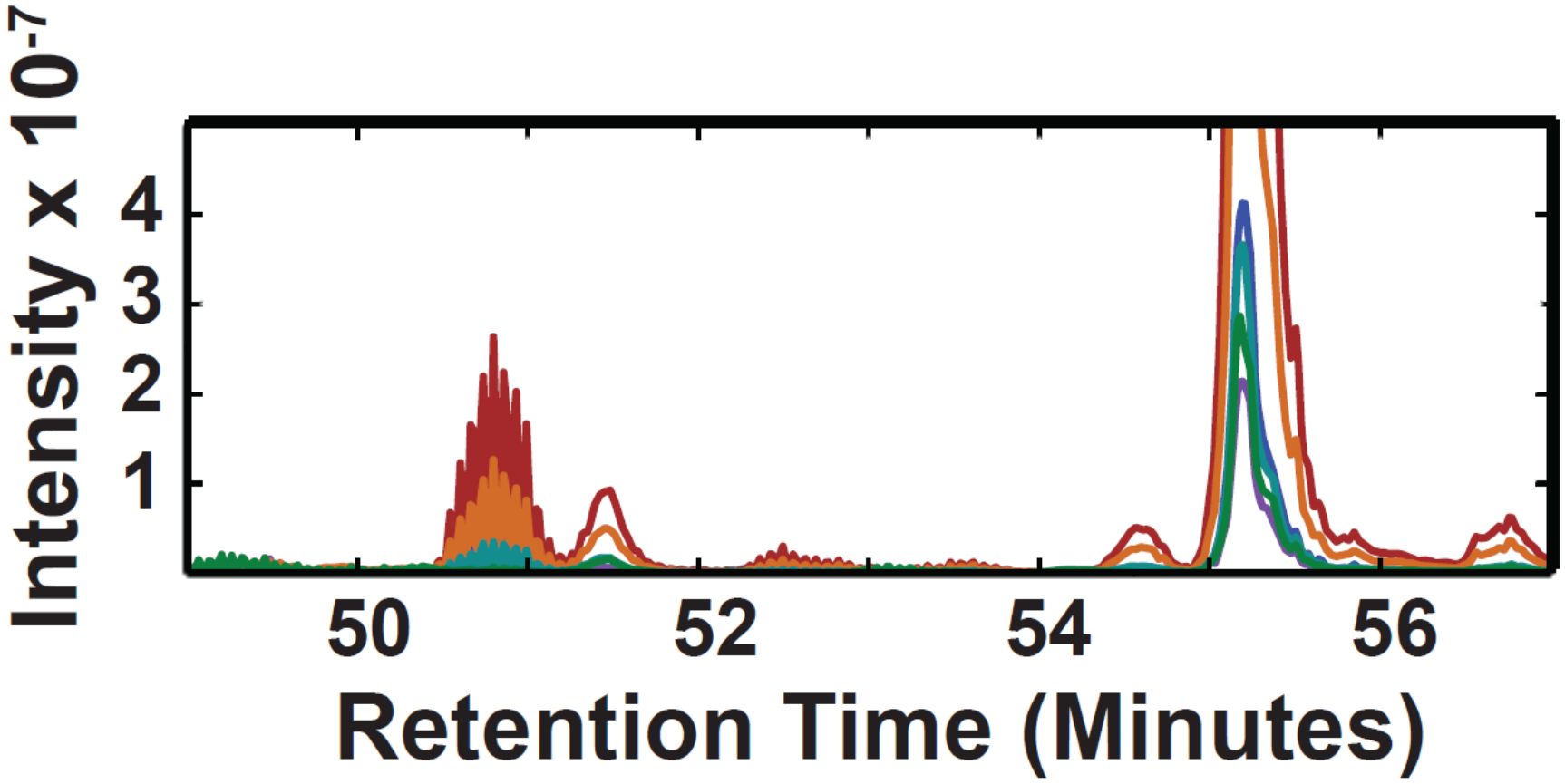
20 *m/z* DIA

GVMNAVNNVNNVIAAAFVK



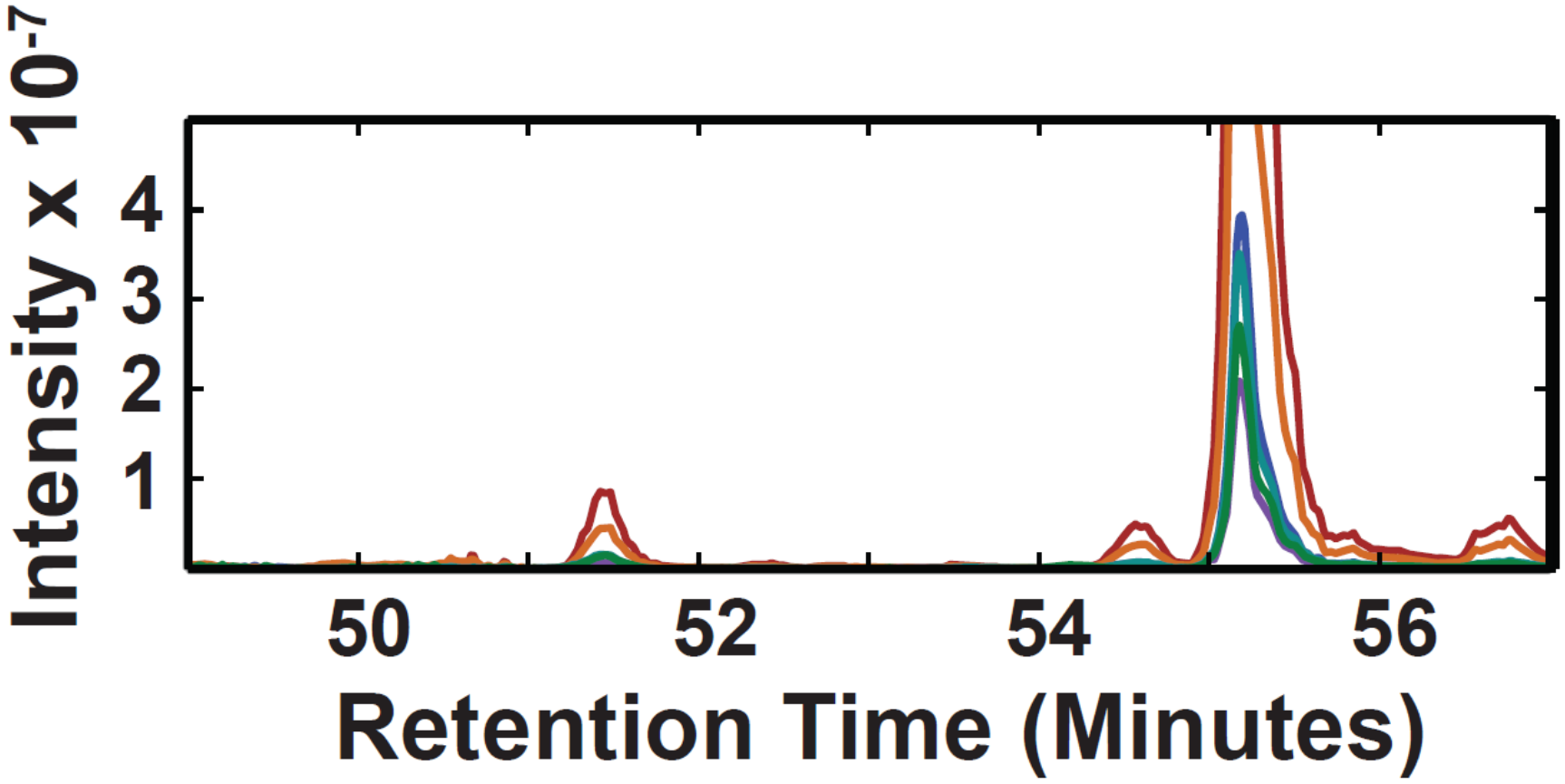
20 *m/z* DIA + Overlap

GVMNAVNNVNNVIAAAFVK



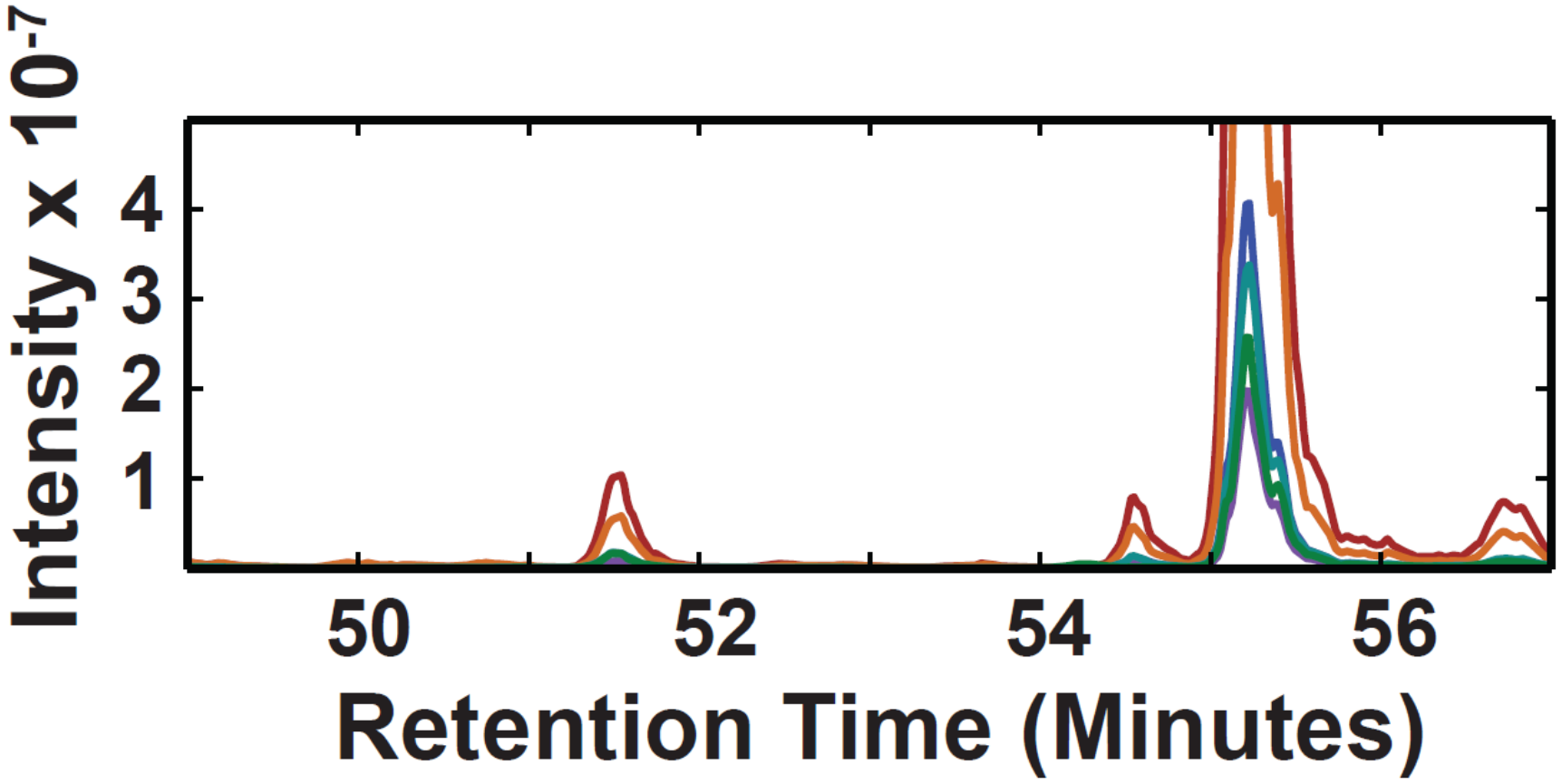
20 *m/z* DIA + Overlap + Demultiplexing

GVMNAVNNVNNNVIAAAFVK



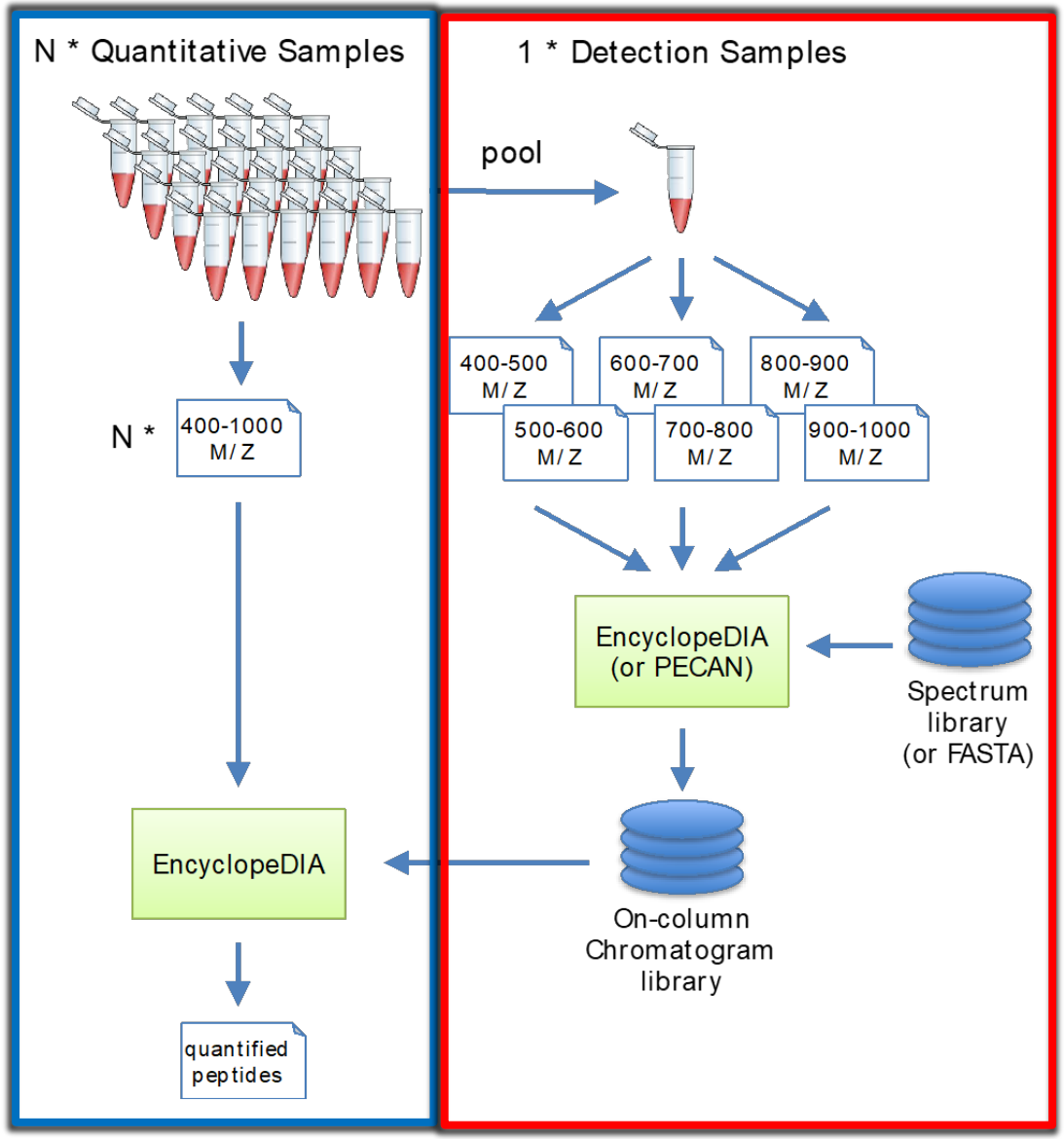
10 *m/z* DIA

GVMNAVNNVVNNVIAAAFVK



Separating Detection from Quantitation

24 m/z overlapping
12 m/z effective



4 m/z overlapping
2 m/z effective

Searle et al, Nature Comm, 2018

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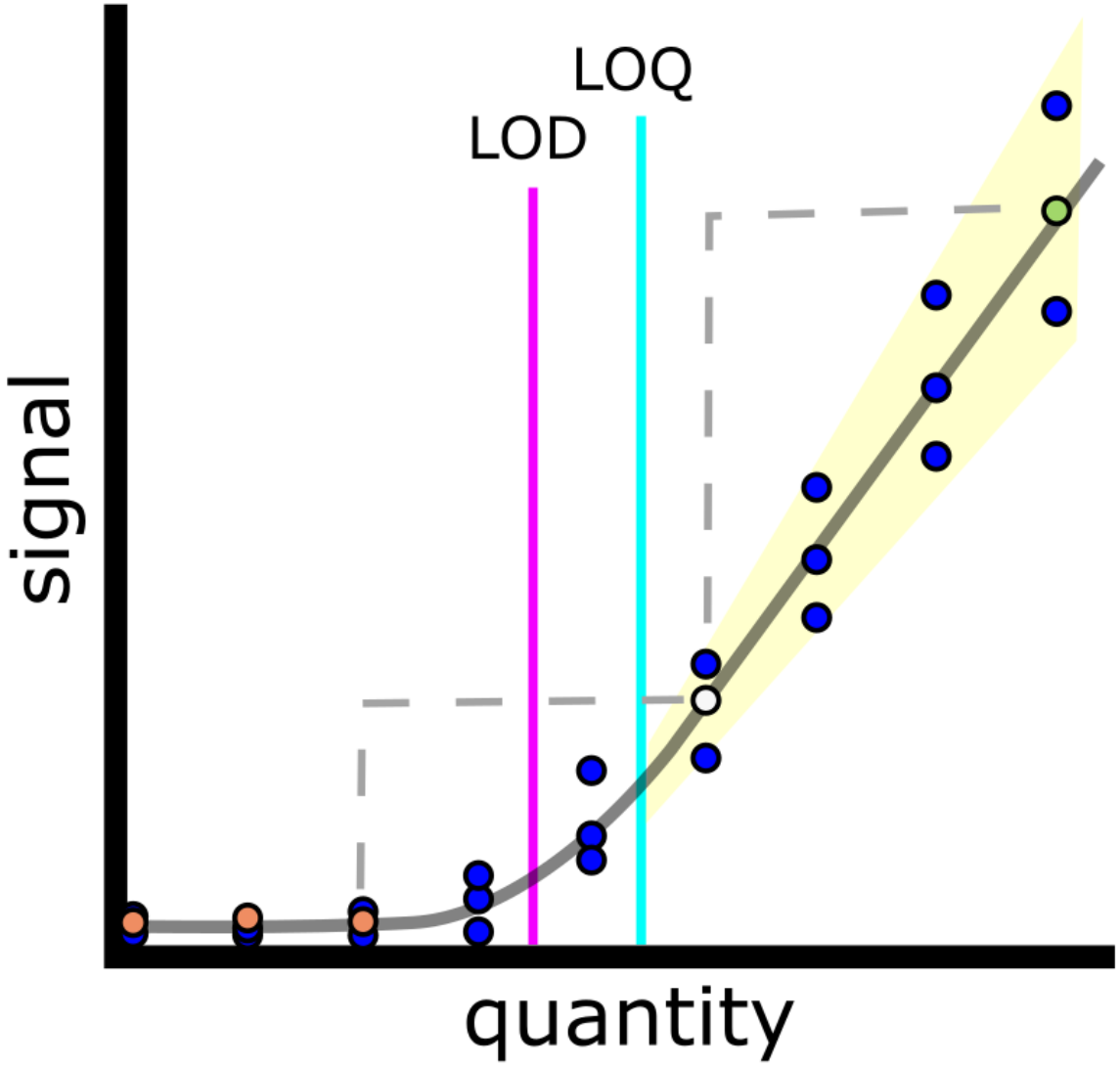
Are the Measurements Quantitative or Differential?

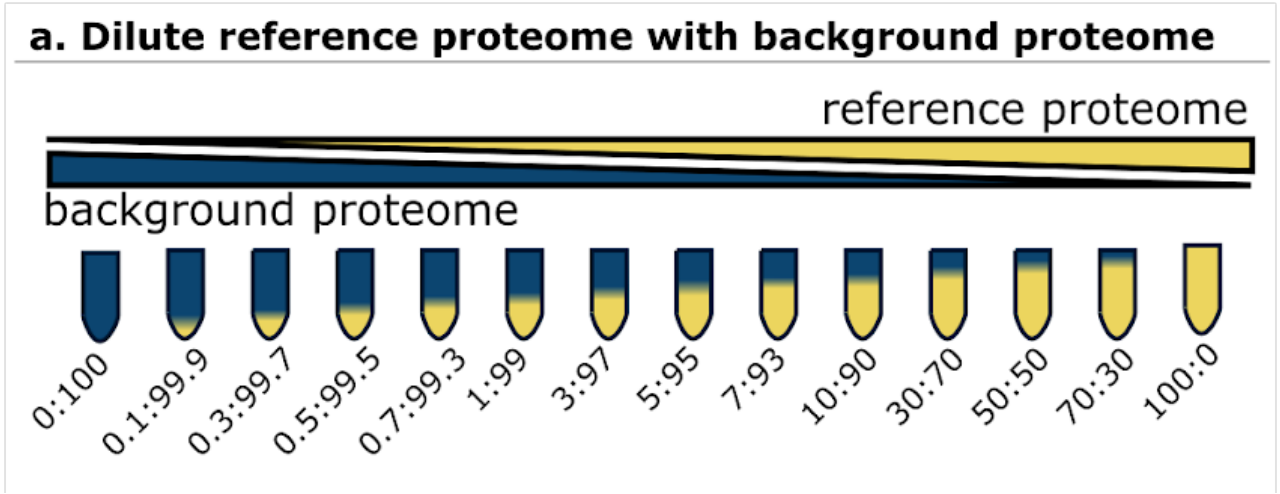
Can we define LOQ and LOD for many peptides at once?

Assessment of LOD and LOQ

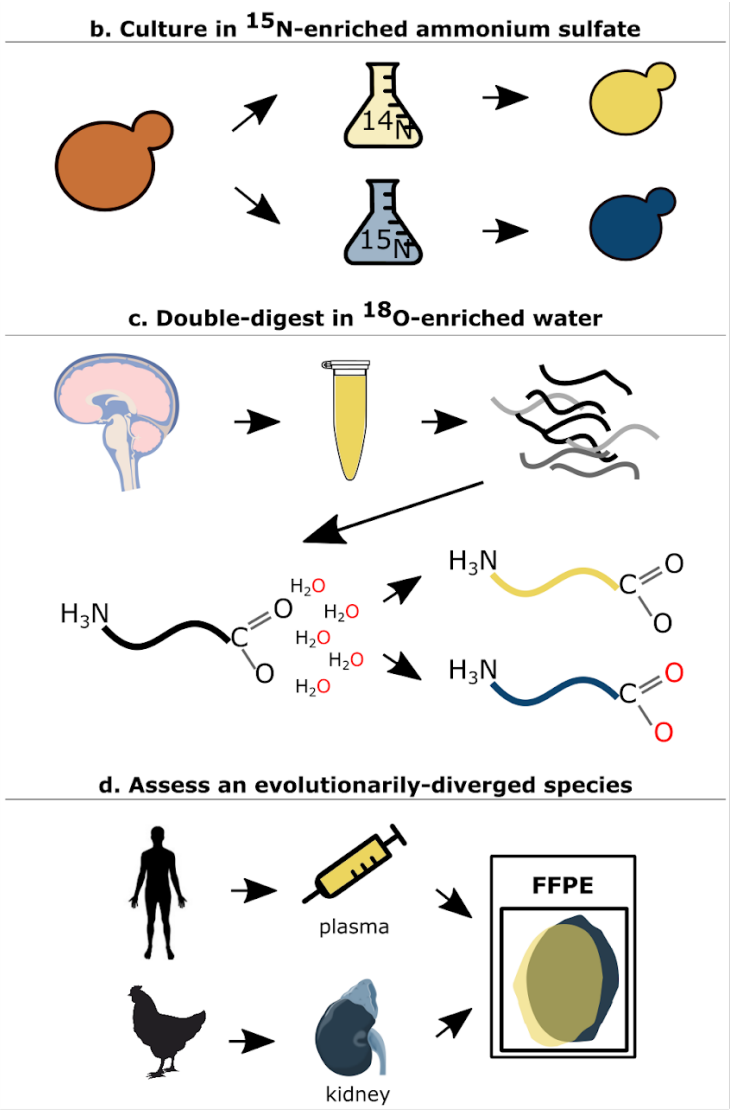


Building on:
Galitzine C et al, MCP 2018

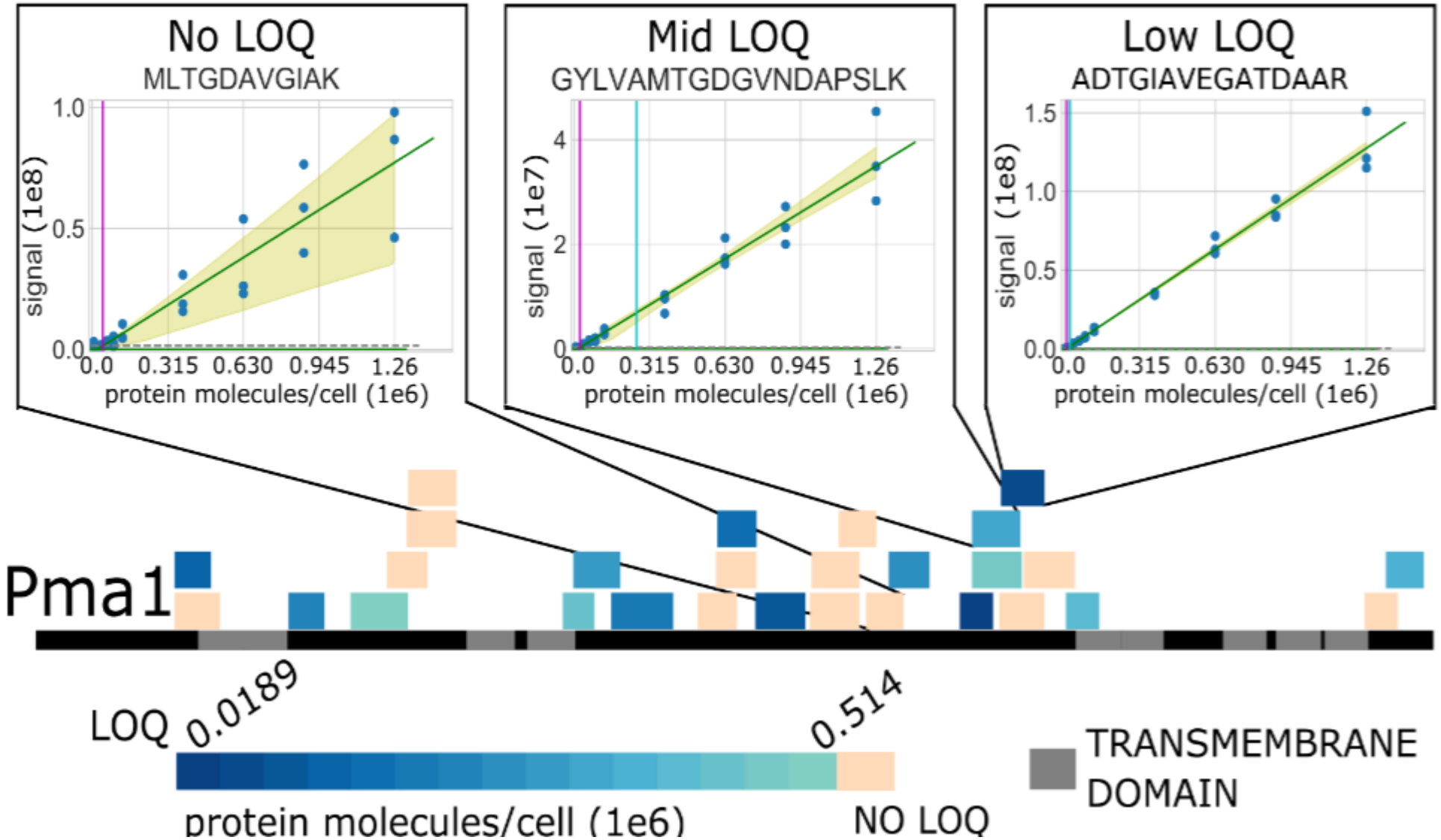
Matched Matrix Dilution Curves



Building on:
Grant and Hoofnagle, Clin Chem 2014

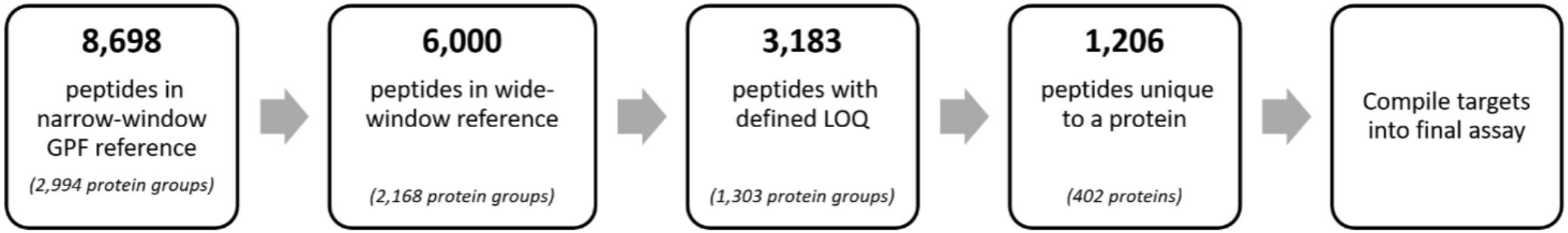


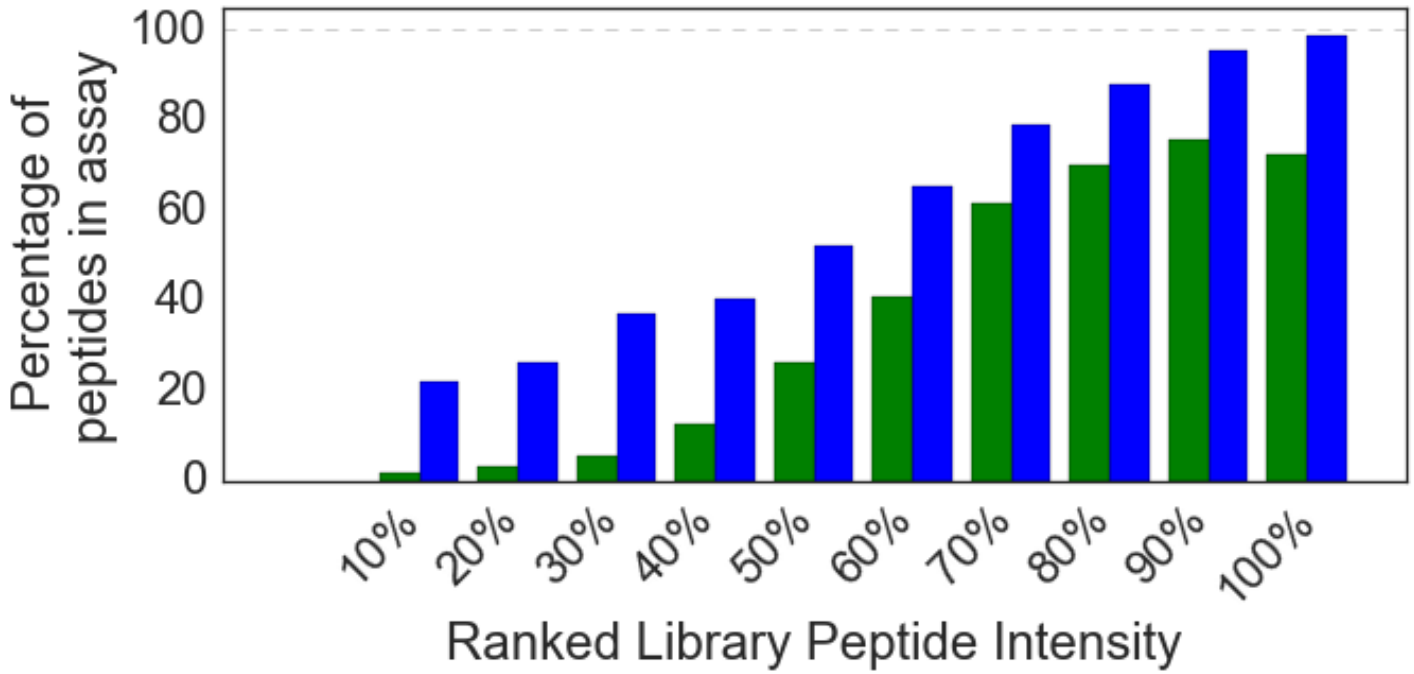
Not all Peptides are Equal Quantitative Proxies for a Protein



Not all Peptides are Quantitative

Cerebrospinal fluid (CSF) method development using DIA-MS





- **Detectable Peptides**
- **Quantitative Peptides**

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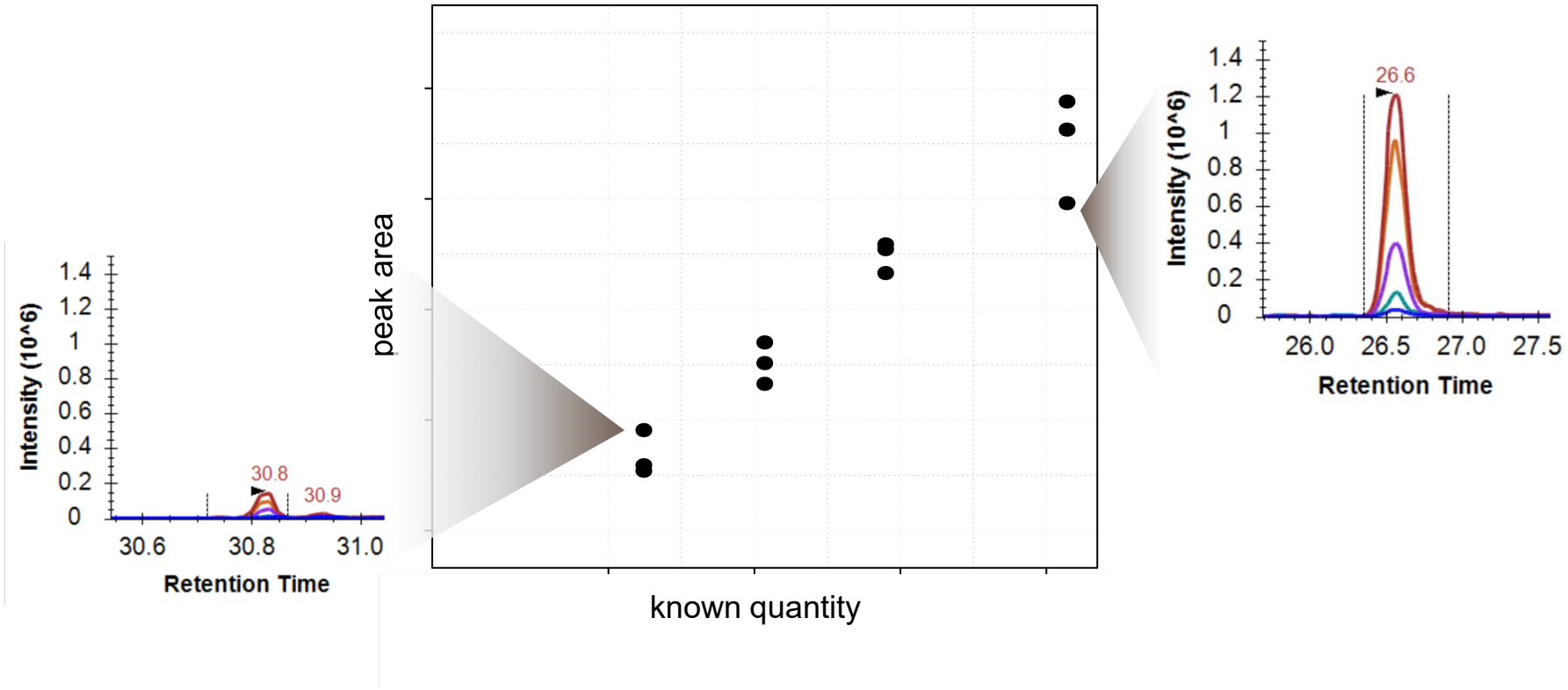
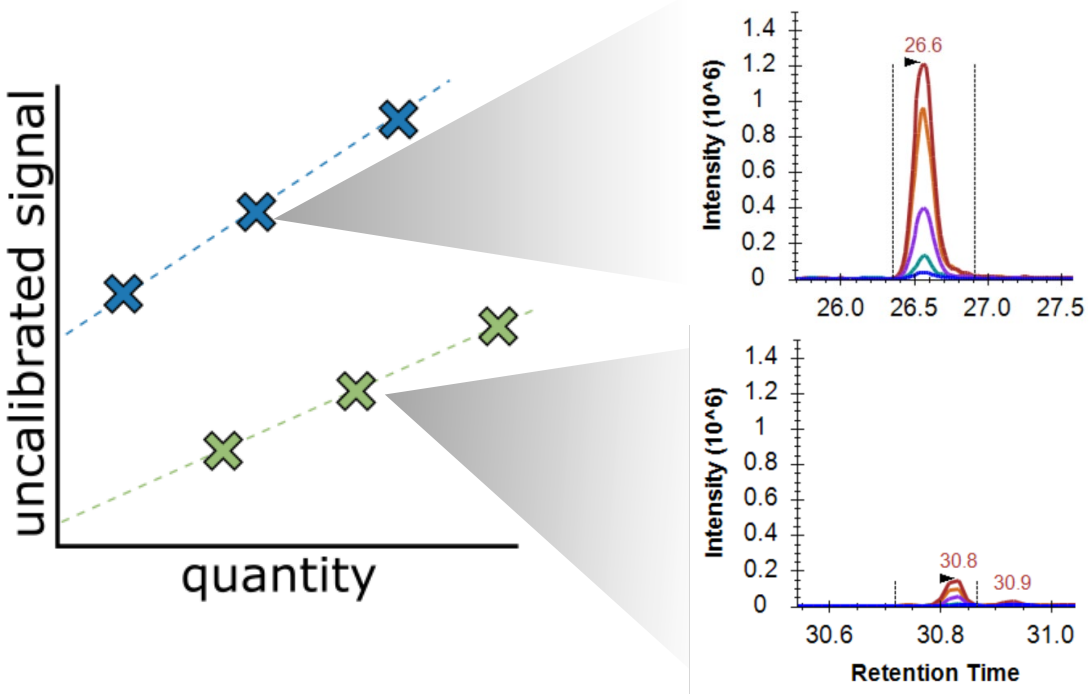
Comparing Signal Intensities Between Labs, Instrument Platforms

Signal Calibration in Proteomics?

Peak area measurements should scale with the amount of peptide in the sample



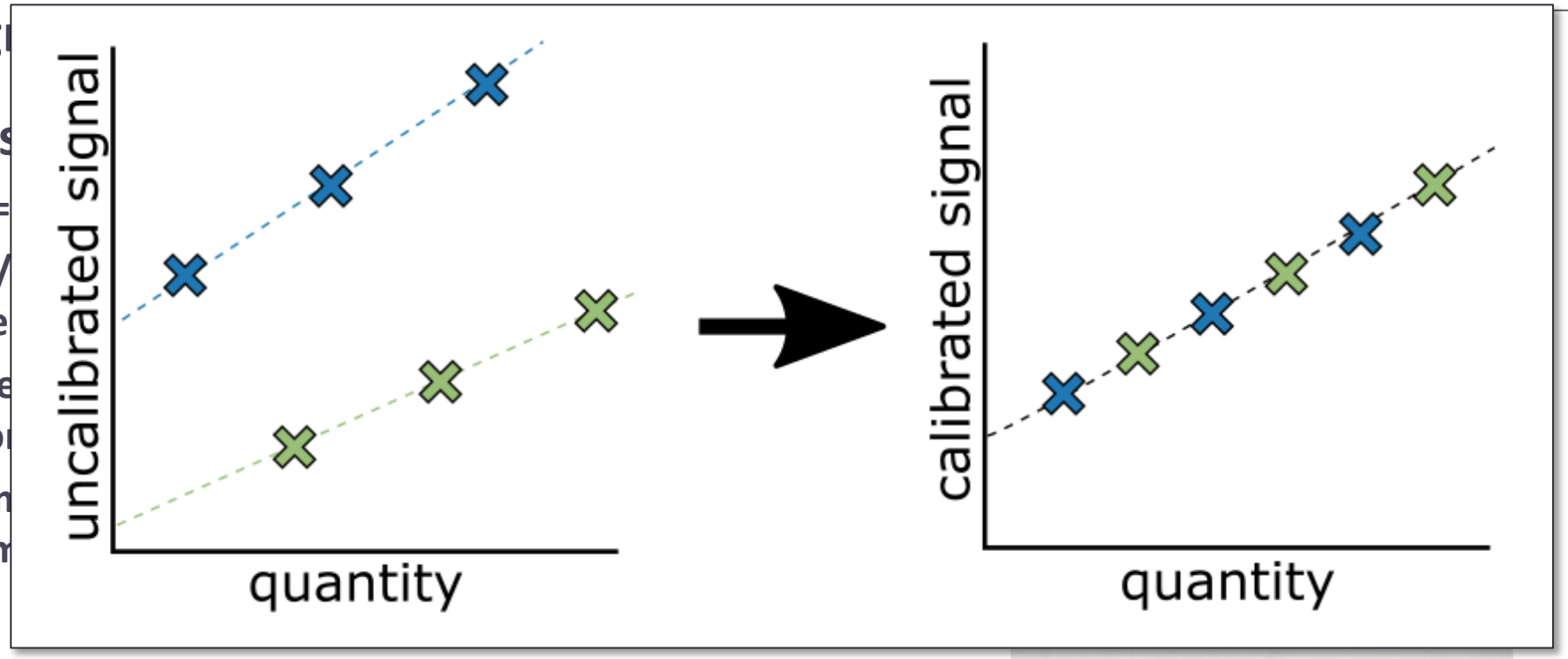
Measurements on different platforms are not measured on the same scale



Between Batch/Platform/Lab Signal Calibration

Purpose of signal calibration

- Placing sig
- Often the s
  - Kilogram =
  - Meter = 1/ north pole
  - Pee dee be Peedee Fo
  - Standard m arbitrary m world.



COLORS

THE ORIGINAL KILOGRAM IS KEPT UNDER GUARD IN SÈVRES, FRANCE. IT IS BASED ON THE WEIGHT OF A LITER OF PURE WATER.

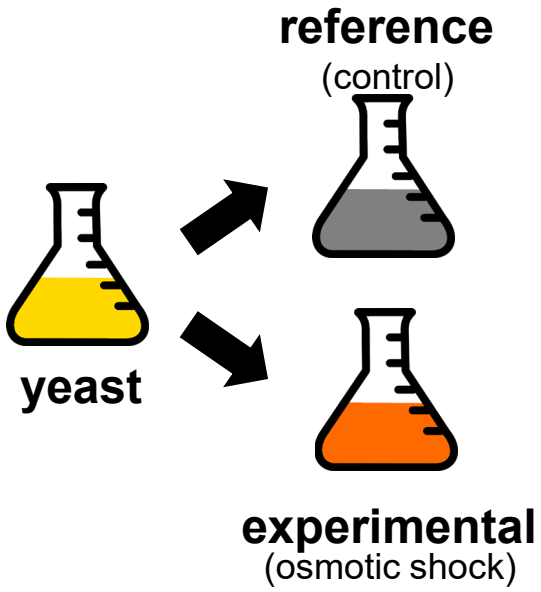
FRANCE

Data harmonization between days, instrument platforms, and laboratories

Technological Innovation and Resources

Reproducible and Consistent Quantification of the *Saccharomyces cerevisiae* Proteome by SWATH-mass spectrometry*

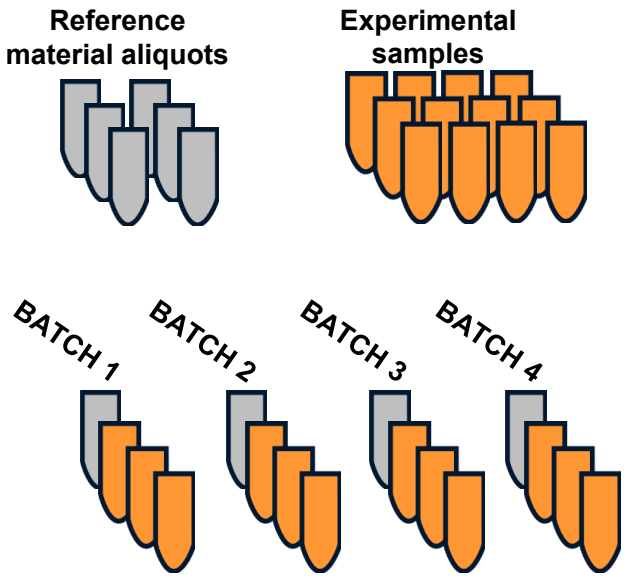
Nathalie Selevsek‡, Ching-Yun Chang§, Ludovic C. Gillet‡, Pedro Navarro‖, Oliver M. Bernhardt¶, Lukas Reiter¶, Lin-Yang Cheng§, Olga Vitek‖, and Ruedi Aebersold‡‡§§



BY4741
Ghaemmaghami et al
Nature 2003

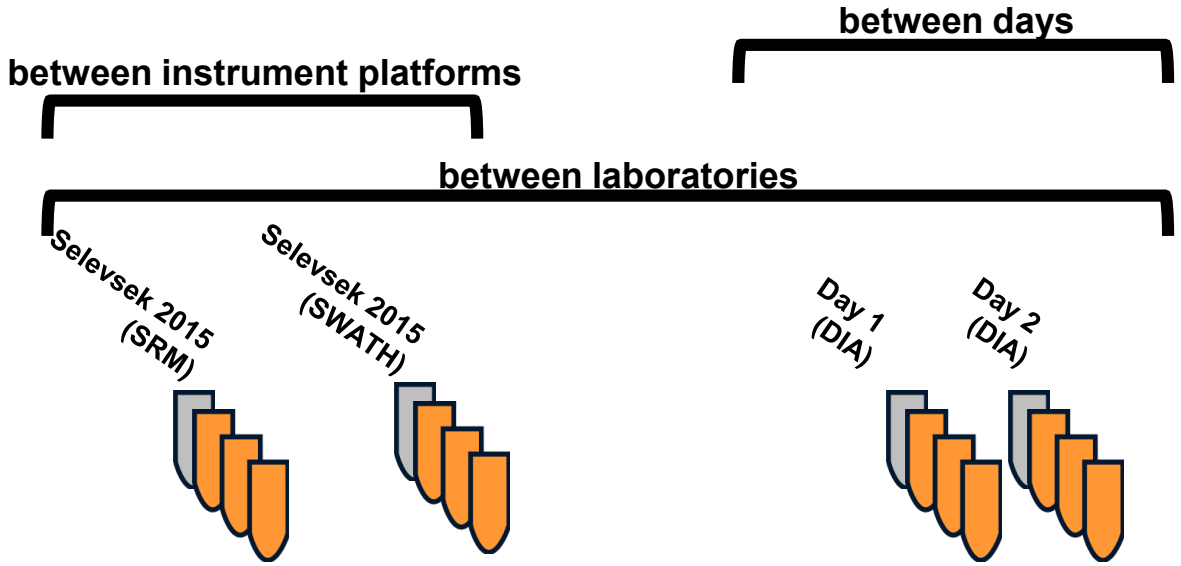
Pino et al, Anal Chem, 2018

Aliquots of reference material are prepared together with each experimental batch



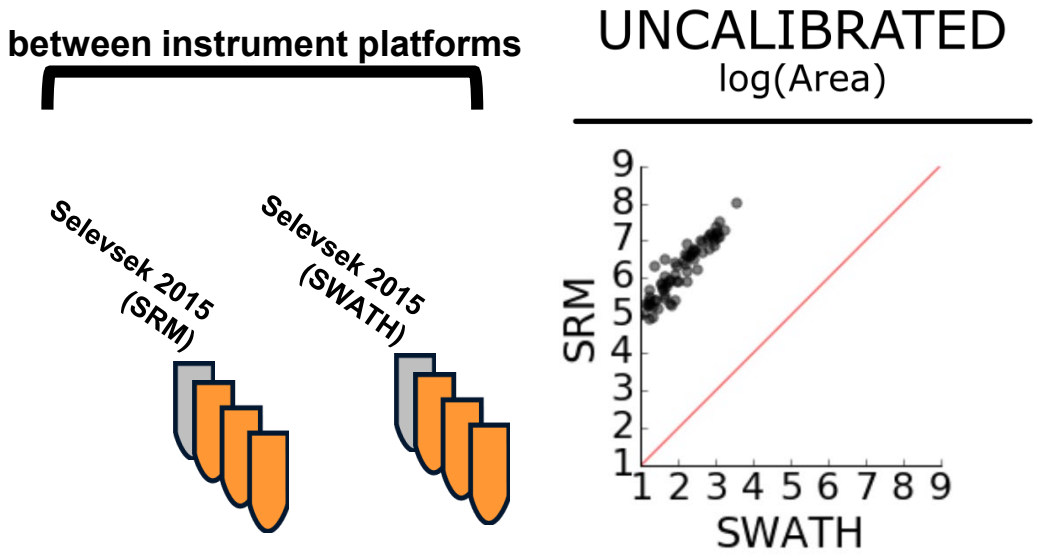
Pino et al, Anal Chem, 2018

Data harmonization between days, instrument platforms, and laboratories



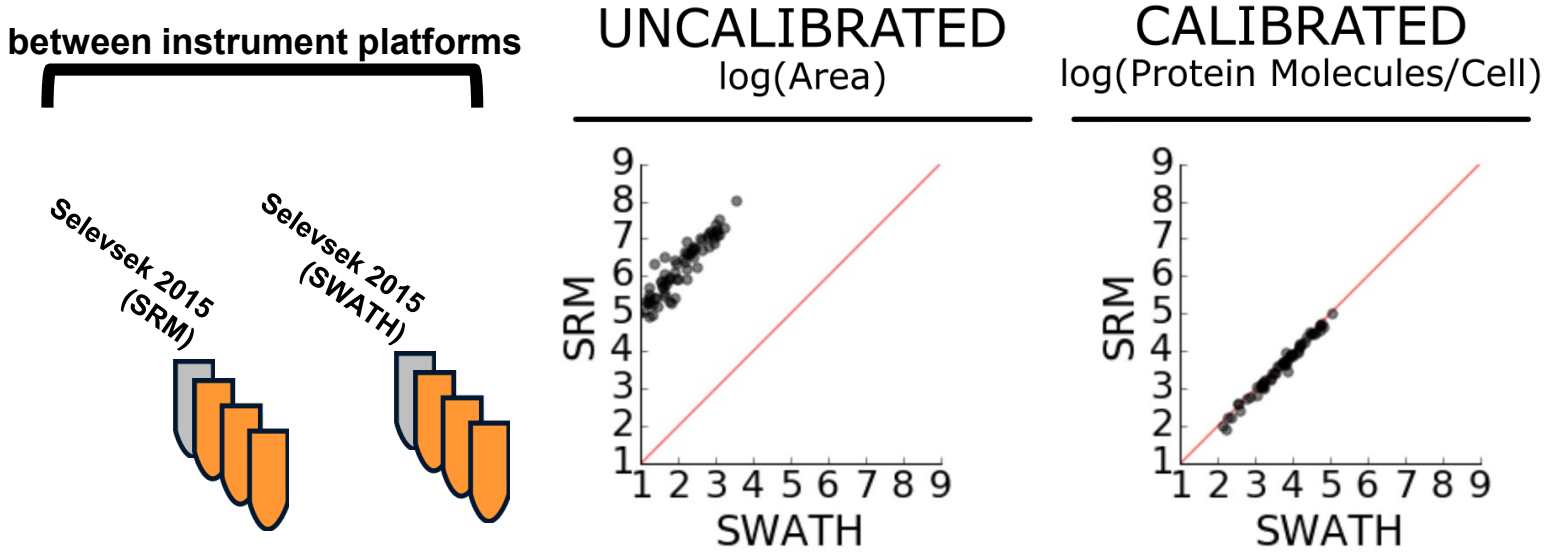
Pino et al, Anal Chem, 2018

Signals measured on different platforms are often on different scales



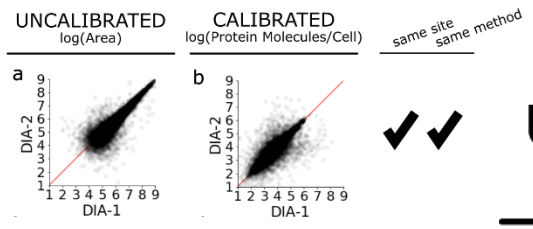
Pino et al, Anal Chem, 2018

External reference calibration places different platforms on the same scale



Pino et al, Anal Chem, 2018

Calibration to the same reference minimizes quantitative variance



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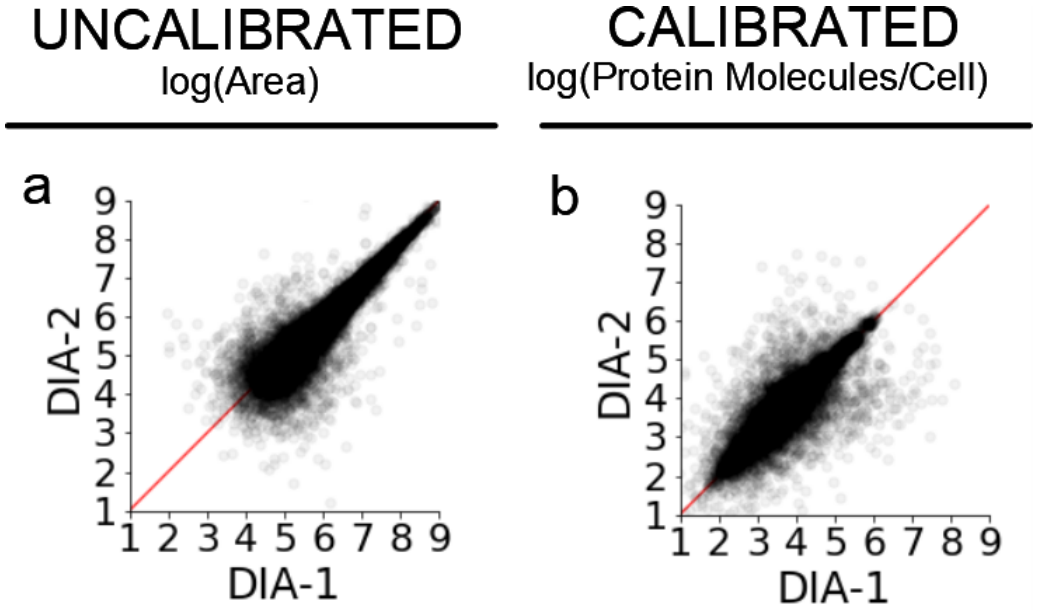

Pino et al, Anal Chem, 2018

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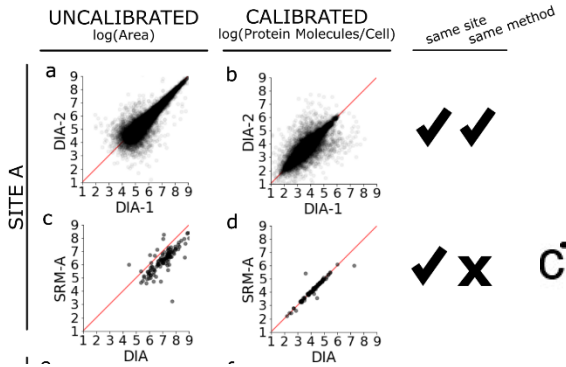

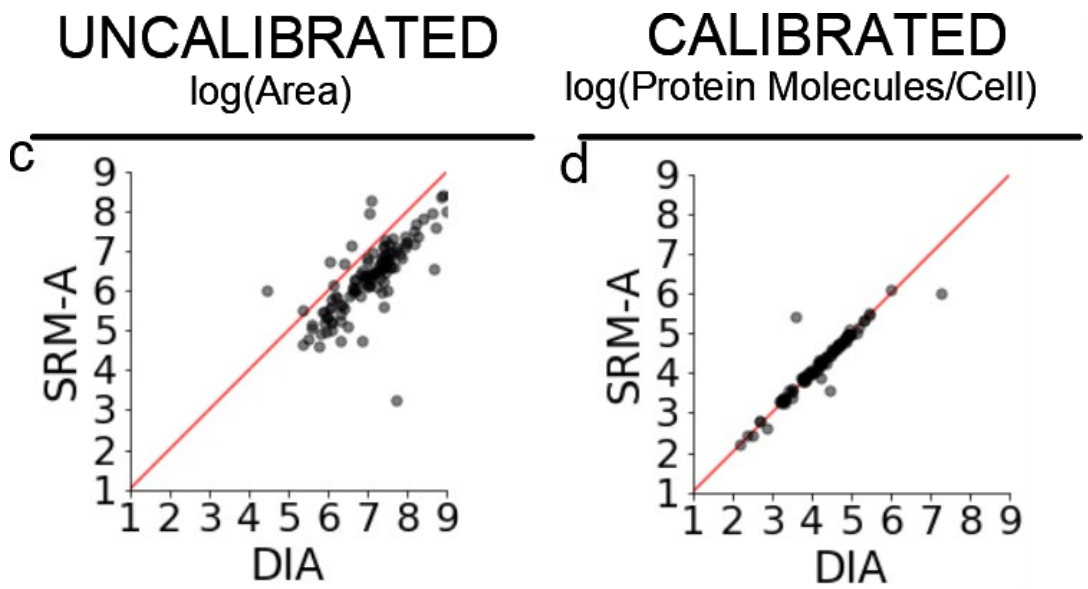

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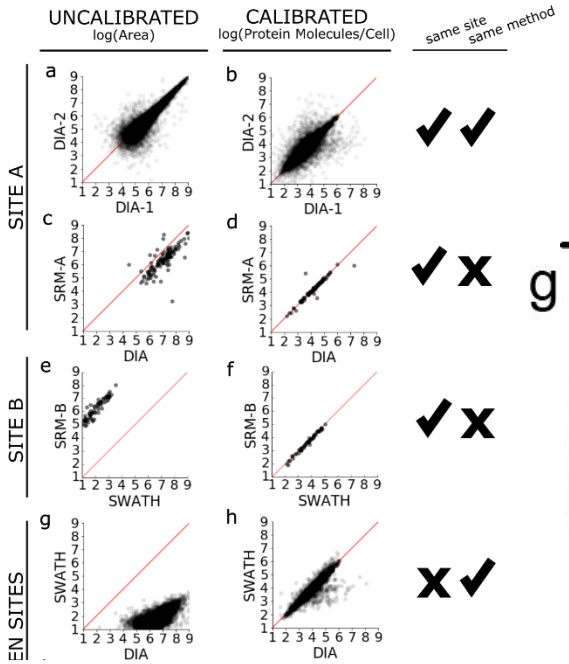

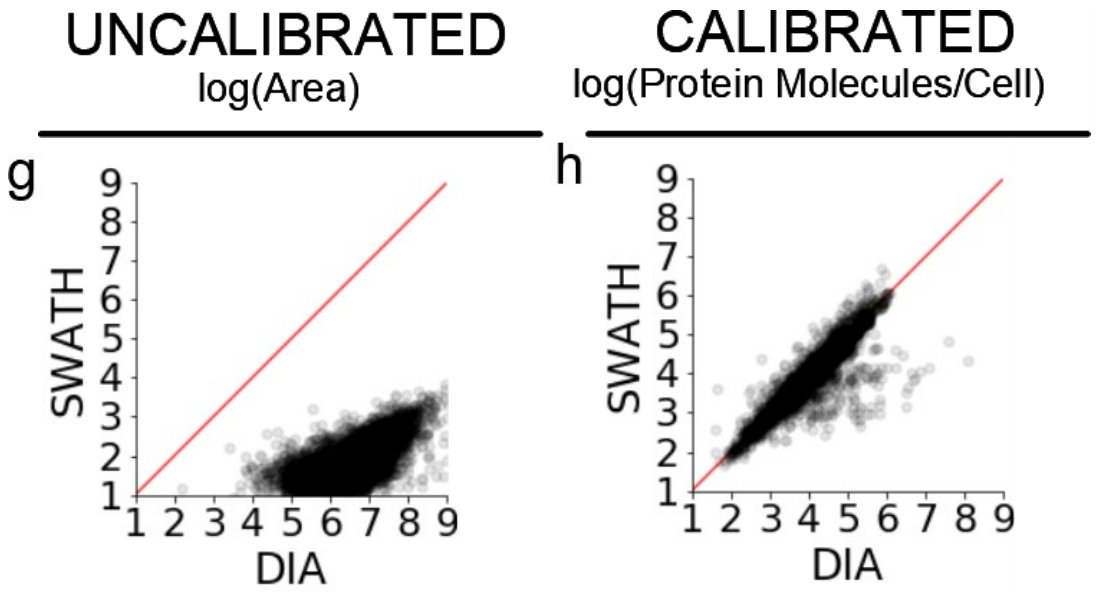

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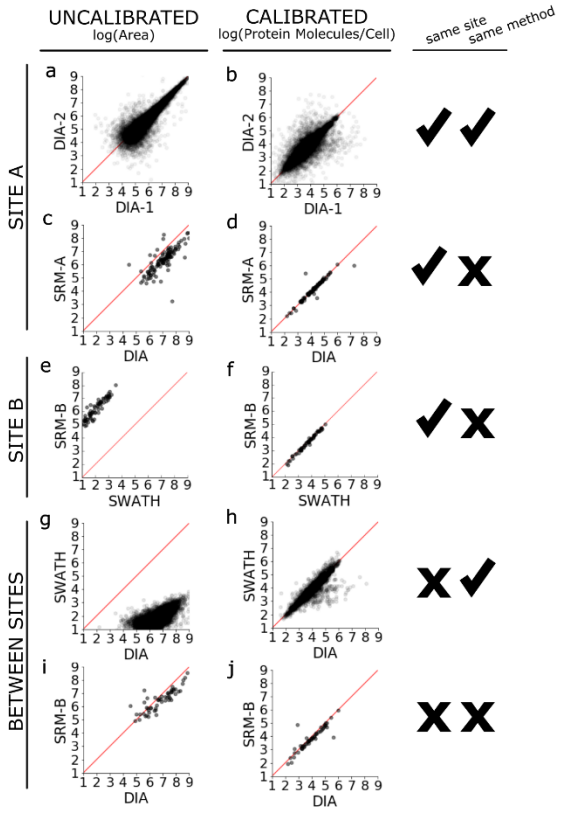

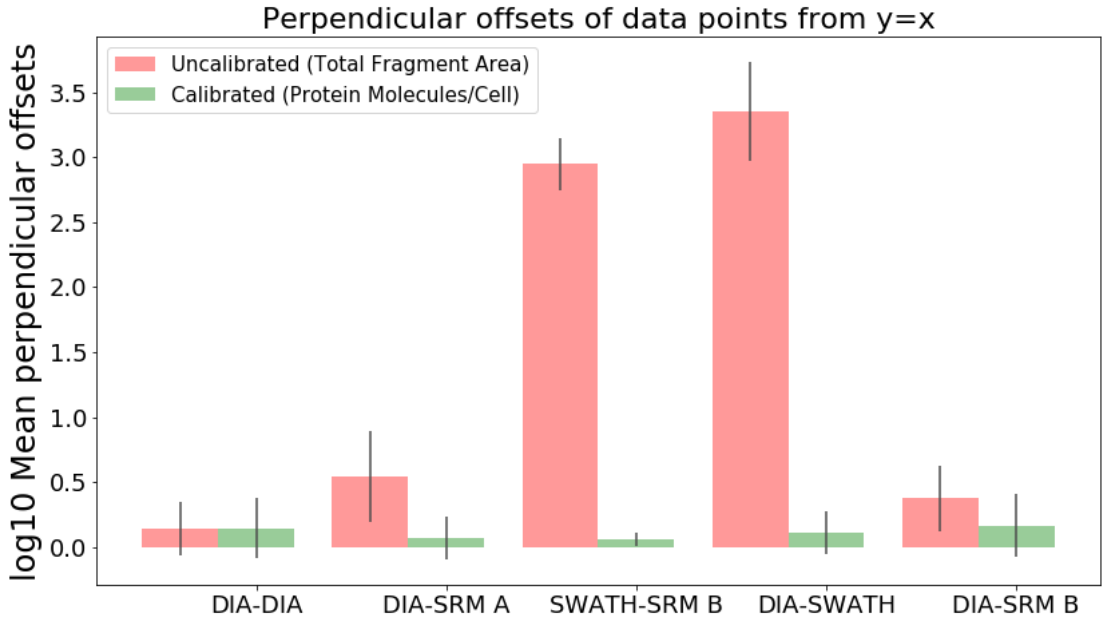

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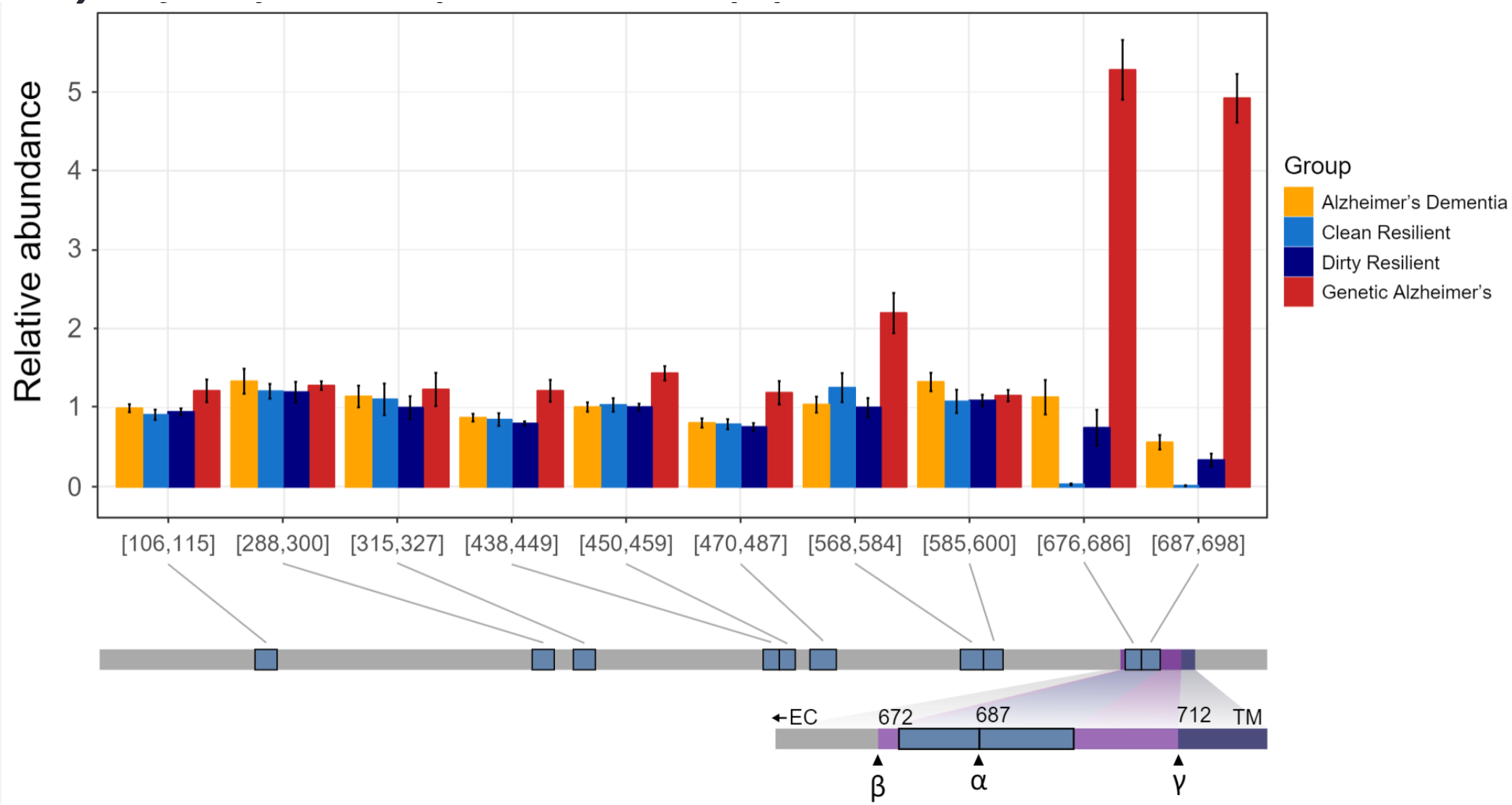
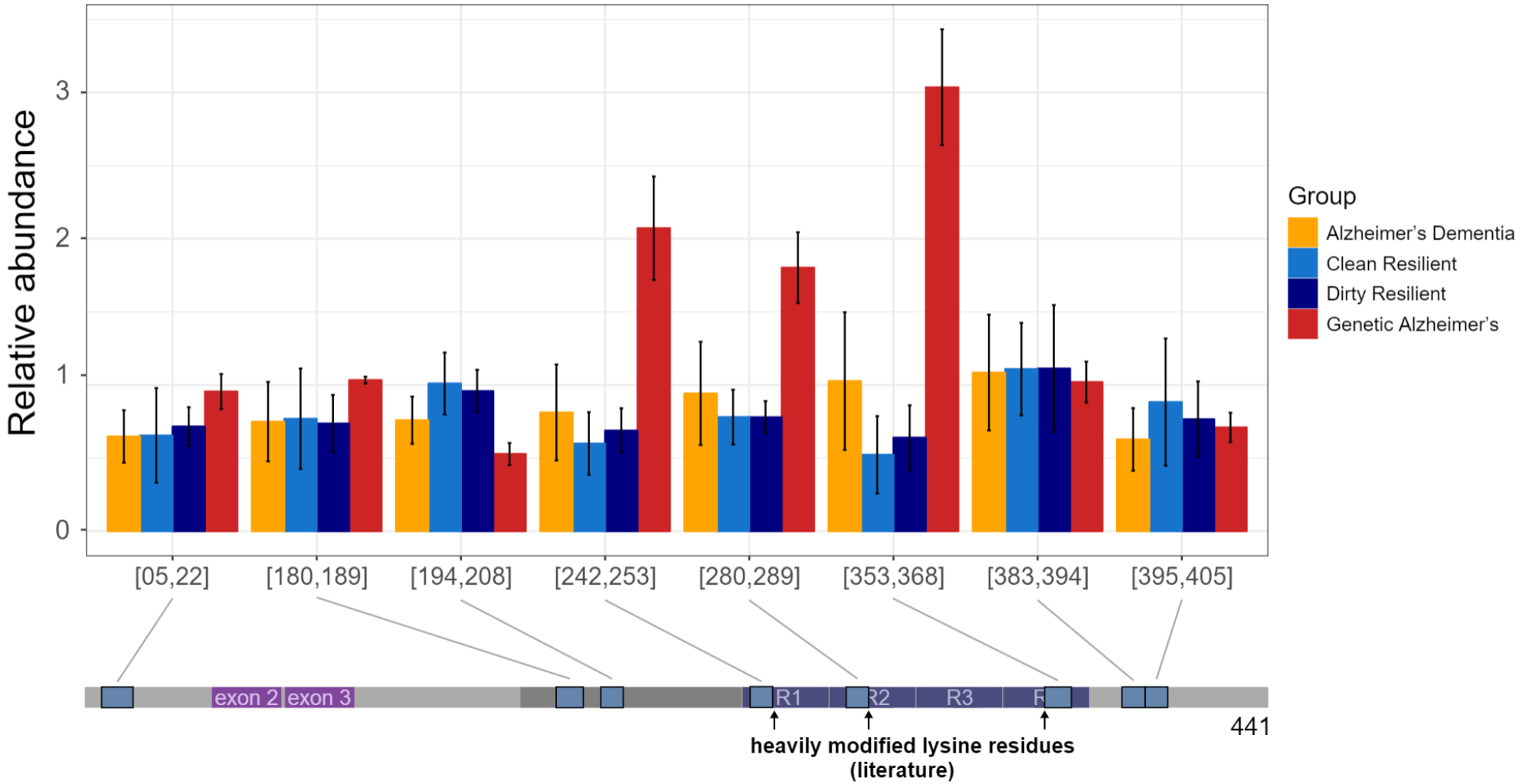
Different Peptides Reflect Different Proteoforms

Amyloid Precursor Protein



Different Peptides Reflect Different Proteoforms

Tau



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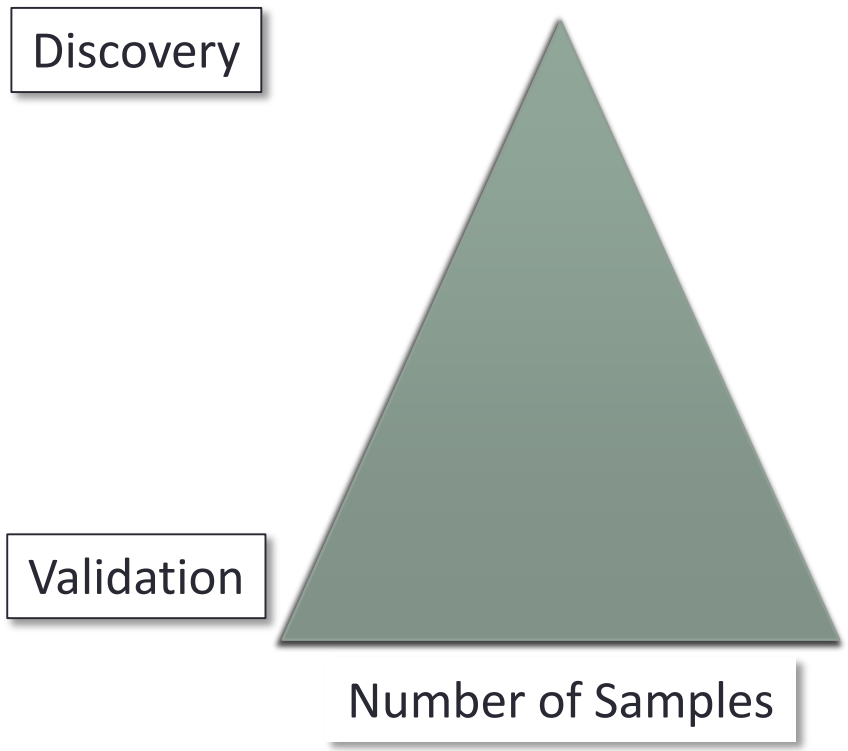
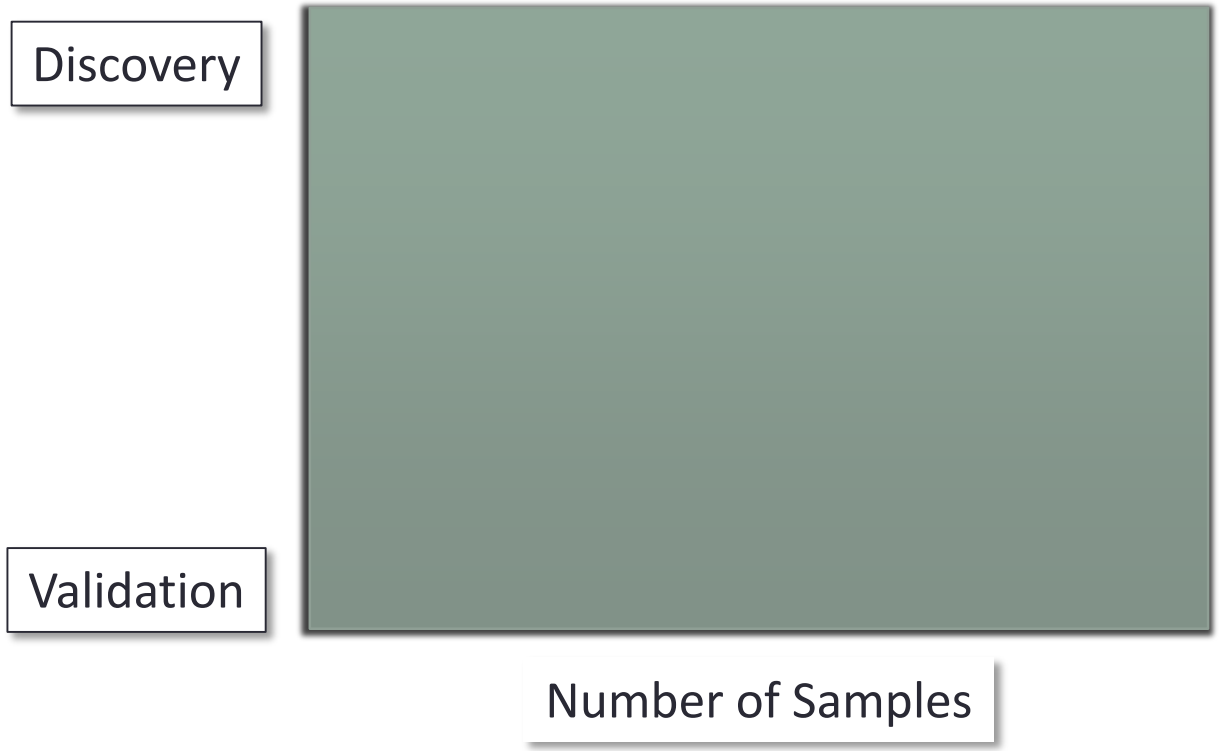
Shift from a Triangular Process to a Rectangular





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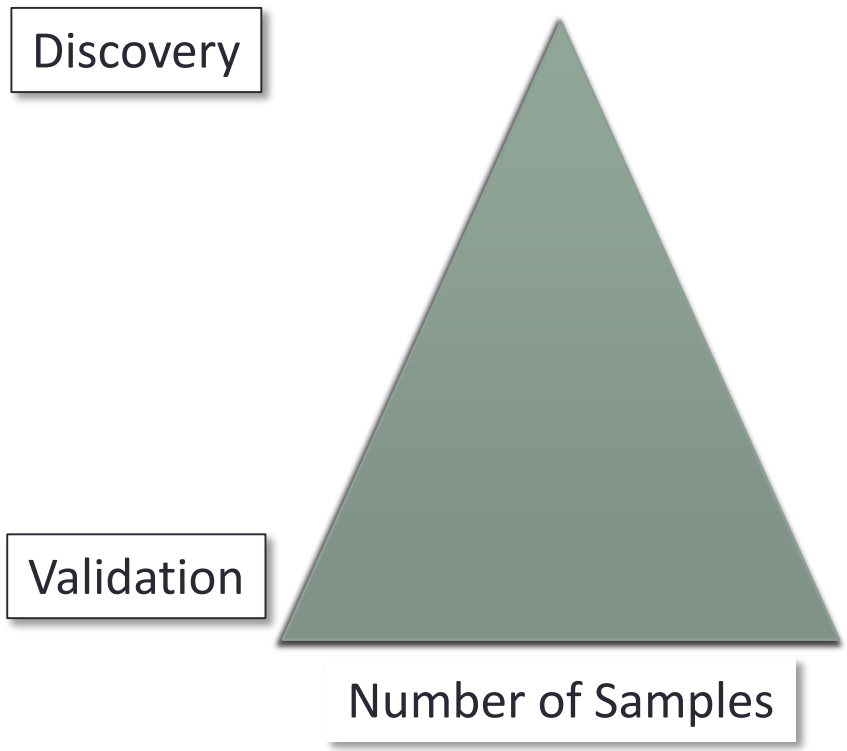
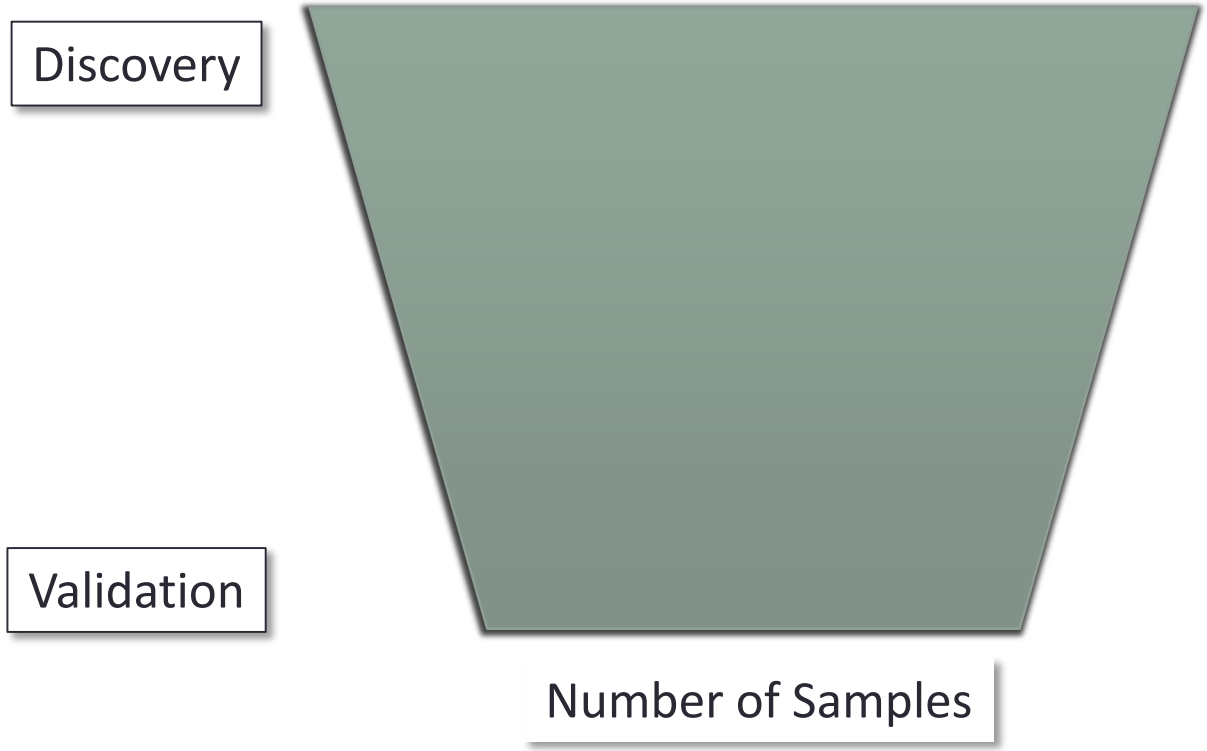
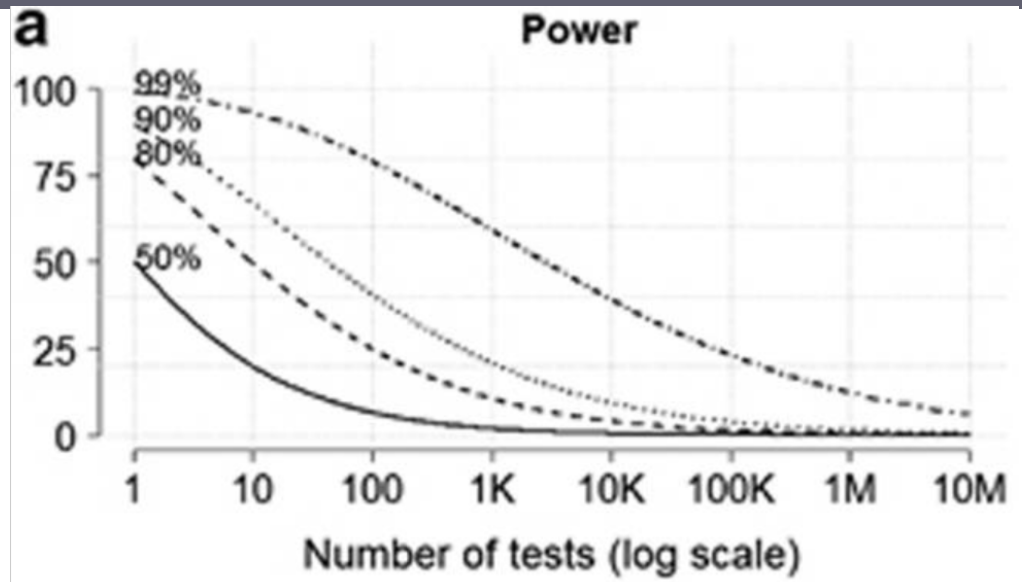
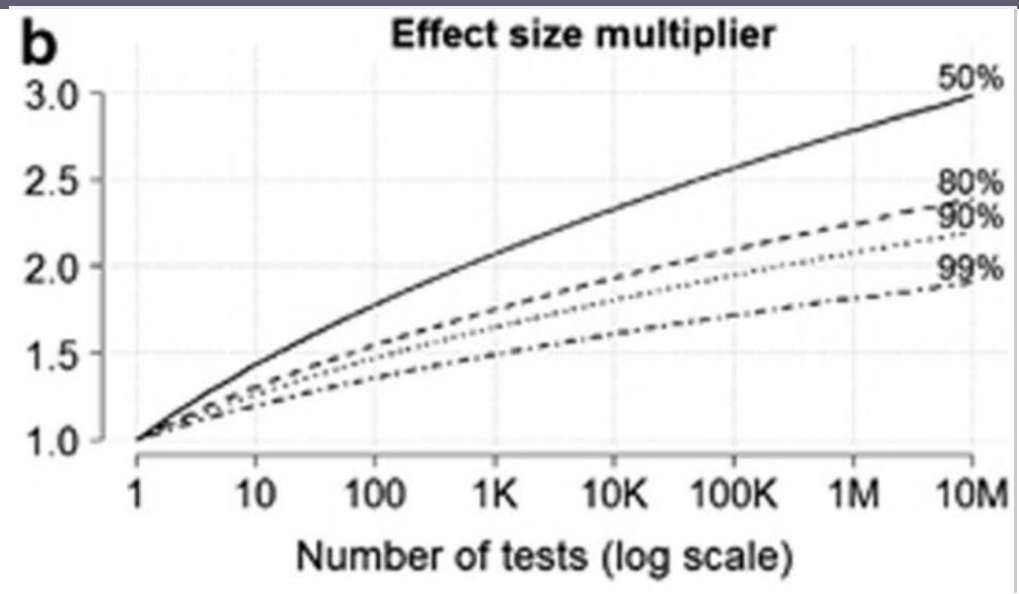
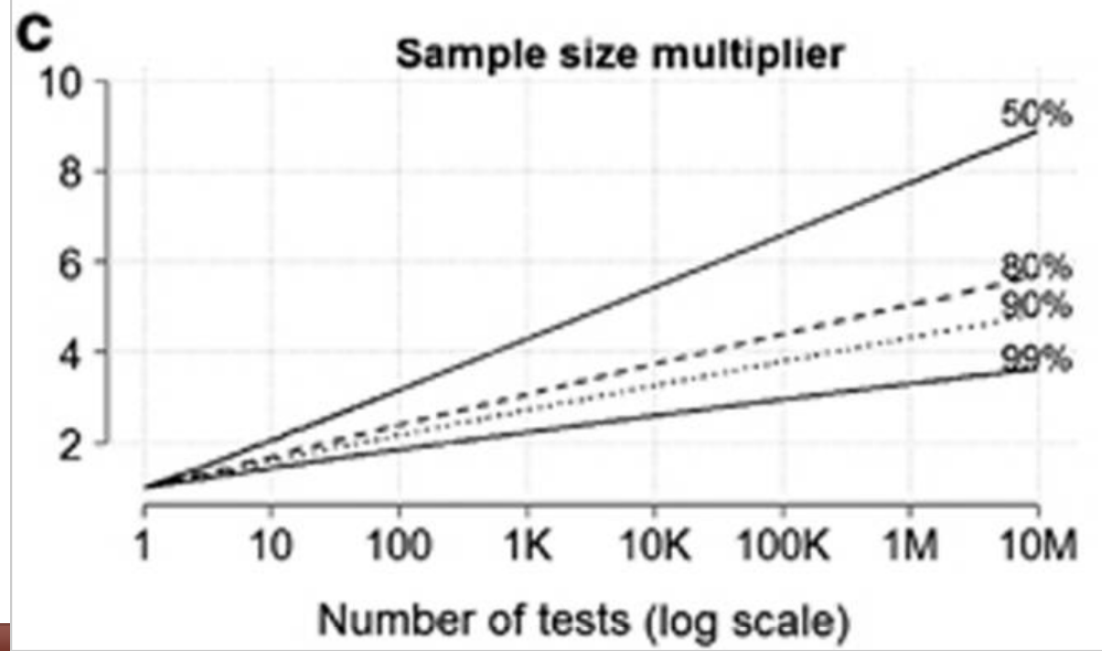




Measuring More Analytes Requires Measuring More Samples







Lazzeroni and Ray Mol Psychiatry.
2012 Jan; 17(1): 108–114.

Conclusions

- Data independent acquisition offers systematic sampling over traditional discovery proteomics.
- We have the ability to perform DIA with selectivity of 50% the precursor isolation window size across the entire m/z range.
- Dynamic range and sensitivity that approximates PRM but is comprehensive
- Signal can be calibrated between labs and instrument platforms using a common external reference sample
- Stop doing protein roll up for bottom-up proteomics.
- Proteomics assays that measure lots of peptides require lots of samples.

Acknowledgement

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Vagisha Sharma
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John Stamatoyannopolous
Judit Villen and Lab

The Broad Institute
Jake Jaffe and Lab

UW Lab Medicine
Andy Hoofnagle

Northeastern
Olga Vitek and Lab

Former Lab Members
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