

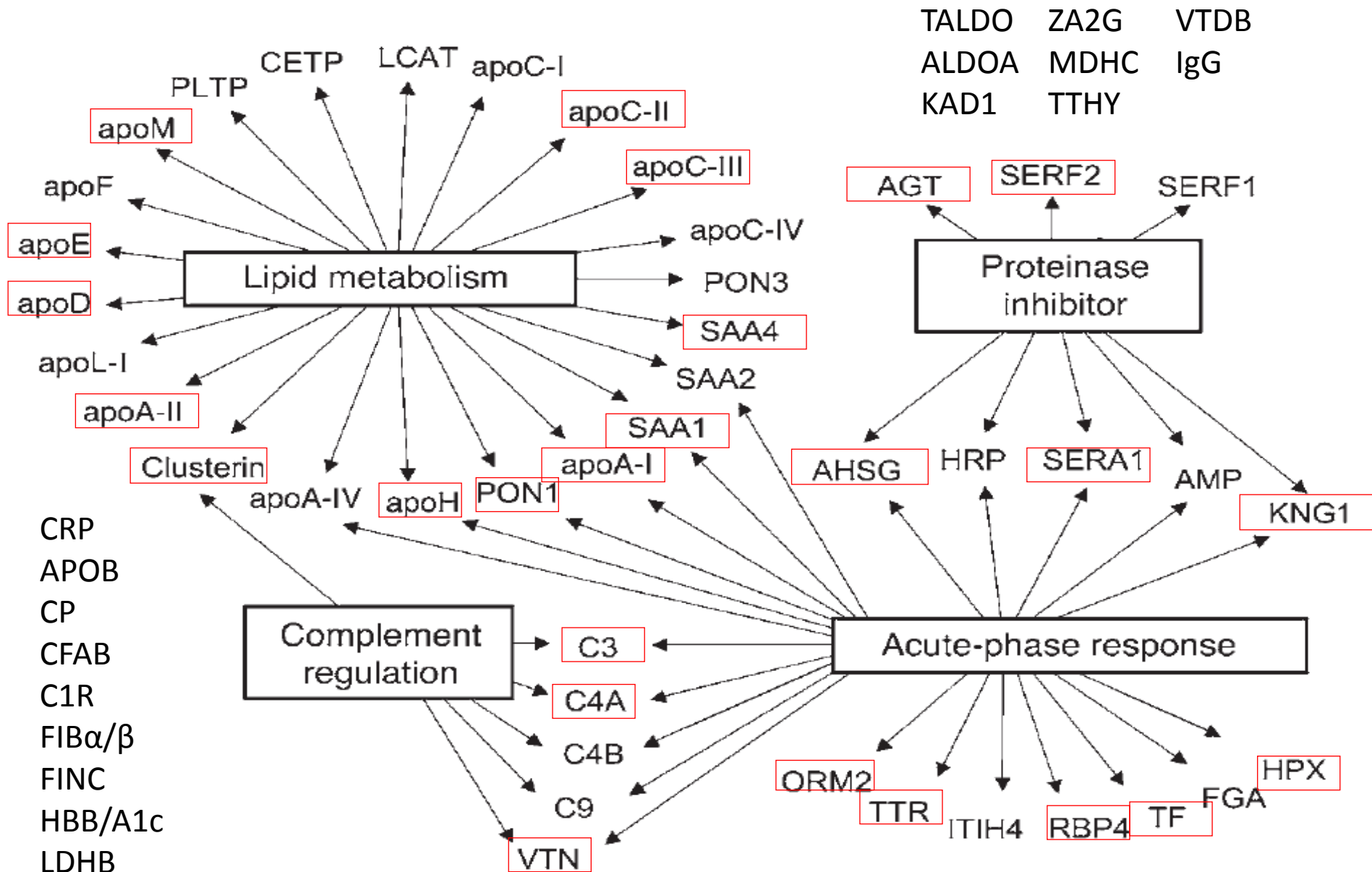
Multiplexing Clinical Protein Diagnostics in Dried Blood Spots

Skyline Webinar – March 10, 2015
James Bollinger - MacCoss Lab
Department of Genome Sciences
University of Washington

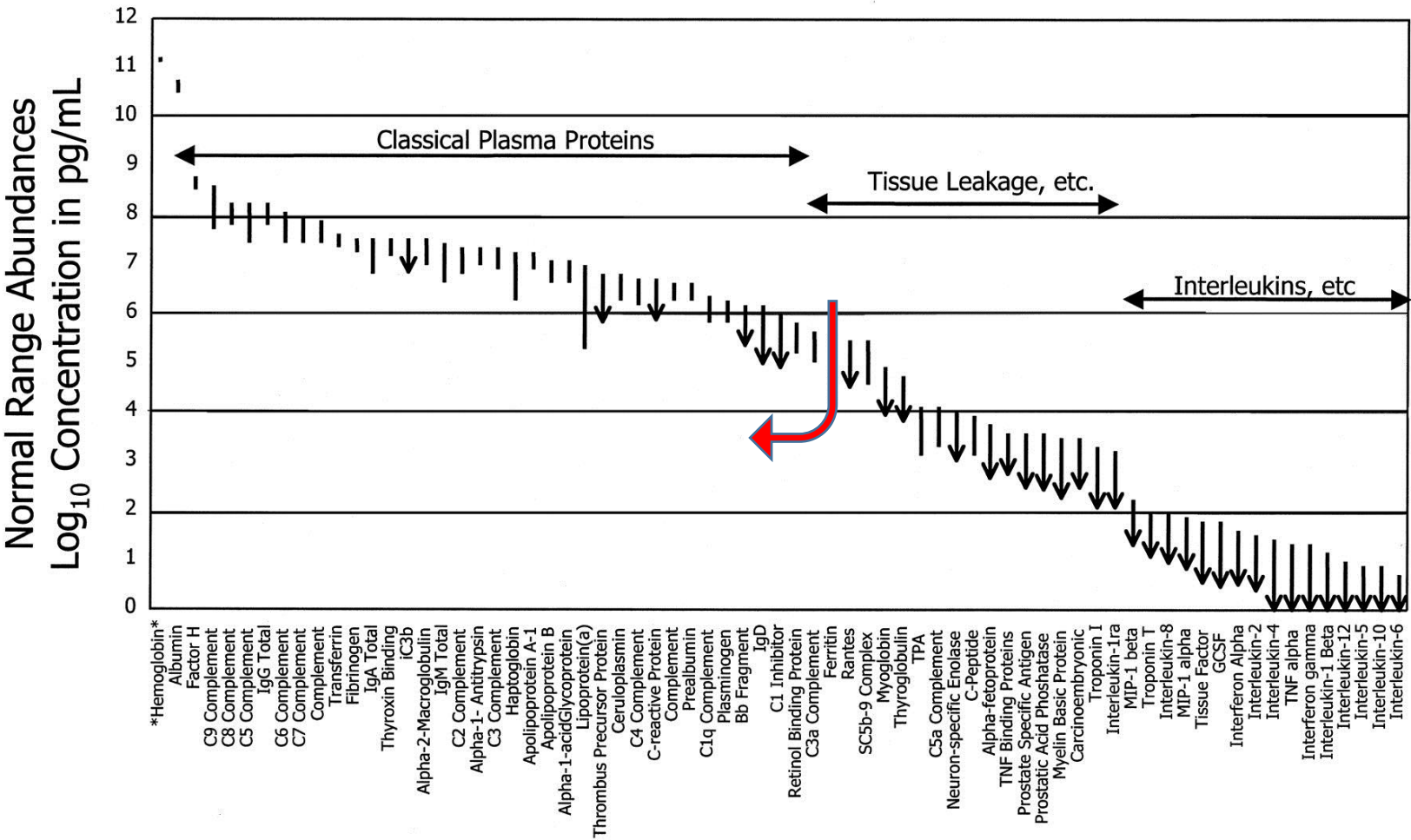
Outline

- Selection of candidate proteins for the multiplex analysis of DBS via targeted proteomics
- The currently employed strategies for the selection of candidate peptides for targeted proteomics
- An empirical refinement process for the selection of optimal peptides and their respective MS/MS transitions

Selection of Protein Targets for DBS Wellness Assay



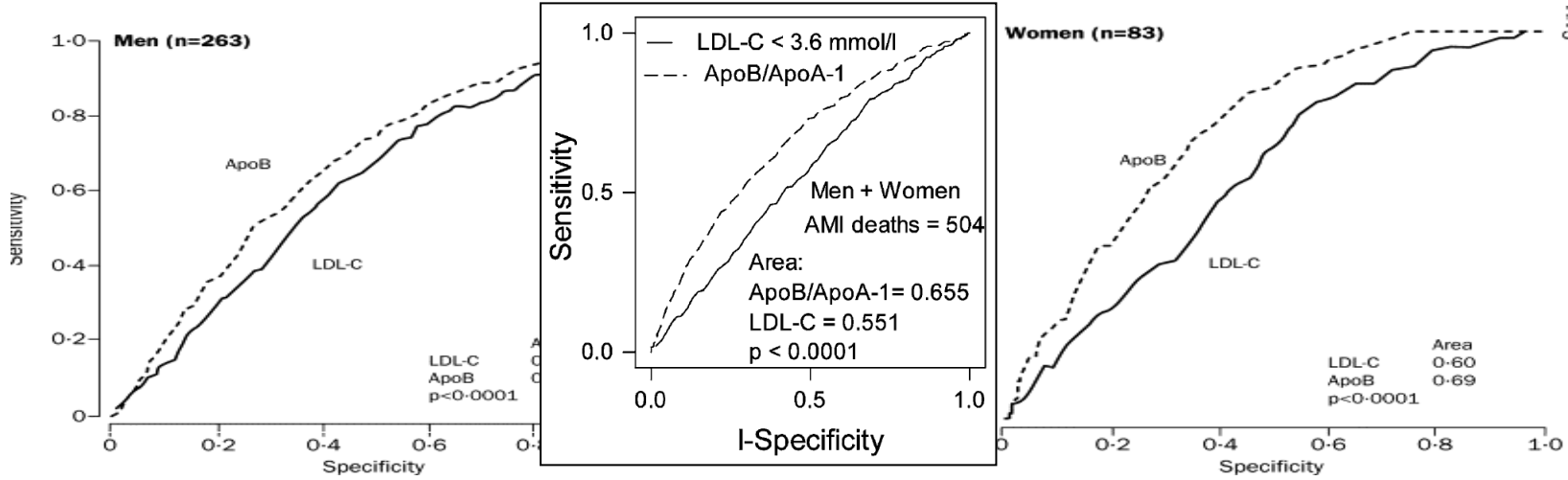
Selection of Protein Targets for DBS Wellness Assay



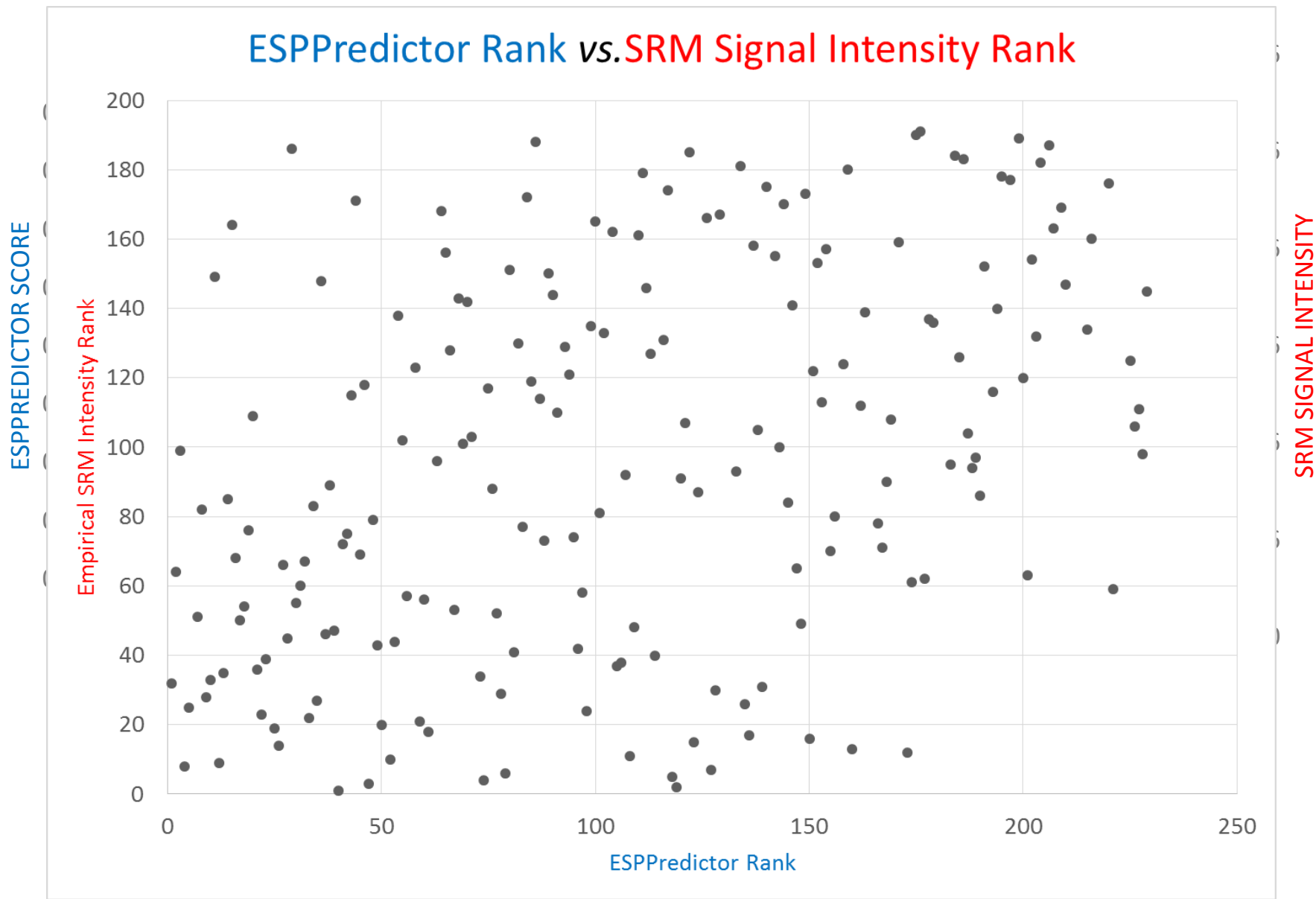
N. Leigh Anderson & Norman G. Anderson *Mol Cell Proteomics* (2002), 1, 845-867
 N. Leigh Anderson. *Clin. Chem.* (2010), 56, 177-185

How Does One Select Optimal Peptides for the Quantitative Analysis of Each Respective Gene Product?

Apolipoprotein B (P04114) – 516 kDa, Serum reference range (~0.5-2 g/L)



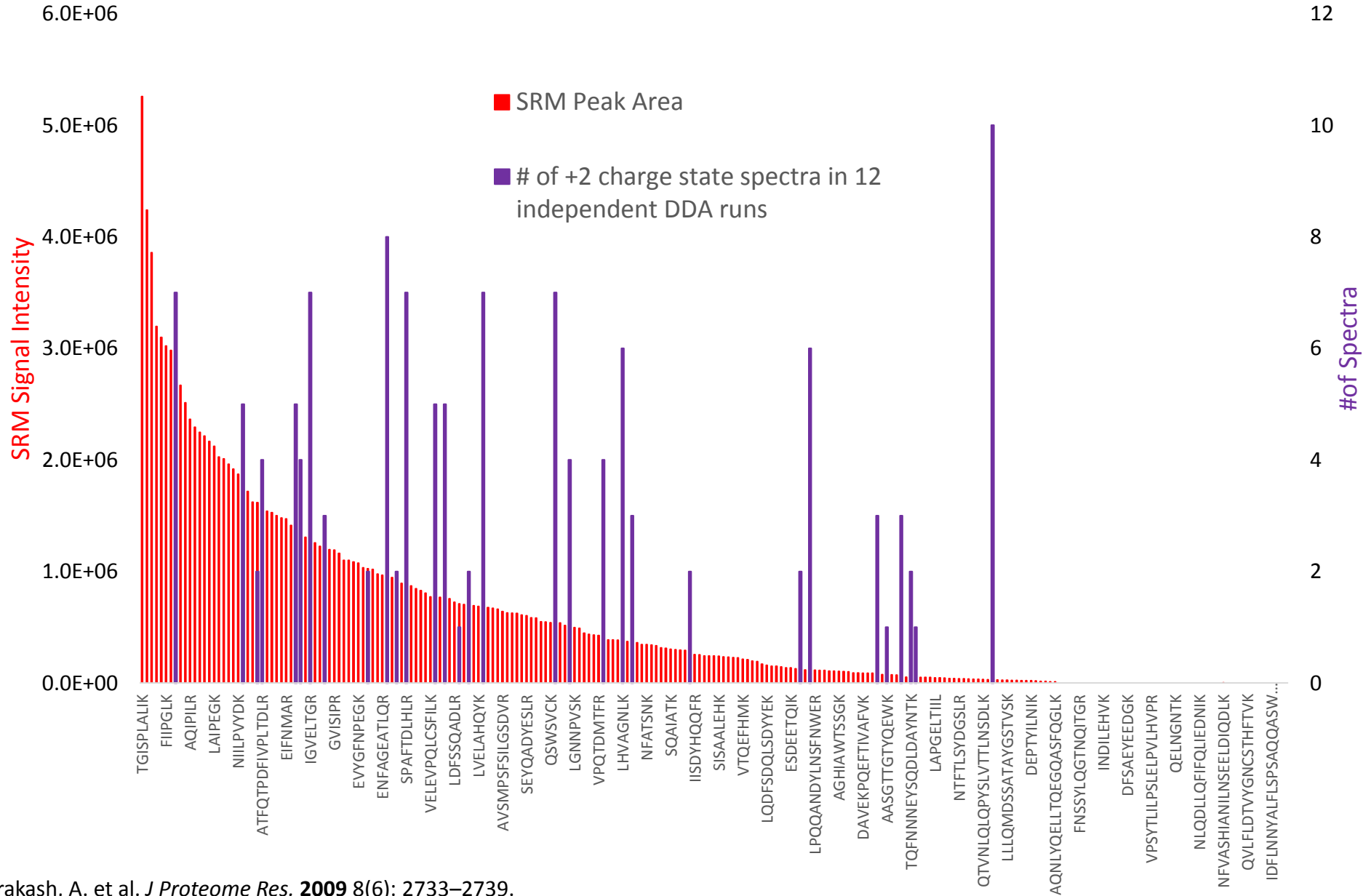
ESPPredictor Score vs. SRM Signal Intensity: Apolipoprotein B100



Mallick, P. & Aebersold, R. et al. *Nature Biotechnology*(2007) 25 (1): 125-131

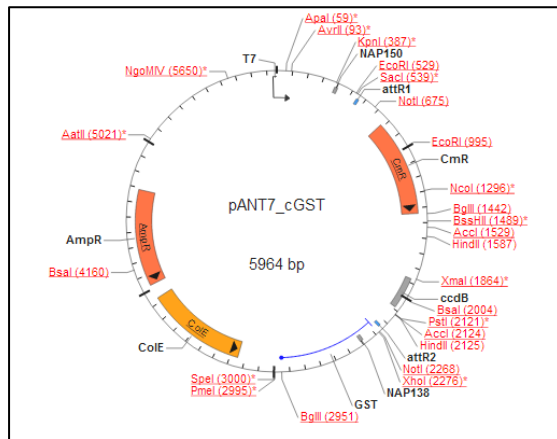
Fusaro, V.A. & Carr, S.A. et al. *Nature Biotechnology* (2009) 27:190-198.

Spectrum Counts vs. SRM Signal Intensity: Apolipoprotein B100



Our Approach.....

Workup of Recombinant/Native Protein Standard

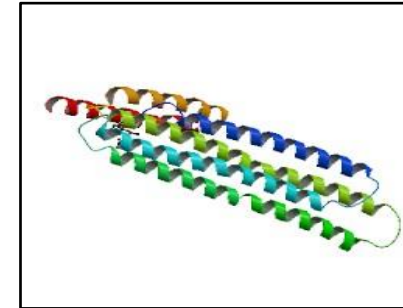


<http://dnasu.org>

Expand, Purify,
& Sequence
cDNA clone

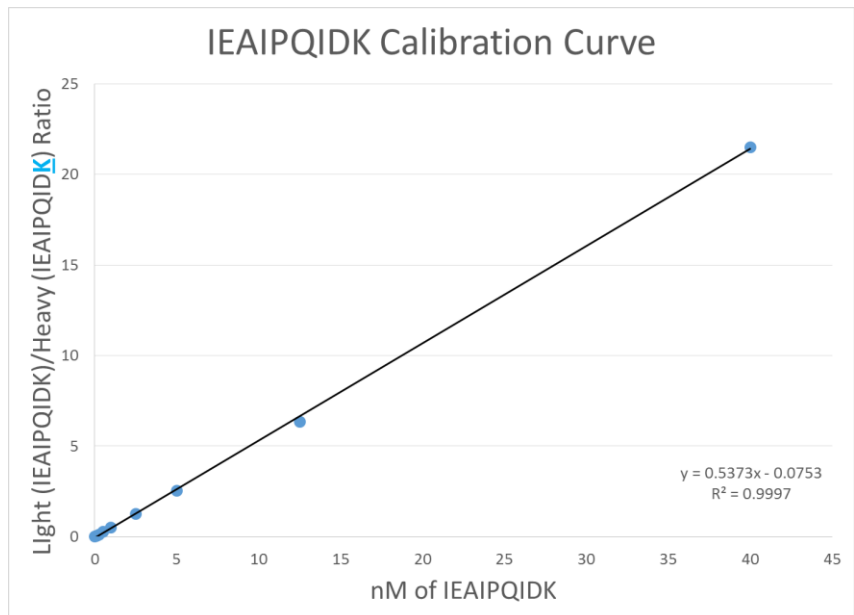
In Vitro
Transcription
and Translation

Enrichment via
Glutathione-
Sephrose Resin



IEAIPQIDK
+
IEAIPQIDK

~25-500 femtomoles/25uL IVT
< \$20.00/Protein



Round 1 nanoLC MS/MS: SRM Signal Intensity Rank and Fragmentation Fingerprint

Peptide Filter #1: The y -ion series (y_3 to y_{n-1}) for all fully tryptic peptides 7 to 25 amino acids in length (+2 charge state) with all cysteines monitored as carbamidomethyl cysteines.



In Silico digestion

Export transition list parameters

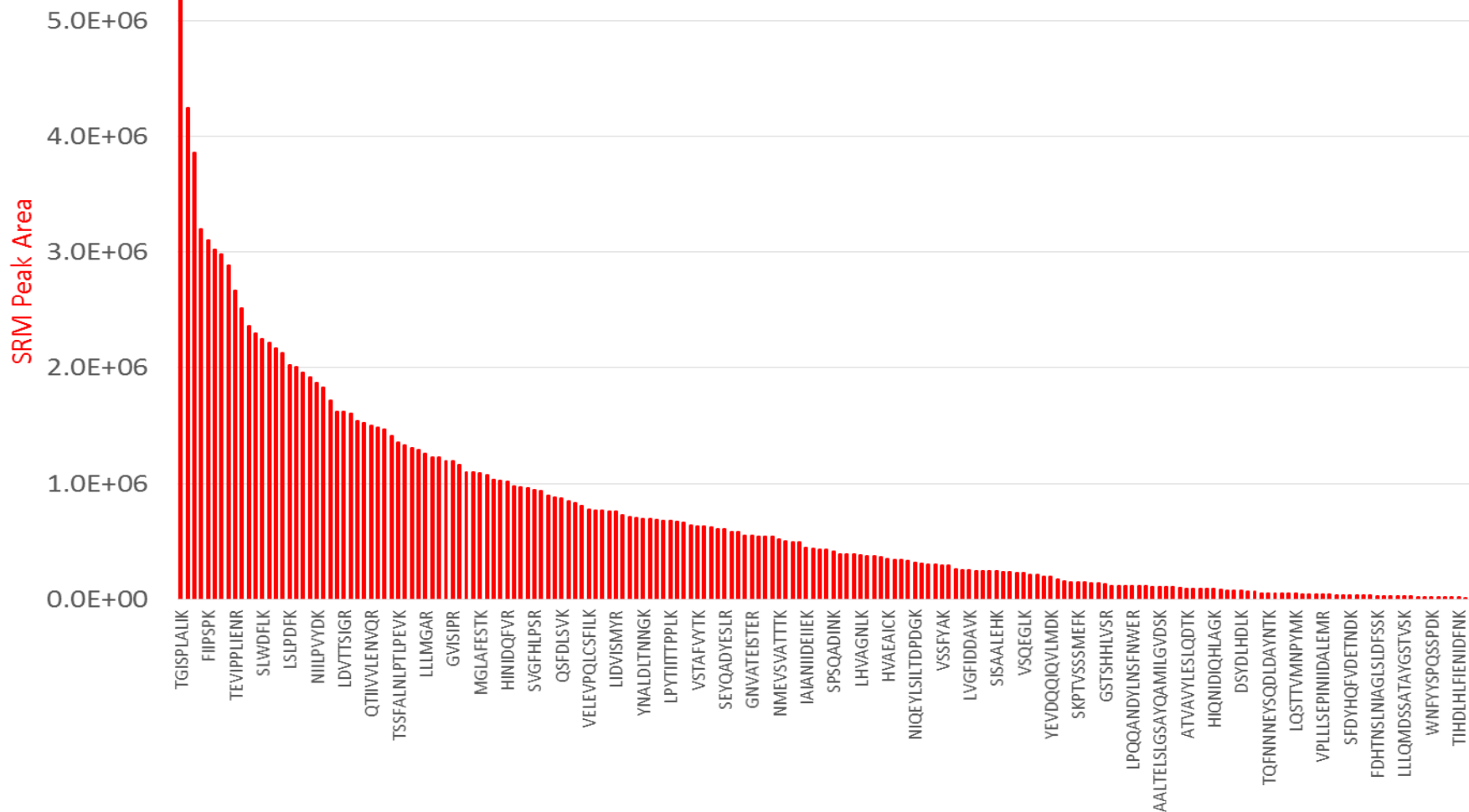
(m/z Precursors, m/z Products, & Collision Energies¹)

Apolipoprotein B100
Peptides

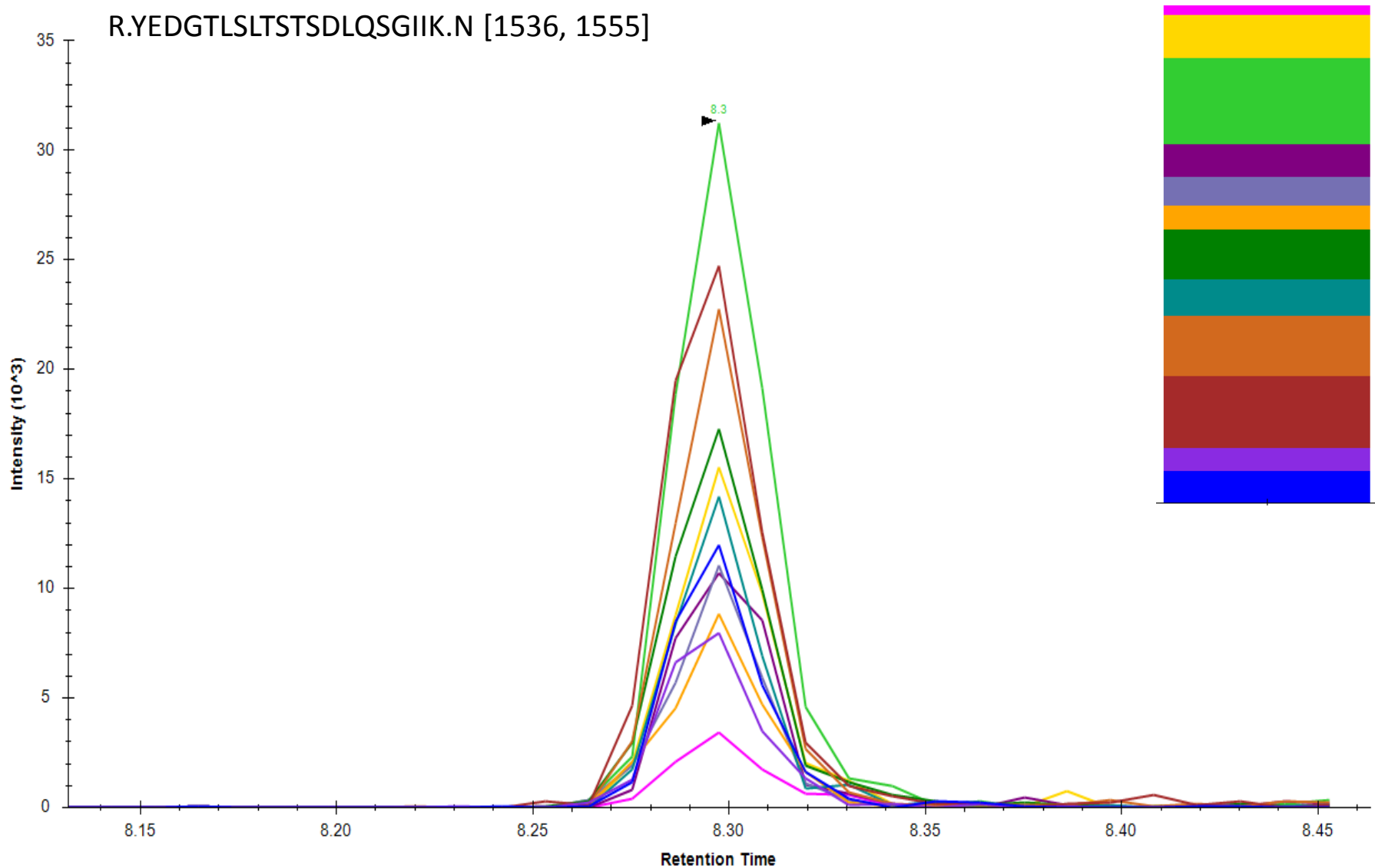
241

Round 1 nanoLC MS/MS: SRM Signal Intensity Rank

Apolipoprotein B100 - SRM Peak Area



Round 1 nanoLC MS/MS: Fragmentation Fingerprint



Round 2 nanoLC MS/MS: iRT Calibration/Peptide Stability Analysis



Peptide Filter #2: Edit and annotate all peptides with non-existent or ambiguous chromatograms



Upload curated assay to Panorama for initial build of chromatogram library



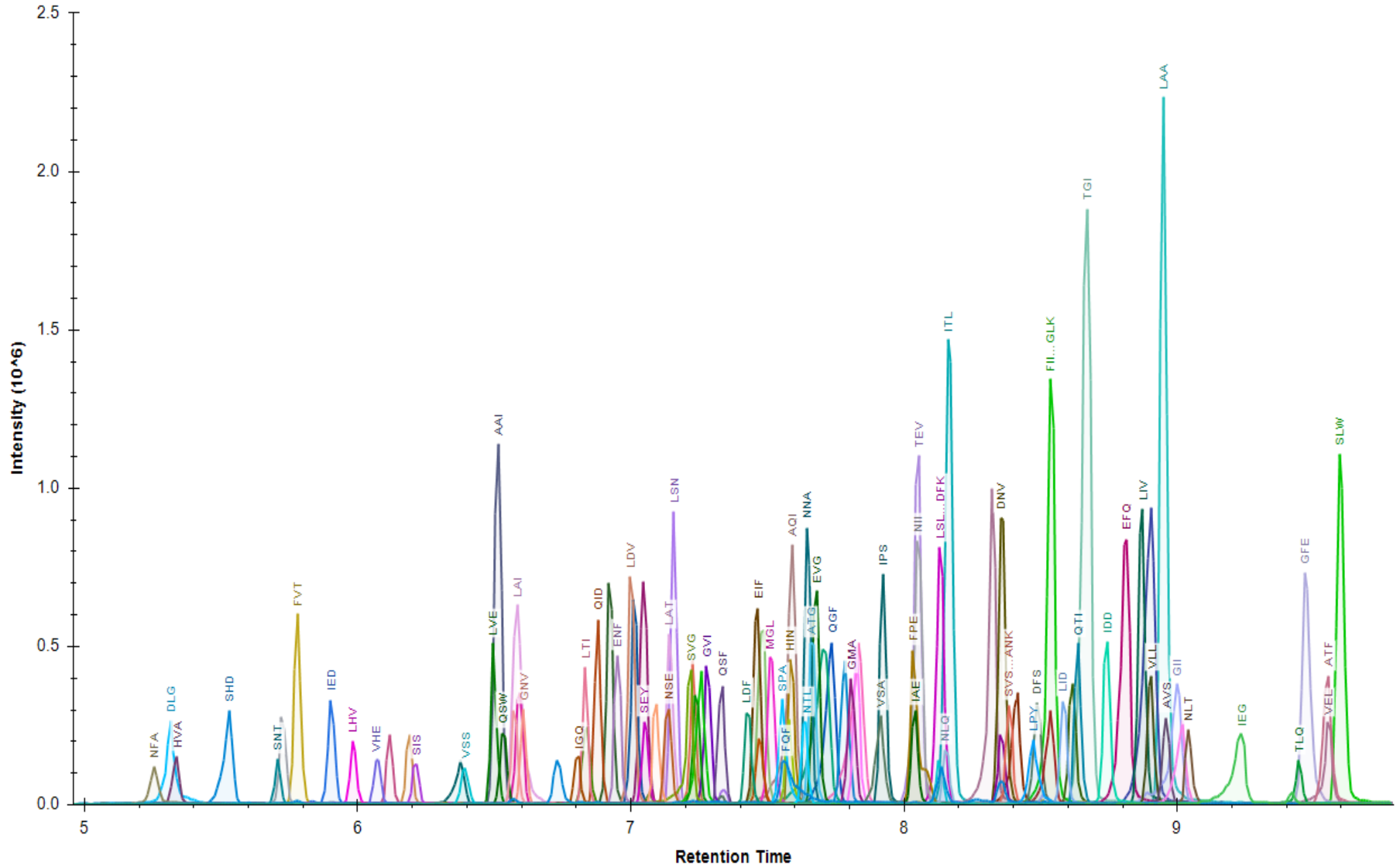
Export methods with top 4 transitions from remaining peptides (Sensitivity and selectivity arguments) for retention time calibration and stability analysis

Apolipoprotein B100
Peptides

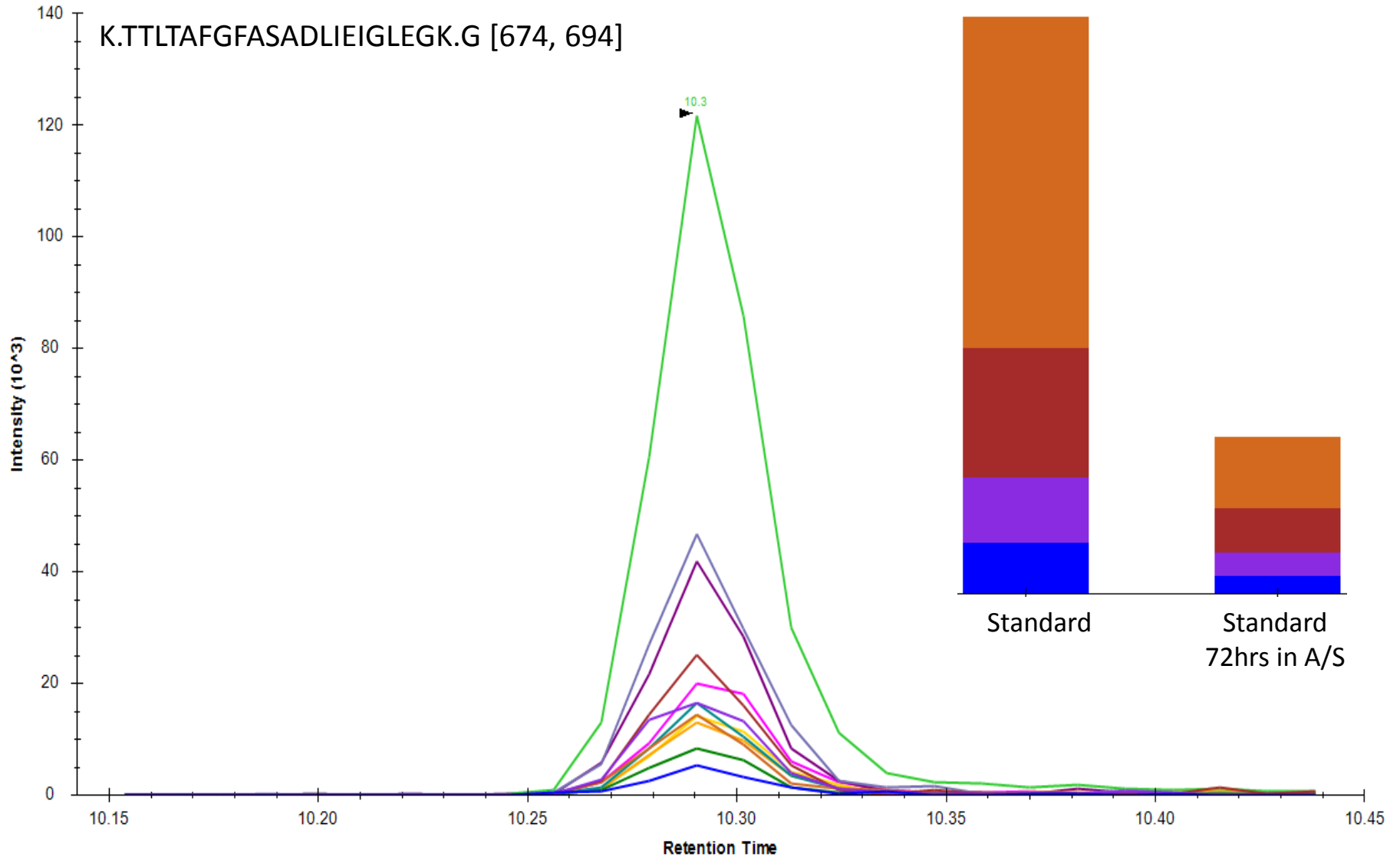
241

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Round 2 nanoLC MS/MS: iRT Calibration



Round 2 nanoLC MS/MS: Peptide Stability Analysis



Round 3 nanoLC MS/MS: Validation of Peptides/Transitions in Matrix



Peptide Filter #3: Edit and annotate all peptides that fail to meet minimum stability requirements.

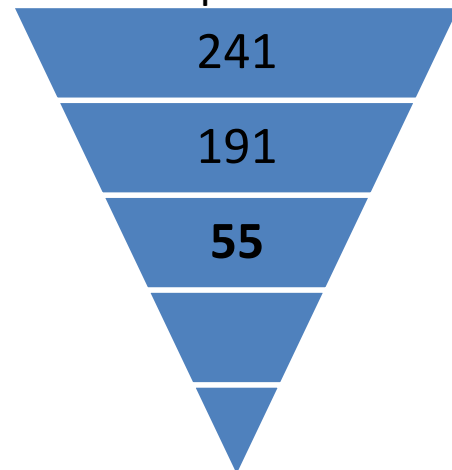


Update chromatogram library files with iRT values

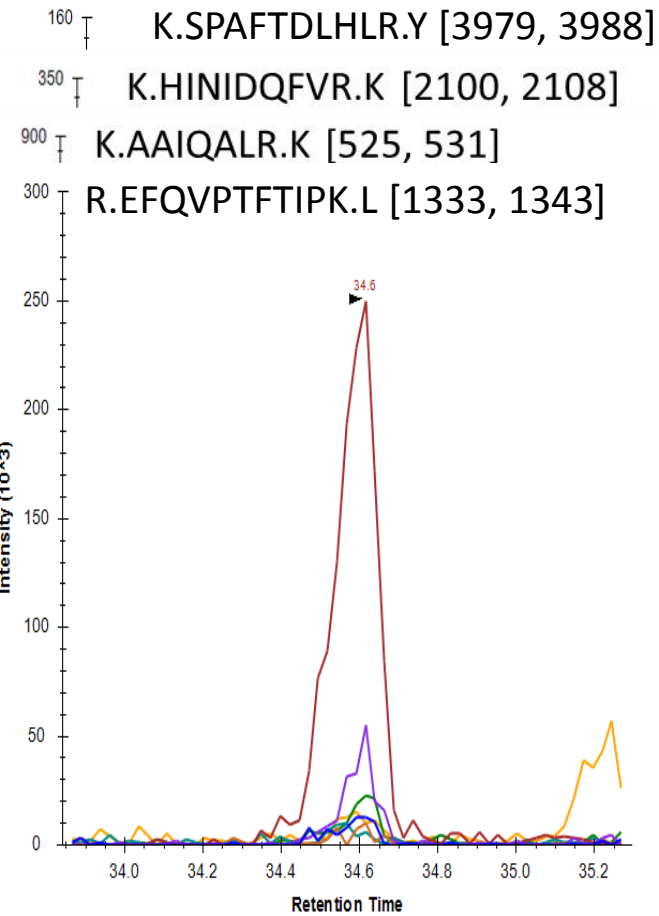
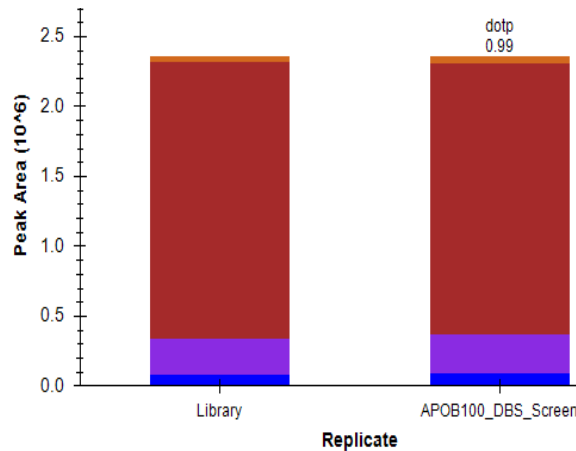
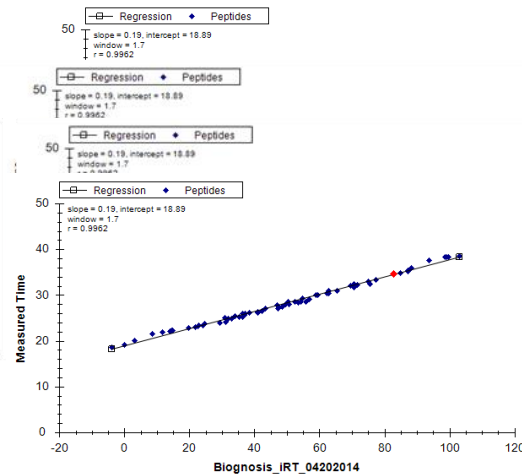
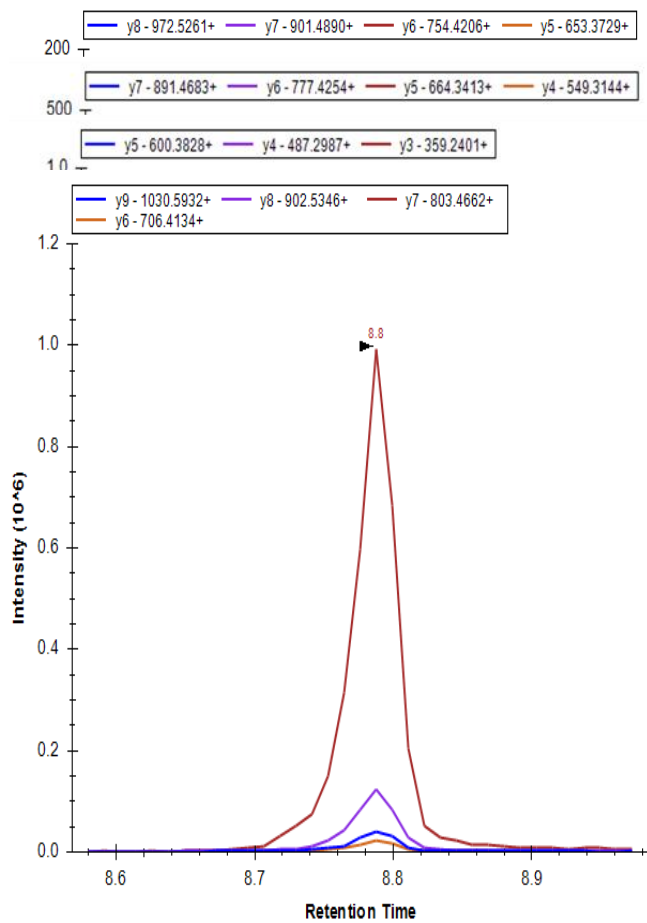


Export scheduled methods with all transitions from all remaining peptides for the screening of a matrix of interest (DBS)

Apolipoprotein B100
Peptides



Round 3 nanoLC MS/MS: Validation of Peptides/Transitions in Matrix



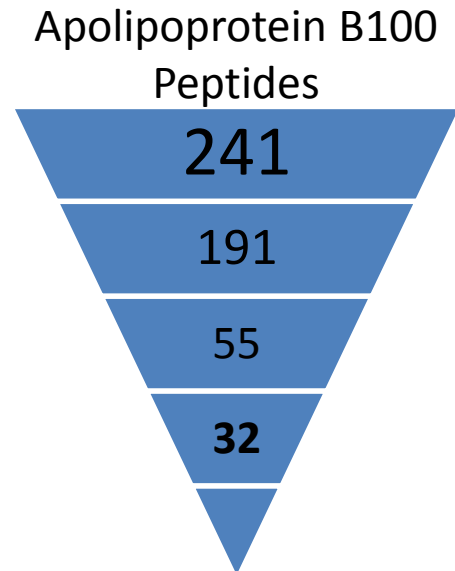
Round 4 nanoLC MS/MS: Digestion Time Course



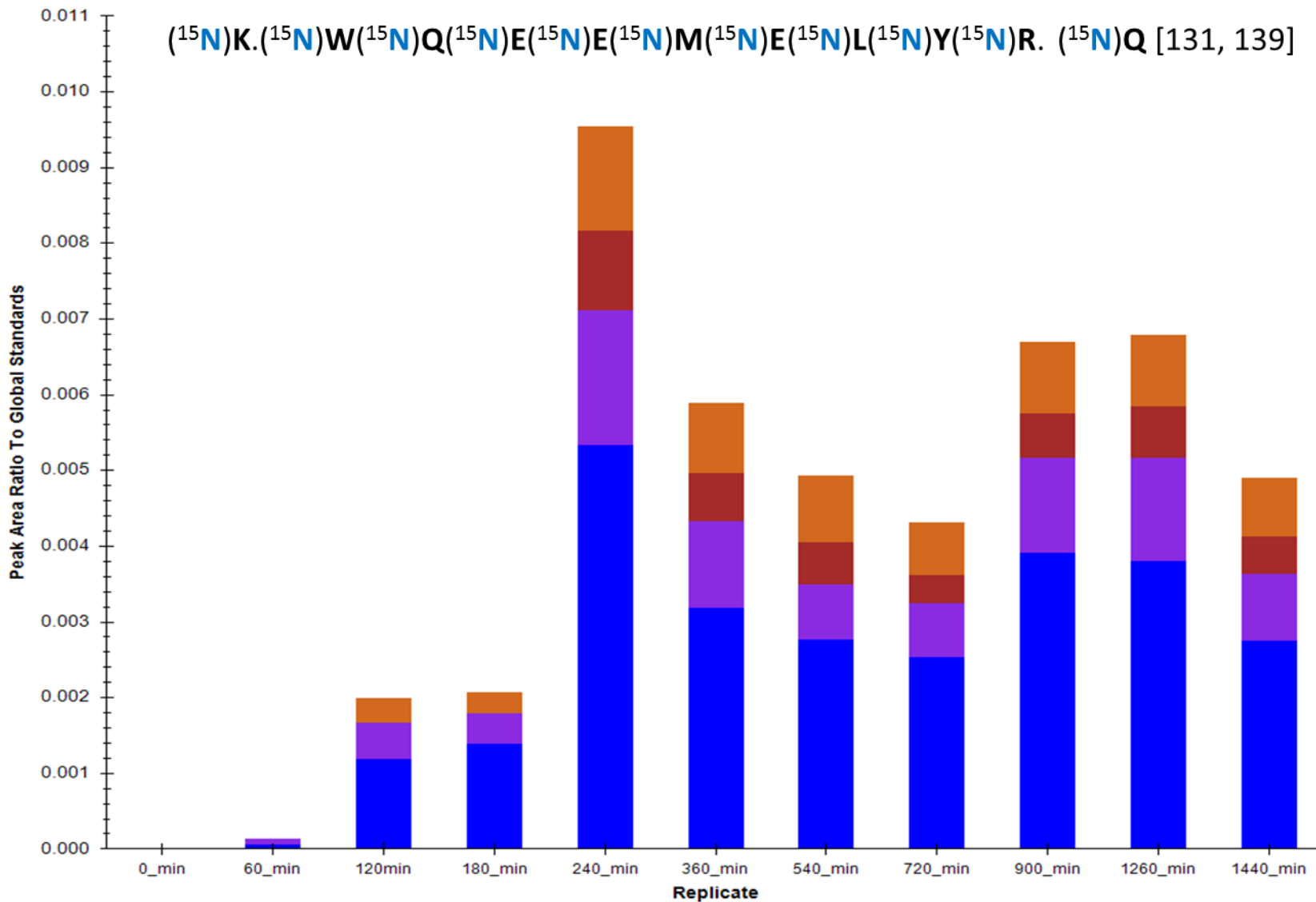
Peptide Filter #4: Edit and annotate all peptides that were not reliably identified in the matrix of interest



Combine and export scheduled methods for all remaining peptides for digestion time course analysis



Round 4 nanoLC MS/MS: Digestion Time Course



Final Method Refinement



Peptide Filter #5: Edit and annotate all peptides that gave a sub-optimal digestion characteristics



Combine and export scheduled methods for all remaining peptides from all target proteins for DBS correlation studies

55 Proteins, 1406 transitions, 281 Peptides

Normalization?

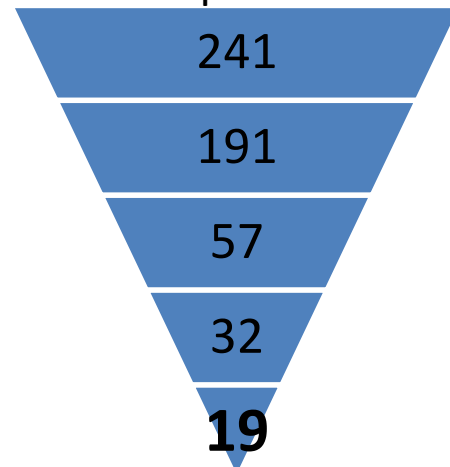
APOA1 Global Internal Standard (^{15}N Protein)¹

Calibration?

Single Point Calibrator²

Assign concentration with clinical immunoassay
Measure in triplicate in each batch

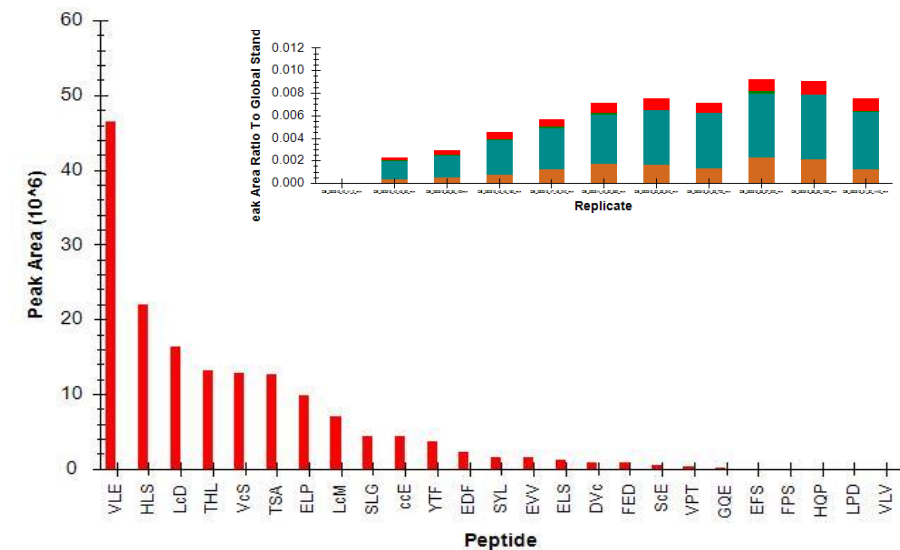
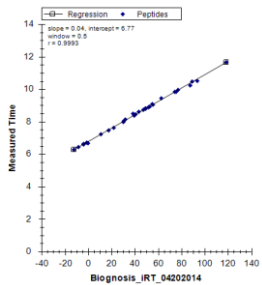
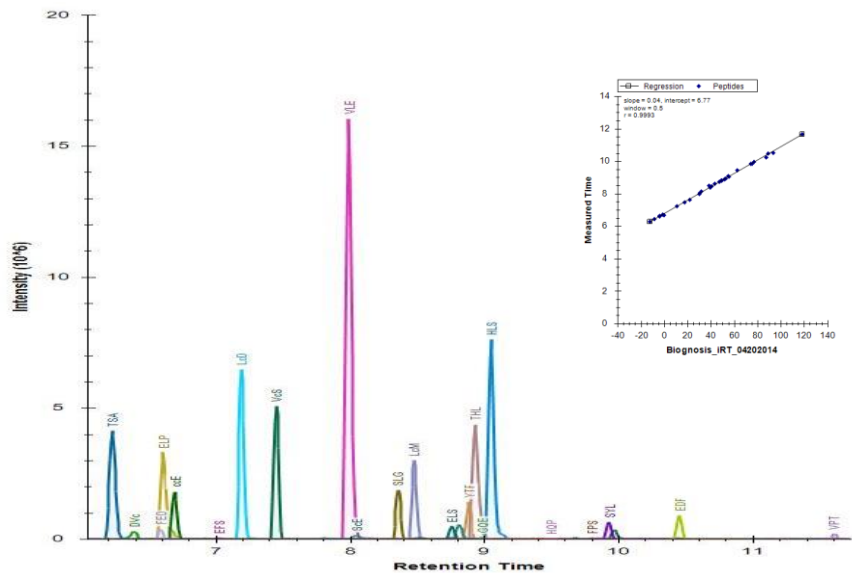
Apolipoprotein B100
Peptides



1) Hoofnagle, A.N. et al. *Clin. Chem.* **2012**, 58(4); 777-781.

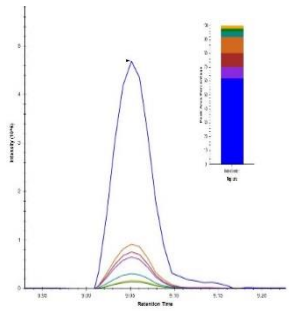
2) Cox, B. et al. *Clin. Chem.* **2014**, 60(3); 541-8.

Summary of Empirical Derivation with Vitamin D Binding Protein (P02774)

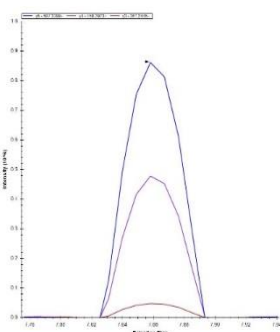
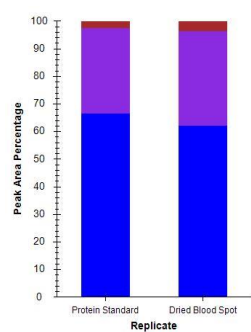
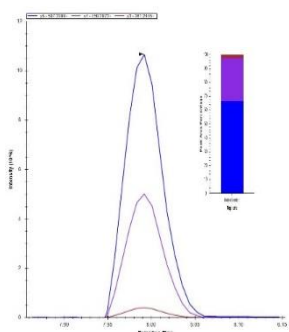
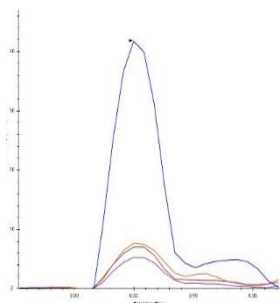
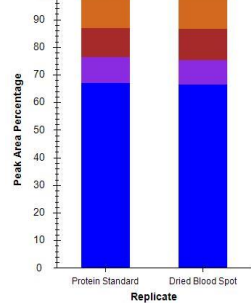


K.HLSLLTILSNR.V [207, 217]

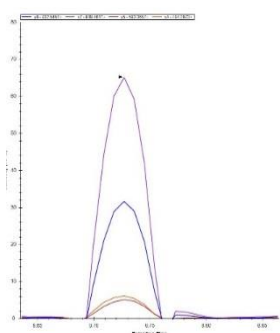
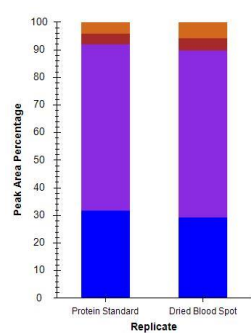
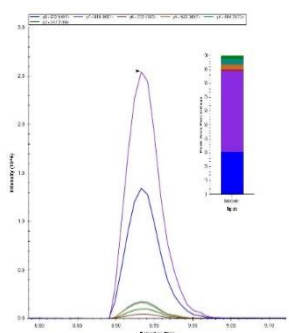
Protein Standard



DBS



K.VLEPTLK.S [363, 369]



R.THLPEVFLSK.V [353, 362]