

Using DIA to inform the development of Cerebrospinal Fluid triple quad assays

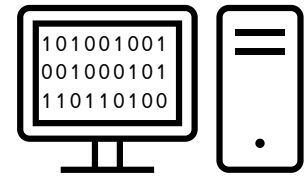
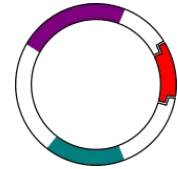
Webinar #22: Using DIA Data To Create SRM Methods

Deanna L. Plubell MS

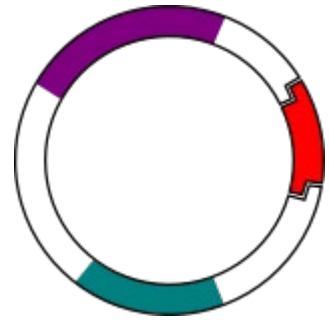
PhD Candidate, MacCoss Lab, University of Washington

Strategies for building a new targeted triple quad assay:

- Analysis of recombinant target proteins
- Search for proteins in existing data
- Use prediction algorithms for selecting peptides



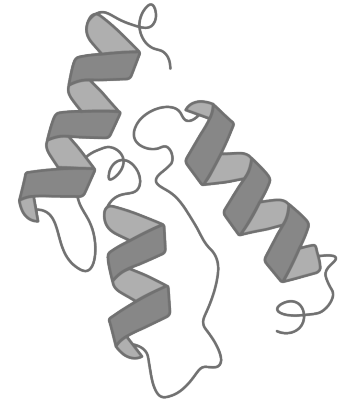
Using recombinant proteins to select peptides



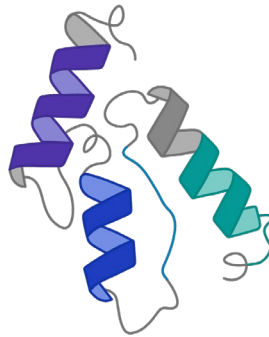
Expand, Purify,
& Sequence
cDNA clone

In Vitro
Transcription
and Translation

Enrichment



digestion

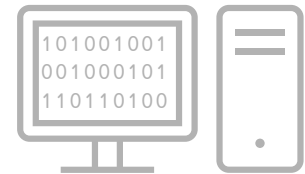
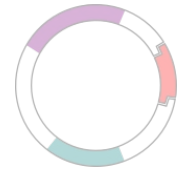


detectable?

Measurement in a background matrix is important!

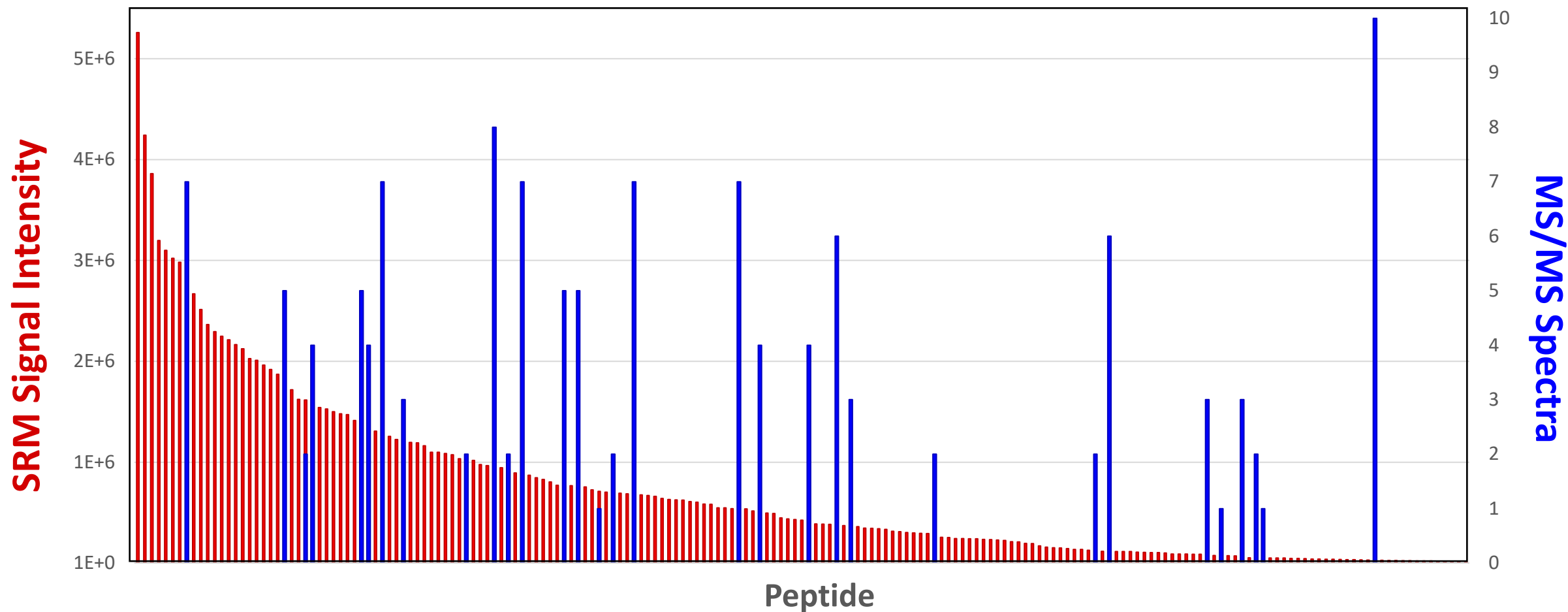
Strategies for building a new targeted triple quad assay:

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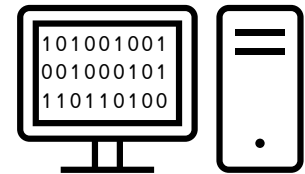
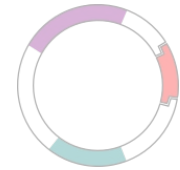
DDA is a poor indicator of SRM peptide performance

SRM **DDA**

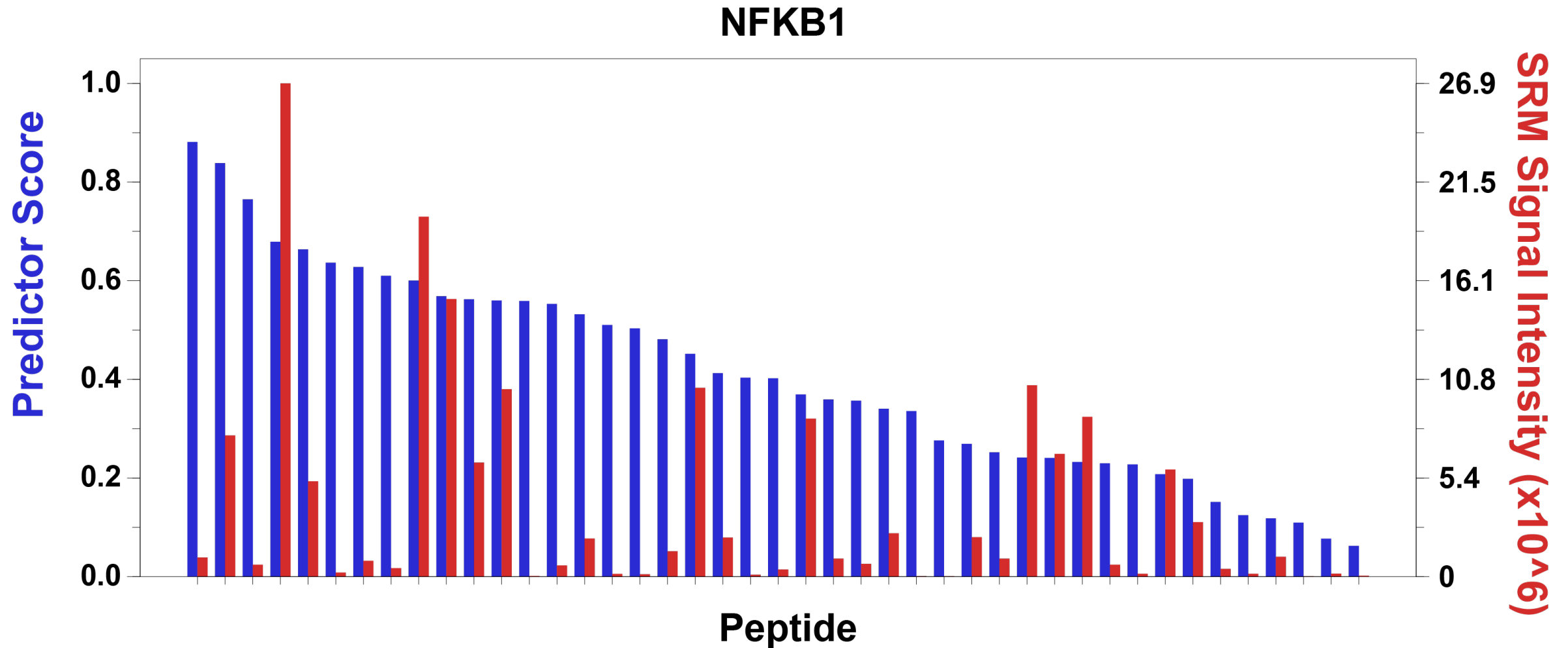


Strategies for building a new targeted triple quad assay:

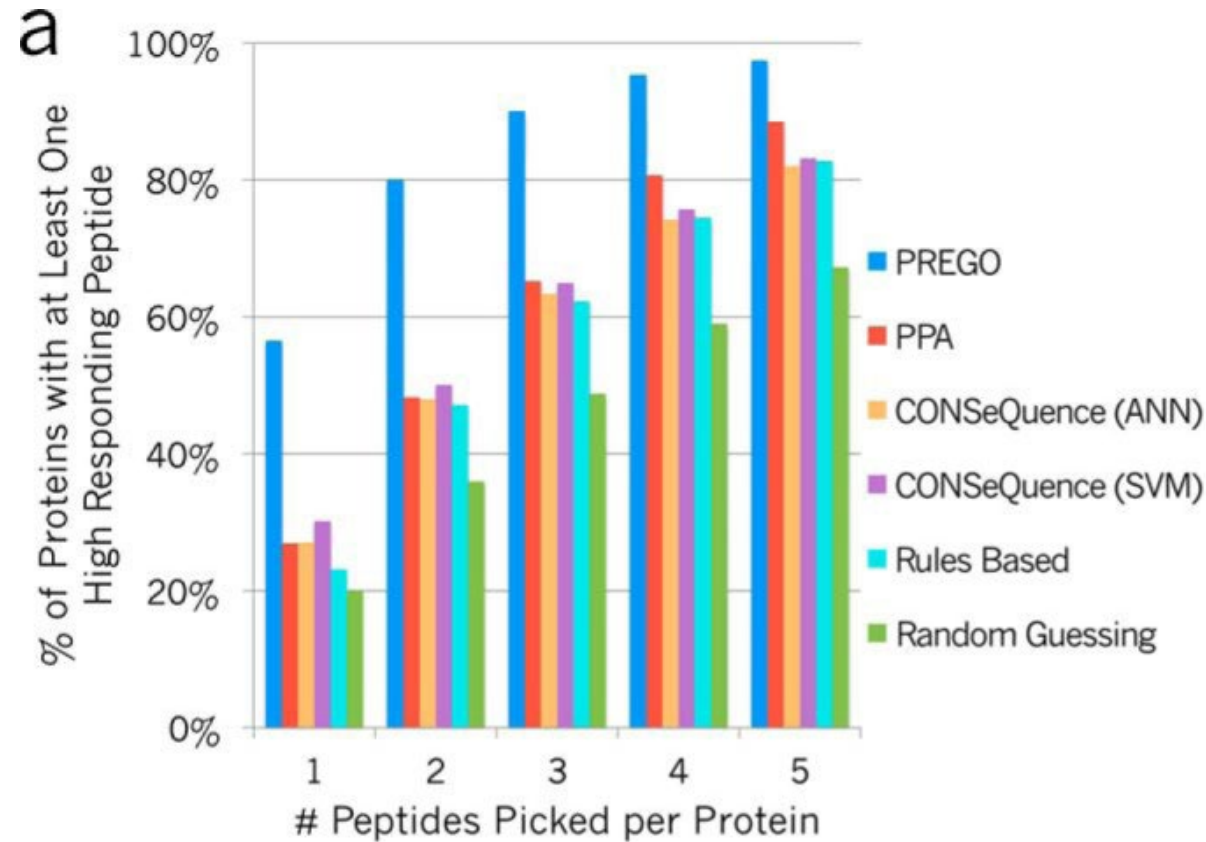
- Analysis of recombinant target proteins
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DDA is a poor predictor of SRM peptide performance

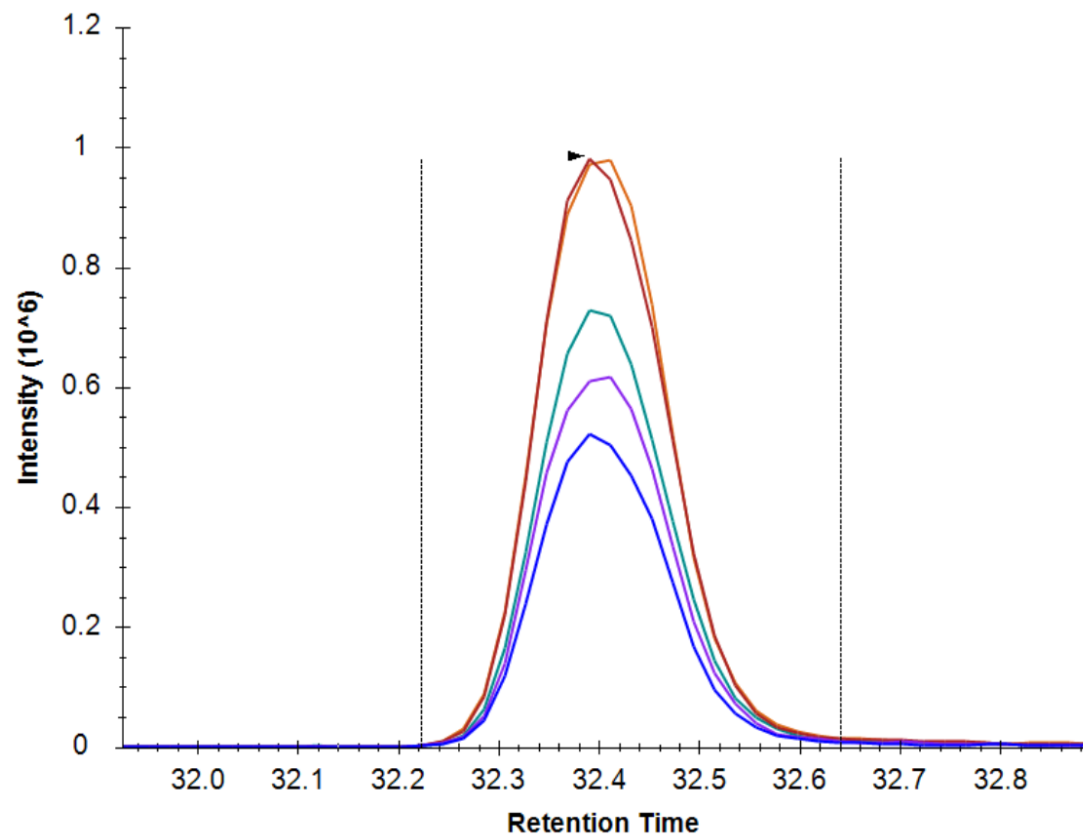


DIA is a better predictor of SRM peptide performance

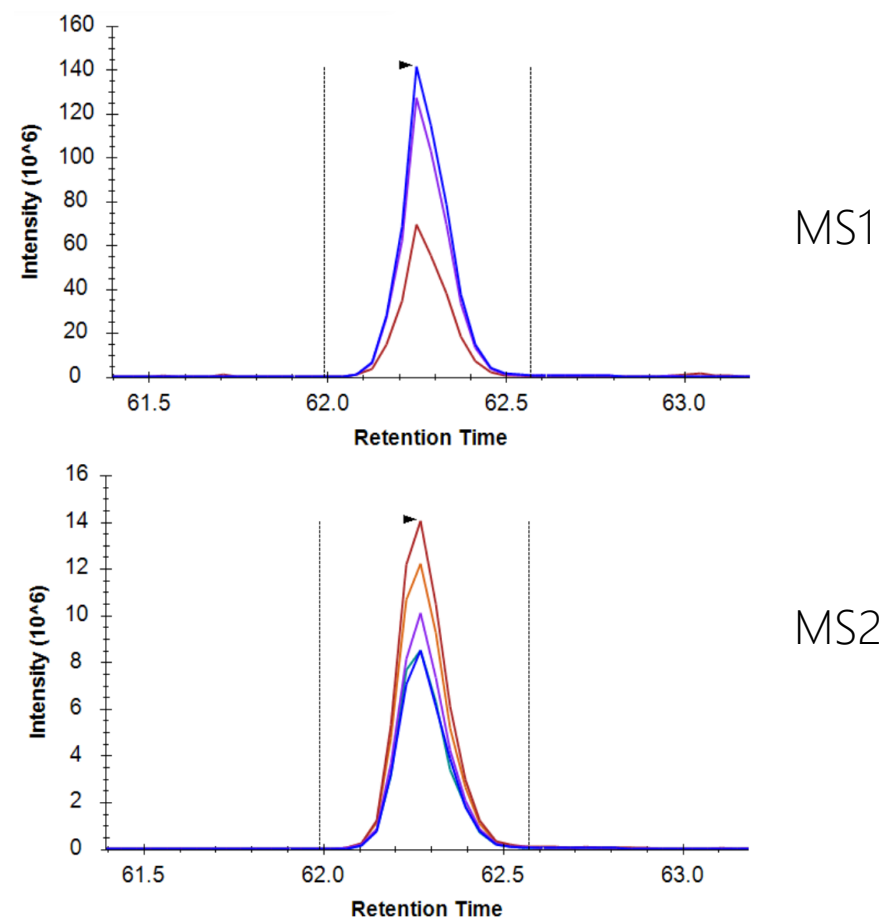


DIA provides evidence useful for SRM peptide selection

End goal: fragment ion quant

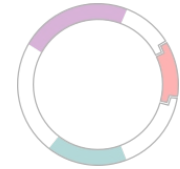


DIA more comprehensively measures fragment ions



Strategies for building a new targeted triple quad assay:

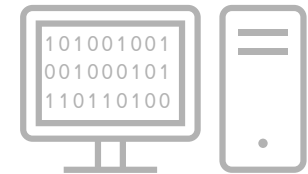
- Analysis of recombinant target proteins



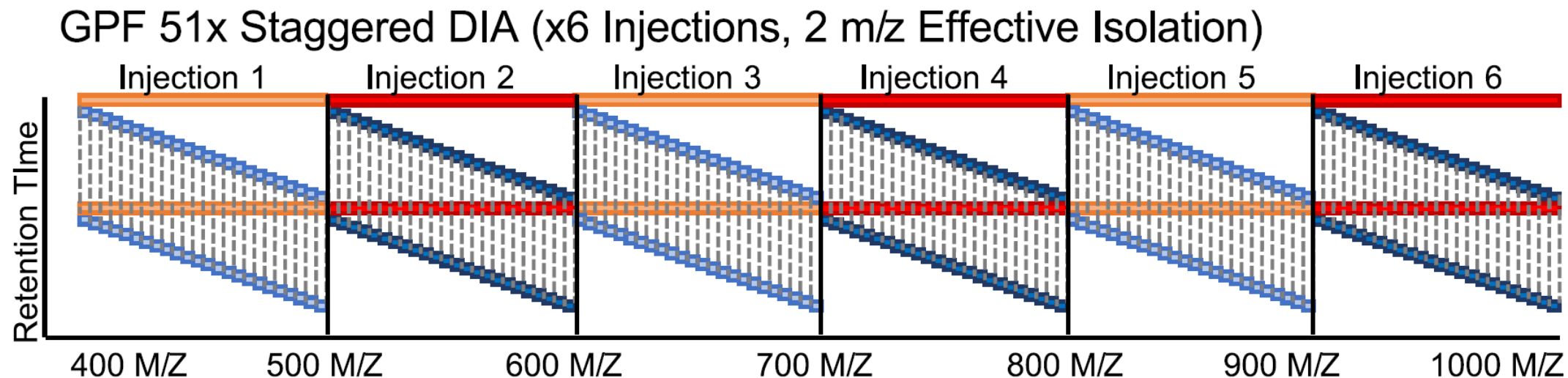
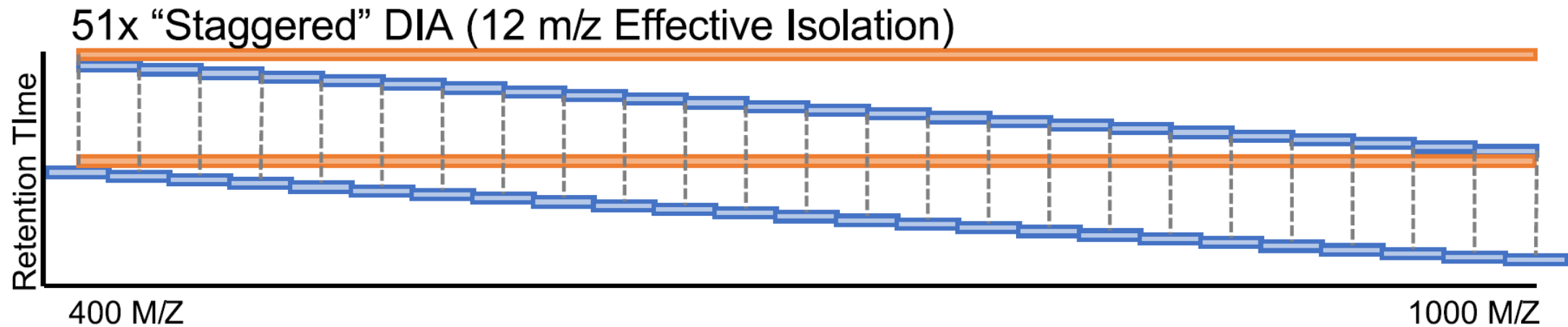
- Search for proteins in existing data *...now with DIA data*



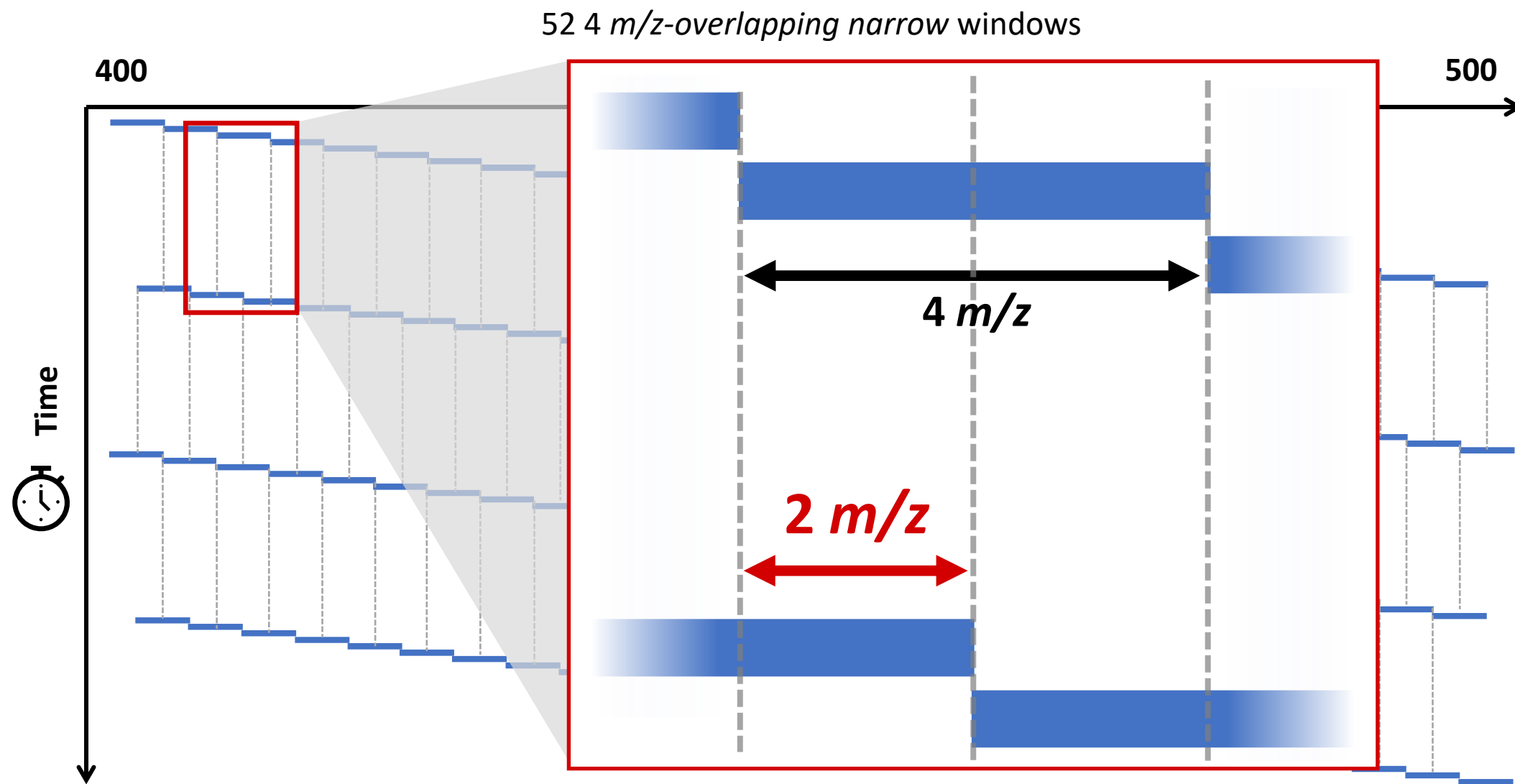
- Use prediction algorithms for selecting peptides



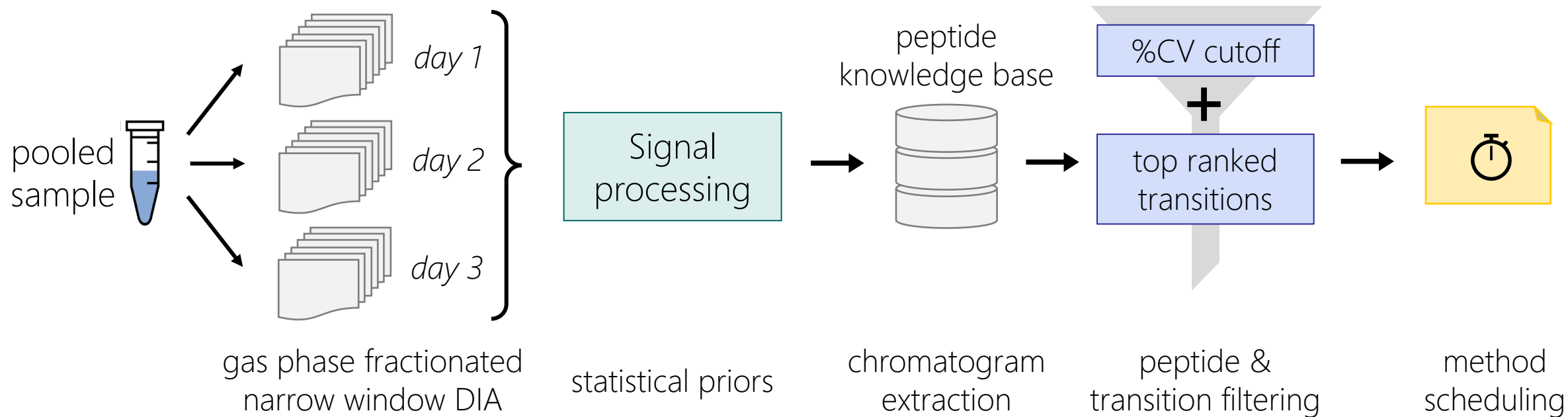
Gas-phase fractionated DIA: narrow isolation



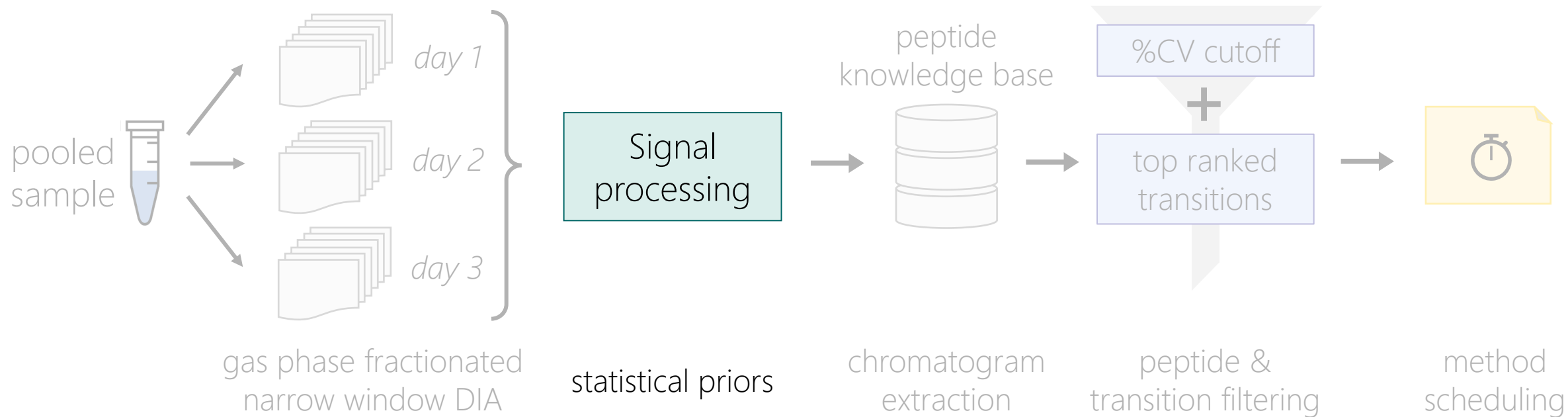
Gas-phase fractionated DIA: narrow isolation



Replicate narrow DIA provide peptide knowledge base

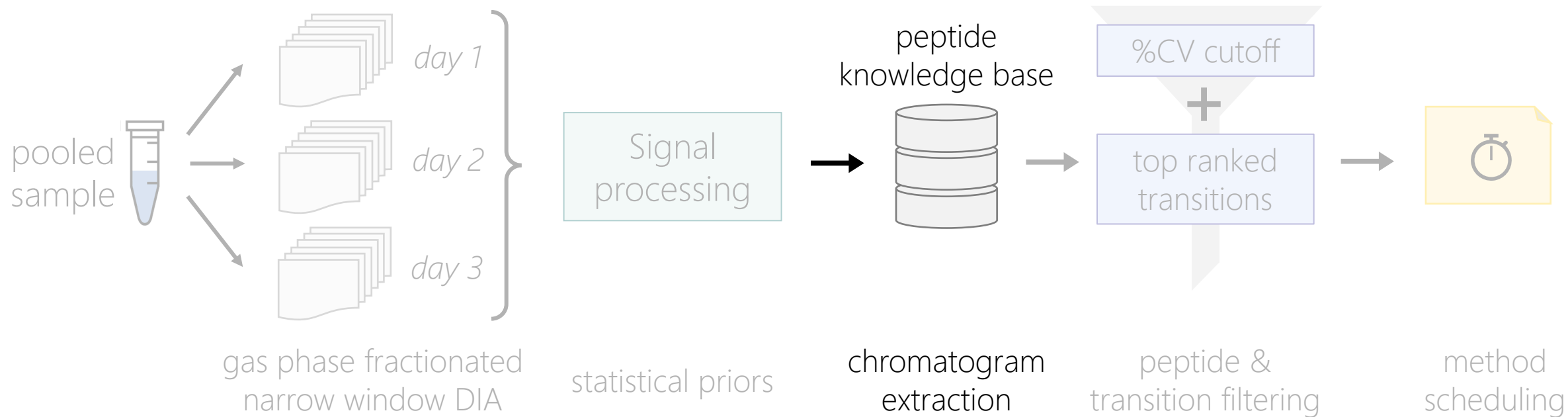


Replicate narrow DIA provide peptide knowledge base



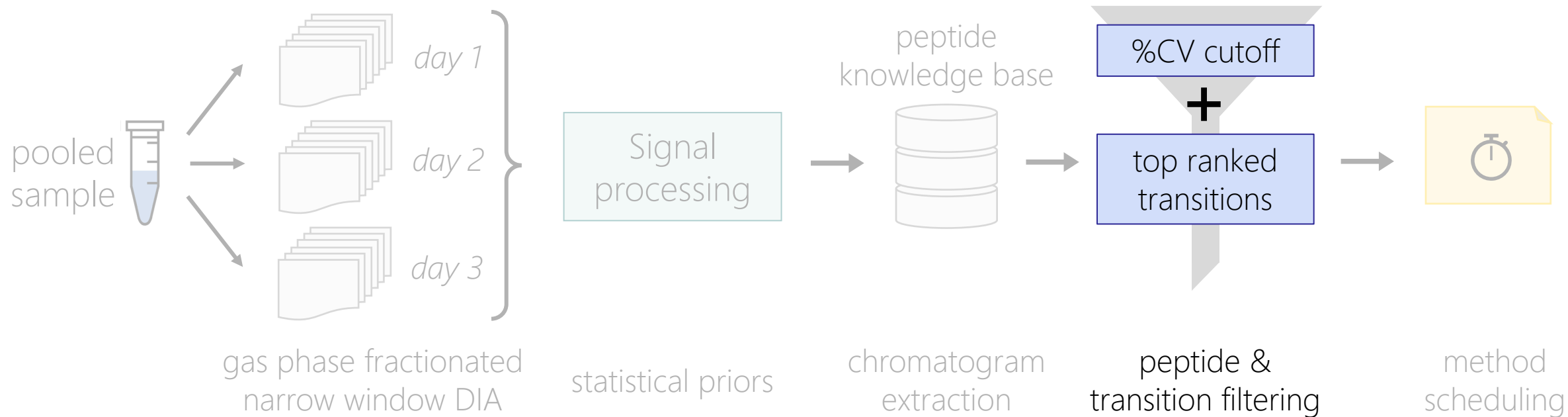
Precursor-product ion pair detection
relative retention time
Co-elution

Replicate narrow DIA provide peptide knowledge base



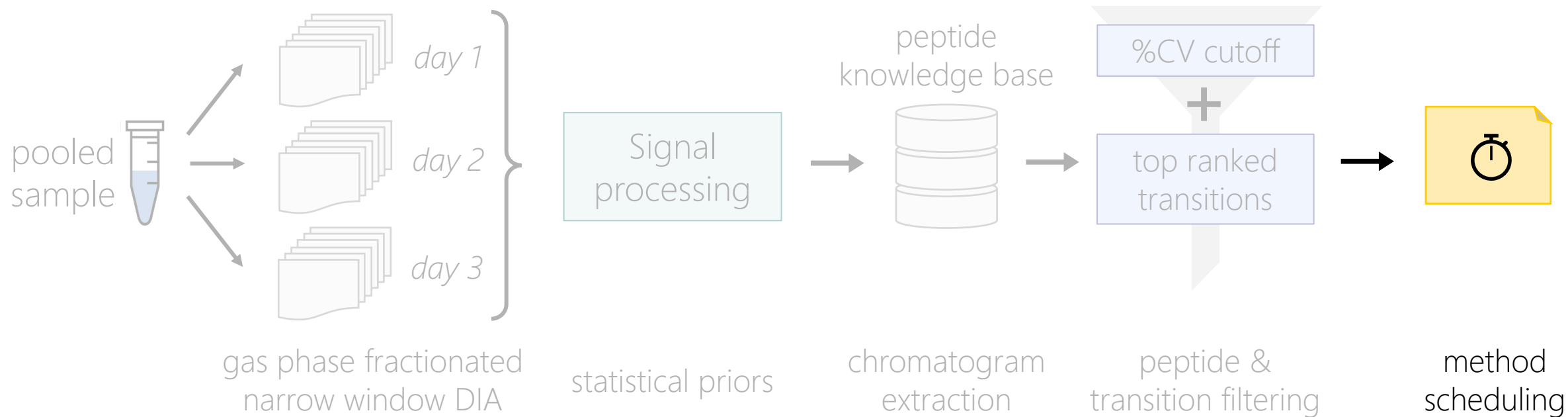
Quantitation of peptides
Visualization of chromatography

Replicate narrow DIA provide peptide knowledge base



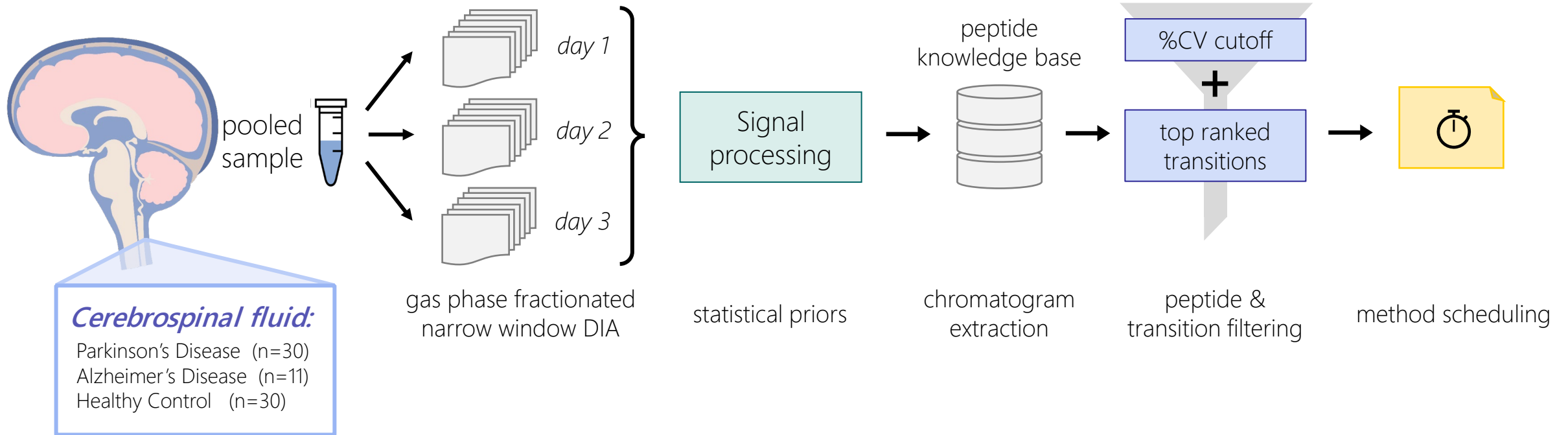
Filtering for well performing peptides & product ions

Replicate narrow DIA provide peptide knowledge base

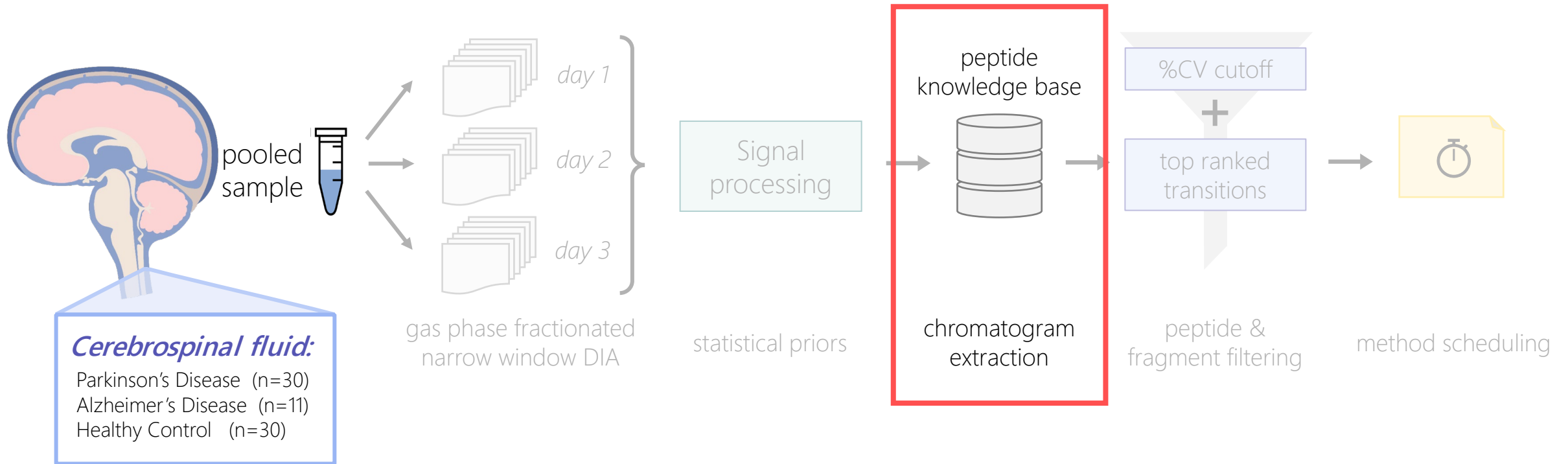


Schedule new triple-quad method with spike-in iRT standard

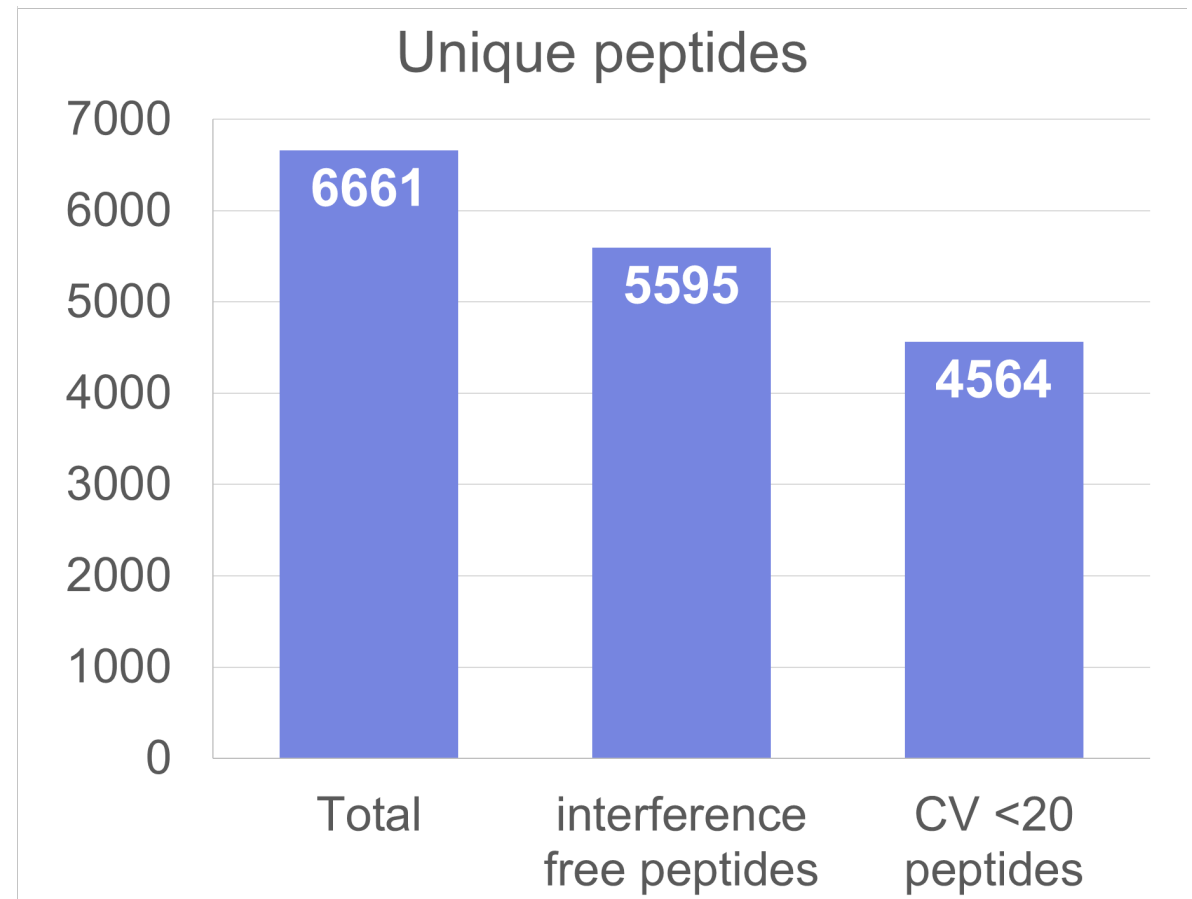
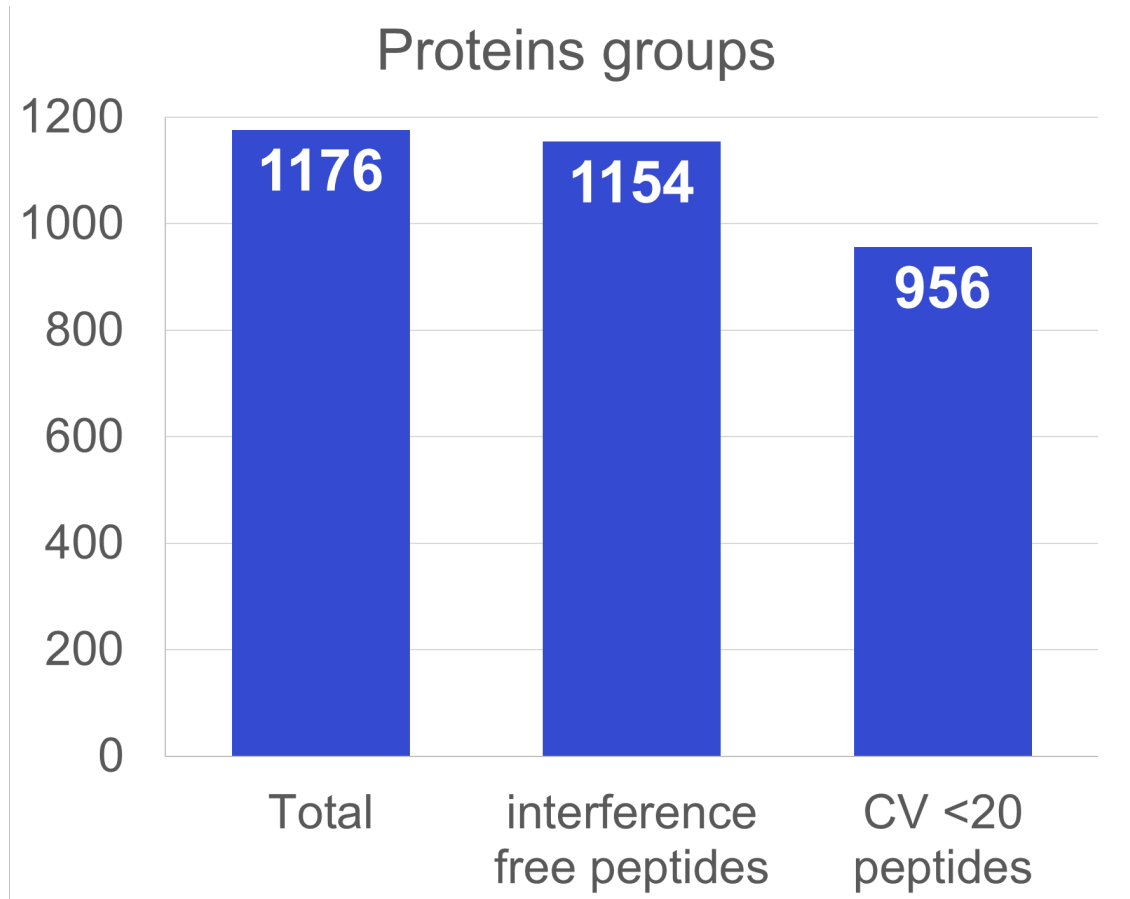
Application to Alzheimer's disease proteins in CSF



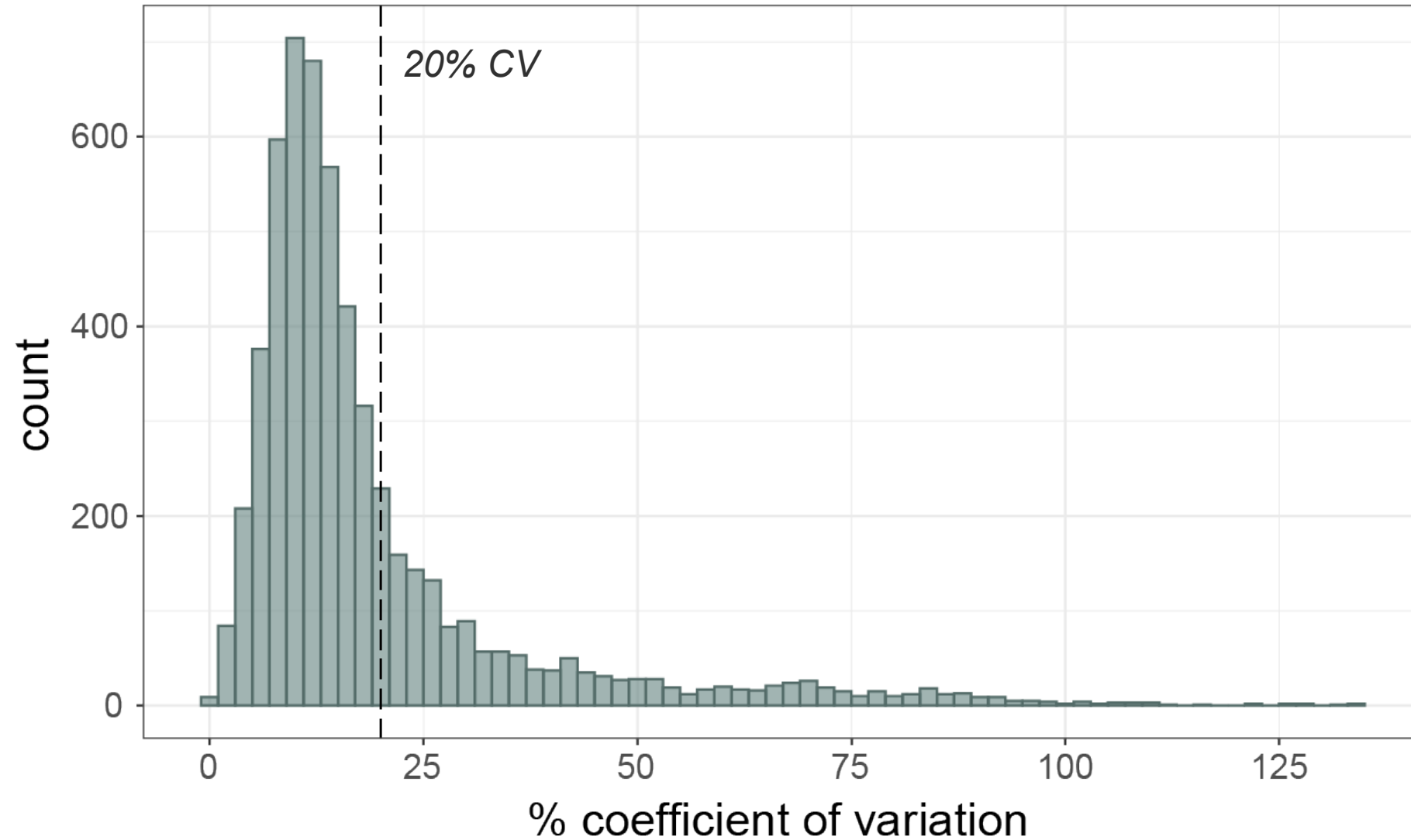
Application to Alzheimer's disease proteins in CSF



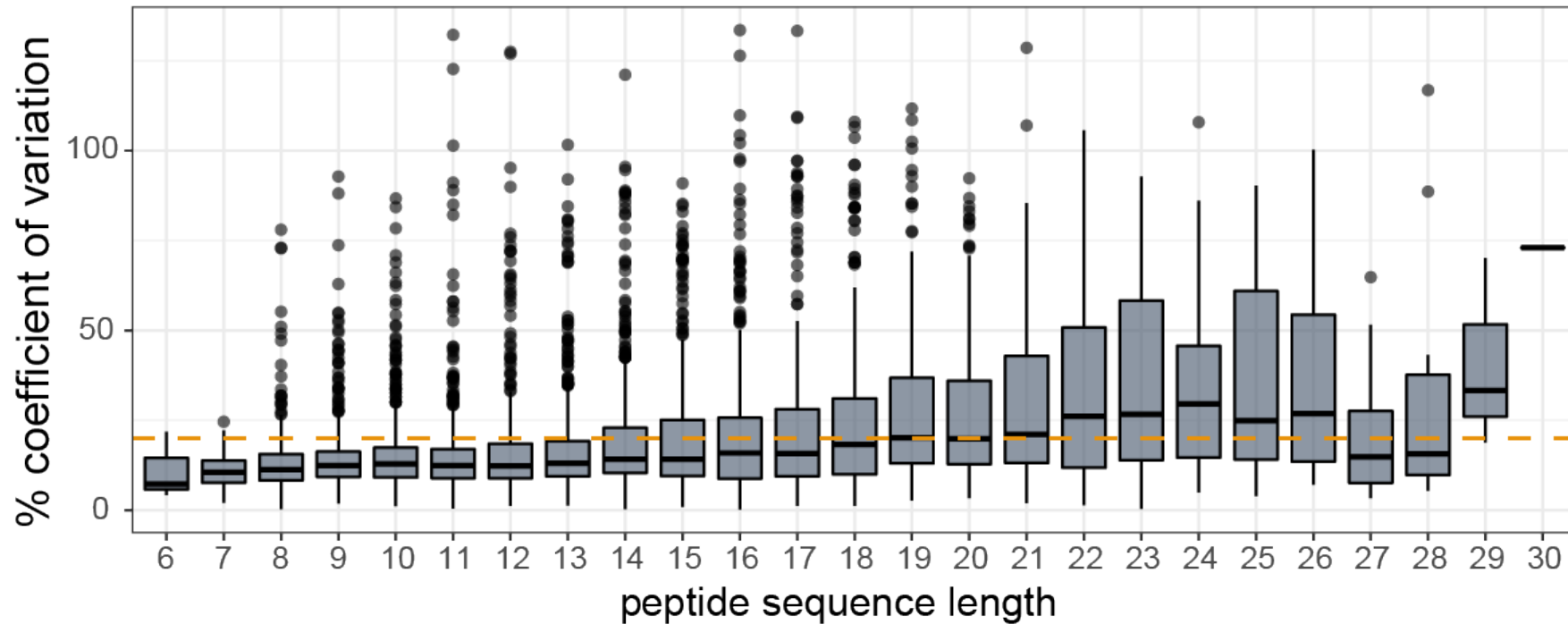
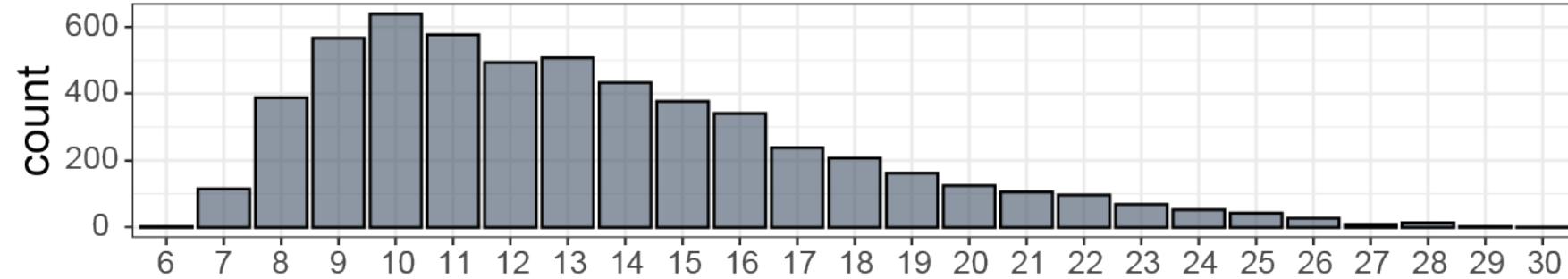
Not all proteins and peptides measured are reproducible



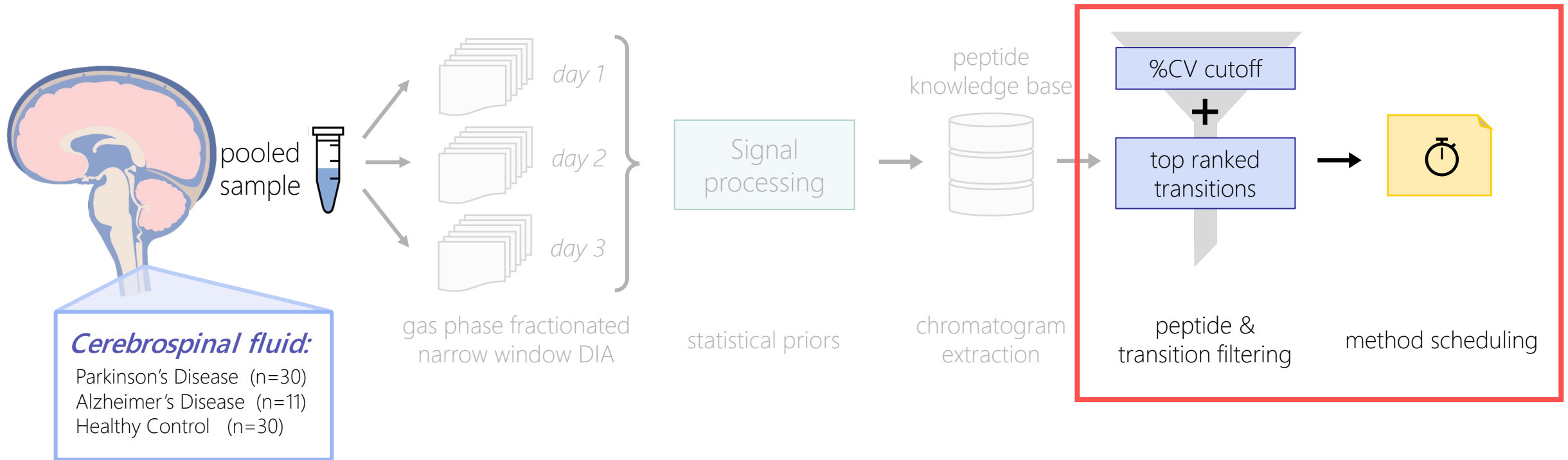
In our DIA a majority of peptides have less than 20% CV



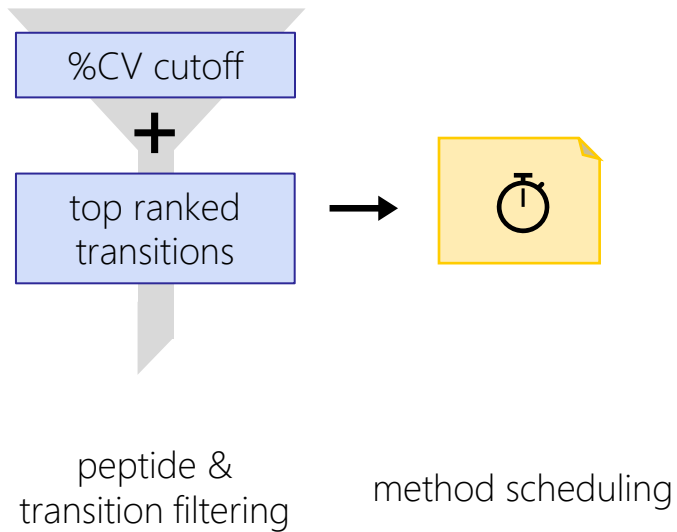
Reproducibility of peptides decreases with increasing sequence length.



Application to Alzheimer's disease proteins in CSF



Building an assay for Alzheimer's disease proteins in CSF



Proteins of interest: 100 from previous Alzheimer's assay
(*Spellman et al., Proteomics Clin. Appl. 2016*)

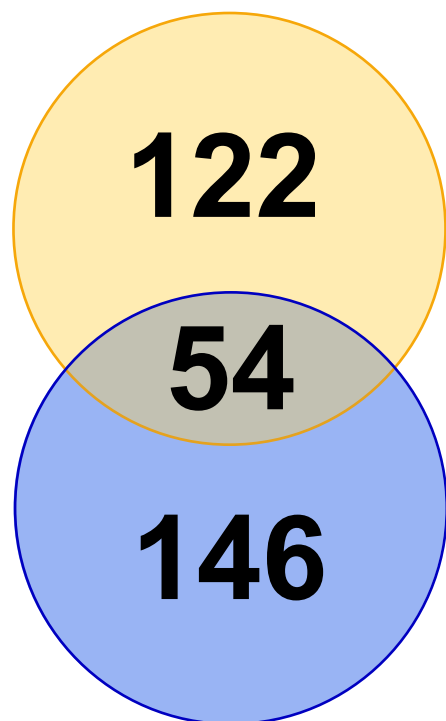
Peptide filtering criteria for 2 peptides/protein of interest:

- 5 interference-free product ions (top ranked intensity)
- <20 %CV
- Rank 1-2 based on total product intensity

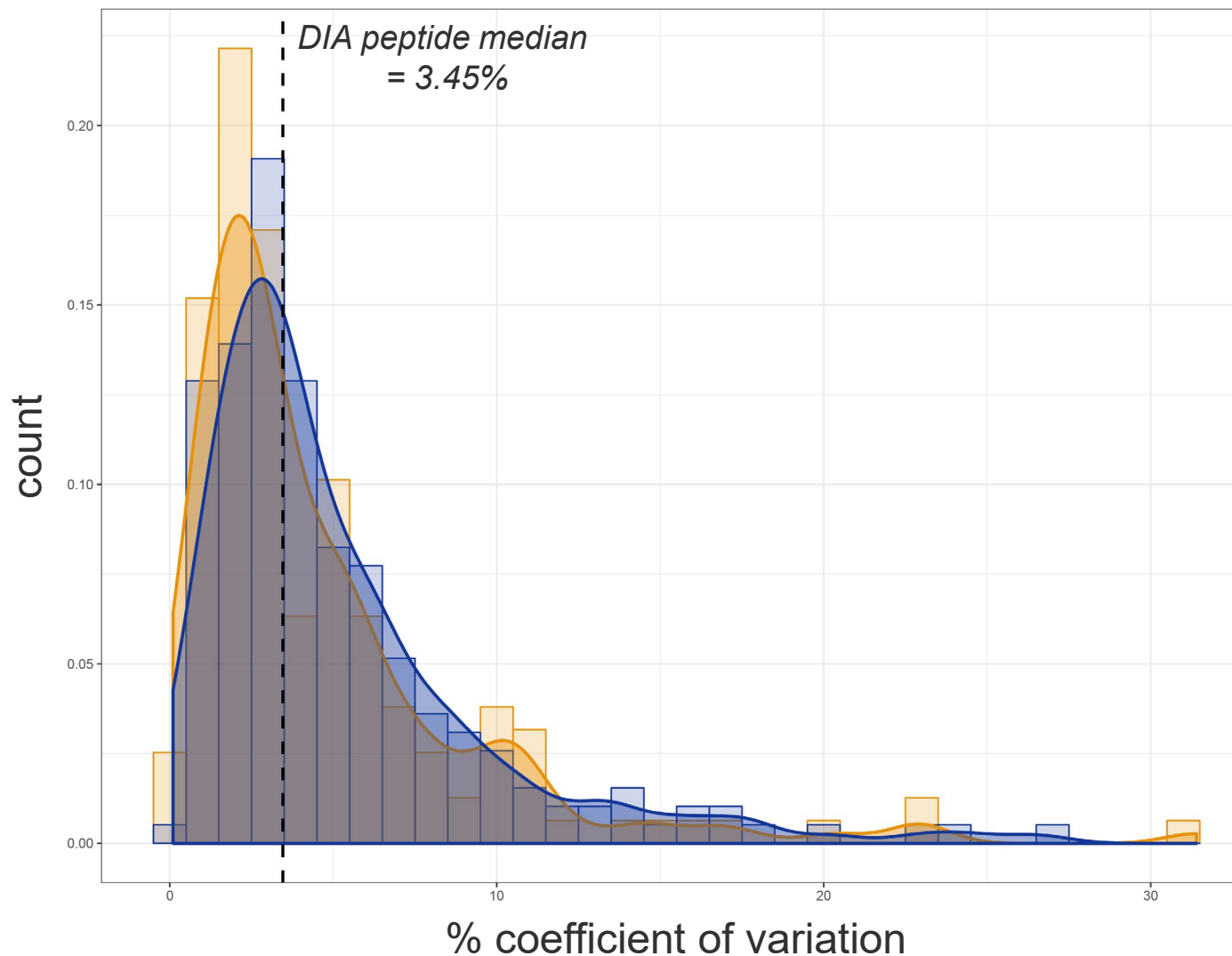
Peptides from both previous assay and selected from DIA experiment **measured in triplicate by SRM** on Thermo Altis

Peptides selected from narrow window DIA results perform similarly to previously characterized Alzheimer's disease assay peptide selections

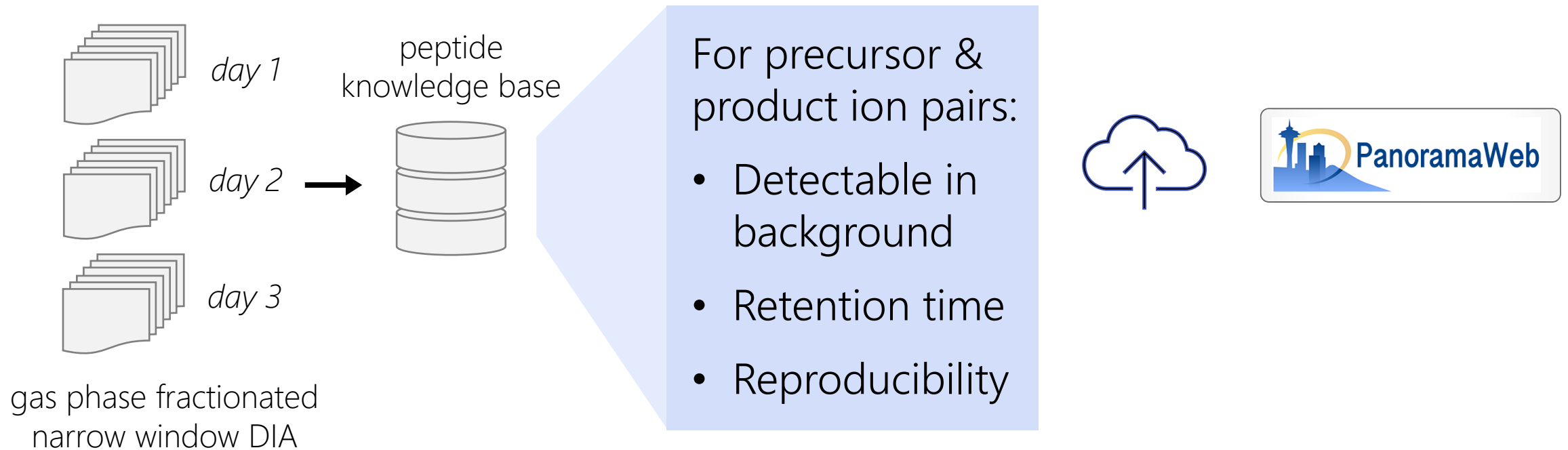
Peptides targeted in published assay



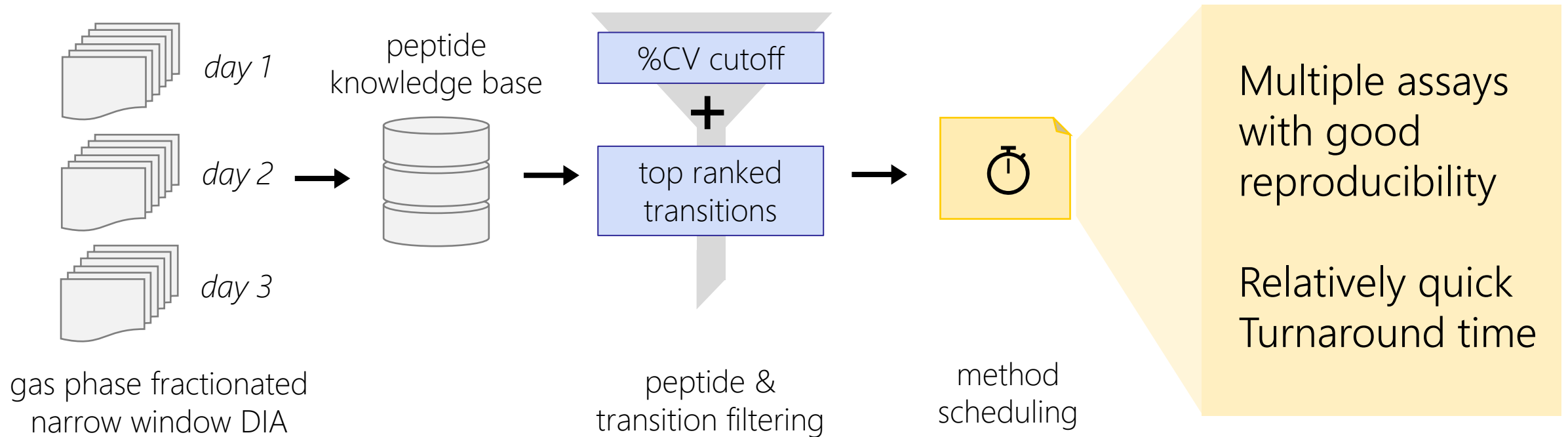
Peptides selected from DIA results



DIA provides useful information for expediting development of multiple triple quadrupole assays



DIA provides useful information for expediting development of multiple triple quadrupole assays



We can mine the same data for different targets

The human CSF pain proteome

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Eva Kosek^d, Diana Kadetoff^{cd}, Per-Johan Jakobsson^b, Kim Kultima^{a,2}, Jon Lampa^{b,*,2}

^a Department of Medical Sciences, Clinical Chemistry, Uppsala University, Sweden

^b Unit of Rheumatology, Department of Medicine, Rheumatology Clinic, Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden

^c Department of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden

^d Department of Clinical Neuroscience, Karolinska Institute, Stockholm, Sweden

New assay: with 3.06 median %CV!

Acknowledgements

MacCoss Lab

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Christine Wu

Julia Robbins

Skyline/Panorama

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Vagisha Sharma

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Josh Eckels

Stanford

Tom Montine

Kathleen Poston

UW

C. Dirk Keene

Andy Hoofnagle



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Overview of our Skyline walk-through:

1. Importing GPF DIA search results into Skyline
2. Filtering peptides based on %CV
3. Filtering target proteins then filtering peptides based on intensity
4. Scheduling & exporting transition lists for SRM methods