Accelerated workflow for targeted SRM assay development from DIA chromatogram library: Targeted assay for Alzheimer's disease markers in cerebrospinal fluid

Deanna Plubell[1], **Eric Huang[1]**, Sandi Spencer[1], Tom Montine[2], Michael MacCoss[1] | [1]*Department of Genome Sciences, University of Washington,* [2]*Stanford University*

Introduction

Targeted mass spectrometry is valuable for the multiplexed measurement of proteins in biofluids. Targeted assays offer distinct advantages in cost and performance for high-throughput targeted measurements, but the effort to establish and validate multiplex assays is non-trivial[1]. Synthetic reference peptide standards in stable isotope–labeled and unlabeled forms are used extensively in targeted proteomics method development and verification. However, choosing which peptides to purchase is often a not straight forward process. Shuford et al. (Anal Chem 2017) recently showed that stable isotope labeled peptides might not be an ideal calibrant for protein quantification, making it an unnecessary expense at the early stage of a project. Here we use data independent acquisition (DIA) experiments to rapidly develop targeted assays in cerebrospinal fluid, and further analyze peptide suitability by selected reaction monitoring (SRM) experiments.

Methods

DIA to SRM Workflow

- Developing targeted assays has typically relied on using data-dependent acquisition or individual recombinant protein analysis to predict or empirically select peptides. Data-independent acquisition (DIA) is a better predictor of peptides that will perform well in SRM experiments[2].
- Peptide fragmentation patterns from resonance CID are generally more different from beam-type CID used in QqQ. However, HCD fragmentation is very similar to that of CID in QqQ.
- DIA data acquired with HCD can improve SRM assay development by identifying the optimal transitions and take advantage of QqQ's robustness, ease-of-use, sensitivity and speed.
- Using chromatogram libraries generated by DIA, we obtain more information about peptides we are detecting.
- Assessing peptide signal and variability from replicate DIA runs allows us to select peptides that will perform well in SRM.

Data-independent Acquisition

Thermo
Q Exactive HF

Peptide/protein detection
- Six gas phase fractionated runs targeting 400 to 1000 m/z
- 4m/z overlapping narrow precursor isolation windows
- Generate Chromatogram Library
- Perform wide window quantitative DIA

filter
Unique
Consistent
Potential targets
filter
Top ranked intensities
Precision: ≤ 15% CV

iRT calibration

Method Transfer to Selected Reaction Monitoring

Thermo
Altis Triple Quadrupole

- Export top 10 peptides of each protein and top 5 transitions of each peptide as Thermo Altis Triple Quadrupole method in Skyline.
- Using the same LC, columns and gradient can help with retention time alignment.

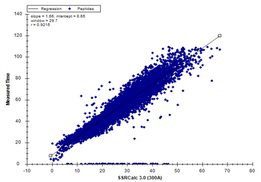
Verify targets using library dot product and retention time.

Using DIA to inform peptide selection for quantitative assays

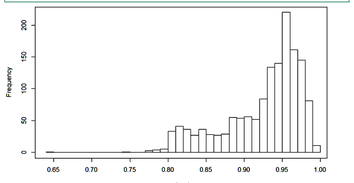
We can schedule SRM using DIA retention times. Using indexed retention time standard measurements from DIA and an SRM QC run we can calculate the expected retention windows for SRM. Even when Library Dot Product is less than 0.85 for selected targets in SRM, DIA to SRM workflow with accurate retention time alignment and calibration correctly identified SRM targets, enabling fast and reliable method transfer.

CSF DIA libraries:
1411 Proteins
9106 Unique peptides
45,471 Transitions

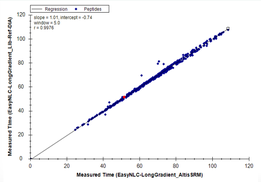
Between DIA retention time alignment



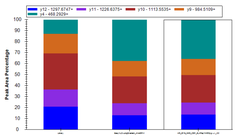
Distribution of Library Dot Product of Selected SRM Targets



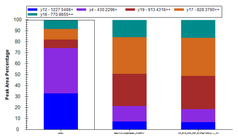
Between DIA & SRM retention time alignment



EWVAIESDSVQPVPR :
Library Dot Product = 0.82



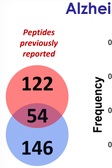
SEALAVDGAGKPGAEEAQDPEGK:
Library Dot Product = 0.66



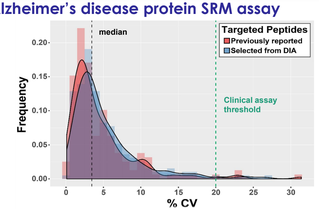
Generating multiple assays using the same DIA experiment

Alzheimer's disease protein SRM assay

Peptides previously reported

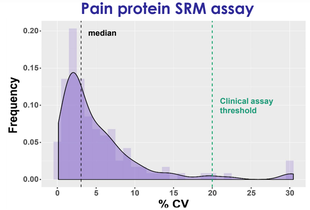


Peptides Selected from DIA



Performance of peptides for AD proteins selected from DIA vs previously reported. To benchmark our peptide selection workflow we compared it to a previously reported assay[3]. Our selection did not have a large overlap with the previously reported assay; however, SRM performance of peptides are comparable for each assay.

Pain protein SRM assay

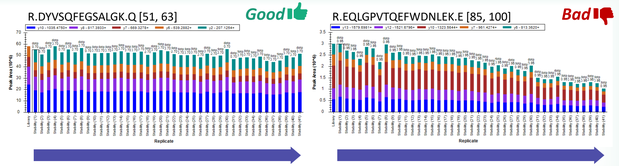


SRM performance of peptides selected from pain proteins. A major advantage of our workflow is the ability to easily generate multiple assays from the same DIA experiment. We generated and ran a targeted assay for pain proteins in 4 days. Selected peptides perform well in SRM experiments.

Assessing peptide suitability for quantitation

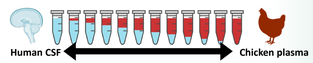
Stability

Not all peptides are stable. For high throughput assays samples may sit in solution at 4°C for extended periods of time. Some peptides retain stable signal, while others do not. *Right:* peptides from the protein APOA1, sampled across 60 hours show different stability profiles

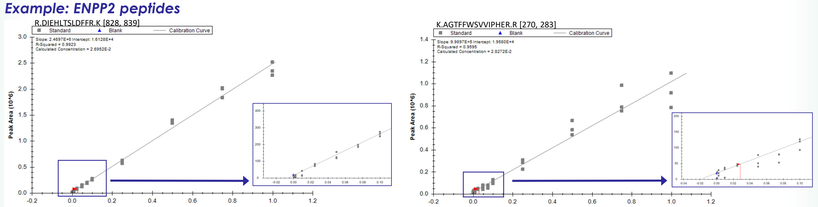


Sensitivity

How low can we go? For a quantitative assay we need to know when we can no longer reliably quantify a peptide (lower limit of quantification) or detect a peptide (lower limit of detection). We determine these figures of merit by analyzing serial dilutions of our sample into a background matrix.

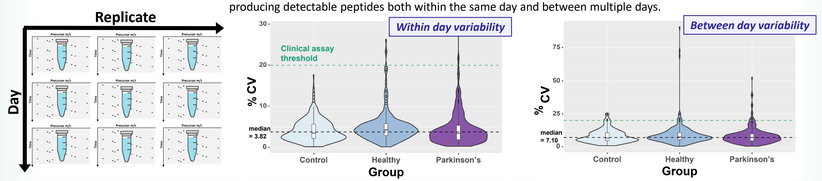


Example: ENPP2 peptides



Reproducibility

Prep. Measure. Rinse. Repeat. For high throughput assays the number of samples may necessitate preparation to be performed in batches. Therefore we need to know how reproducible our sample preparation is at producing detectable peptides both within the same day and between multiple days.



Conclusion

- DIA to SRM workflow provides a faster and cheaper approach for SRM assay development in biological matrix.
- The AD protein assay will be used to study changes in a cohort of patients with varying Parkinson's disease classification or Alzheimer's disease

References

1. Bereman, M. S., MacLean, B., Tomazela, D. M., Liebler, D. C. & MacCoss, M. J. The development of selected reaction monitoring methods for targeted proteomics via empirical refinement. *Proteomics* 12, 1134–1141 (2012).
2. Searle, B. C., Egertson, J. D., Bollinger, J. G., Stergachis, A. B. & MacCoss, M. J. Using data independent acquisition to model high-responding peptides for targeted proteomics experiments. *Mol. Cell. Proteomics* 14, 1–40 (2015).
3. Spellman, D. S. *et al.* Development and evaluation of a multiplexed mass spectrometry based assay for measuring candidate peptide biomarkers in Alzheimer's Disease Neuroimaging Initiative (ADNI) CSF. *PROTEOMICS - Clin. Appl.* 9, 715–731 (2015).