

MS/MS to Targeted Proteomics (MRM)

How it worked on the Human Lens Proteome

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Genes Show Limited Value in Predicting Diseases

“With only a few exceptions, what the genomics companies are doing right now is recreational genomics,” Dr. Goldstein said in an interview. “The information has little or in many cases no clinical relevance.”

The undiscovered share of genetic risk for common diseases, he said, probably lies not with rare variants, as suggested by Dr. Goldstein, but in unexpected biological mechanisms.

Nicholas Wade, New York Times, April 15th 2009

Genomics has already arrived

Commercial Products

Affymetrix: Genome Wide Human SNP Array 6.0 (906,600 SNPs)

Illumina: human1m-duo bead chip (1.1 million evenly distributed loci)

Table 1: GWAS published per year as listed in the NIGRI/NIH www.genome.gov resource.

| | 2006 | 2007 | 2008 | 2009 |
|-------------------------------|------|------|------|-------------------|
| <i>Number of GWAS Studies</i> | 14 | 441 | 901 | 137 (as of March) |

Commercial Services

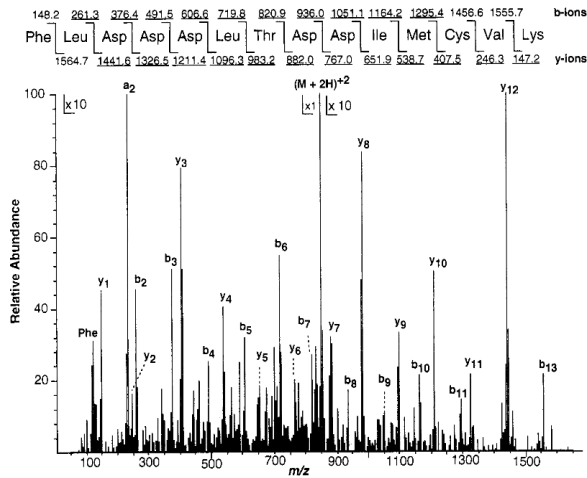
deCODEme: <http://www.decodeme.com/>

“Complete Scan”, “Cardio Scan”, “Cancer Scan”

23andMe: <https://www.23andme.com/>

“Time Magazine’s 2008 Invention of the Year, now \$399.”

Proteomics is arriving

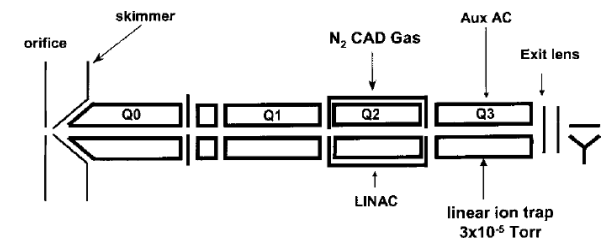


Tandem Mass Spectrometry (MS/MS)

Discovery of proteins and post-translational modifications in a sample.

Targeted Proteomics (MRM aka SRM)

Rapid and sensitive monitoring of proteins and post-translational modifications in a sample.



Proteomics Timeline

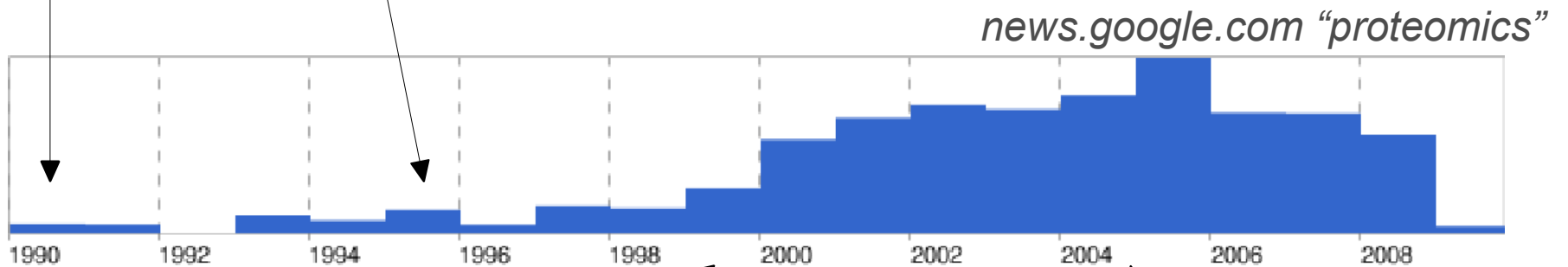
Peptide Ionization: ESI and MALDI

Fenn et al. (1989). "Electrospray ionization for mass spectrometry of large biomolecules". Science (journal) 246: 6471.

Tanaka et al (1988). "Protein and Polymer Analyses up to m/z 100 000 by Laser Ionization Time-of flight Mass Spectrometry". Rapid Commun Mass Spectrom 2 (20): 151–153.

MS/MS: LCQ and Sequest

Eng et al. (1994). "An Approach to Correlate Tandem Mass Spectral Data of Peptides with Amino Acid Sequences in a Protein Database". J Am Soc Mass Spectrom 5: 976–989.



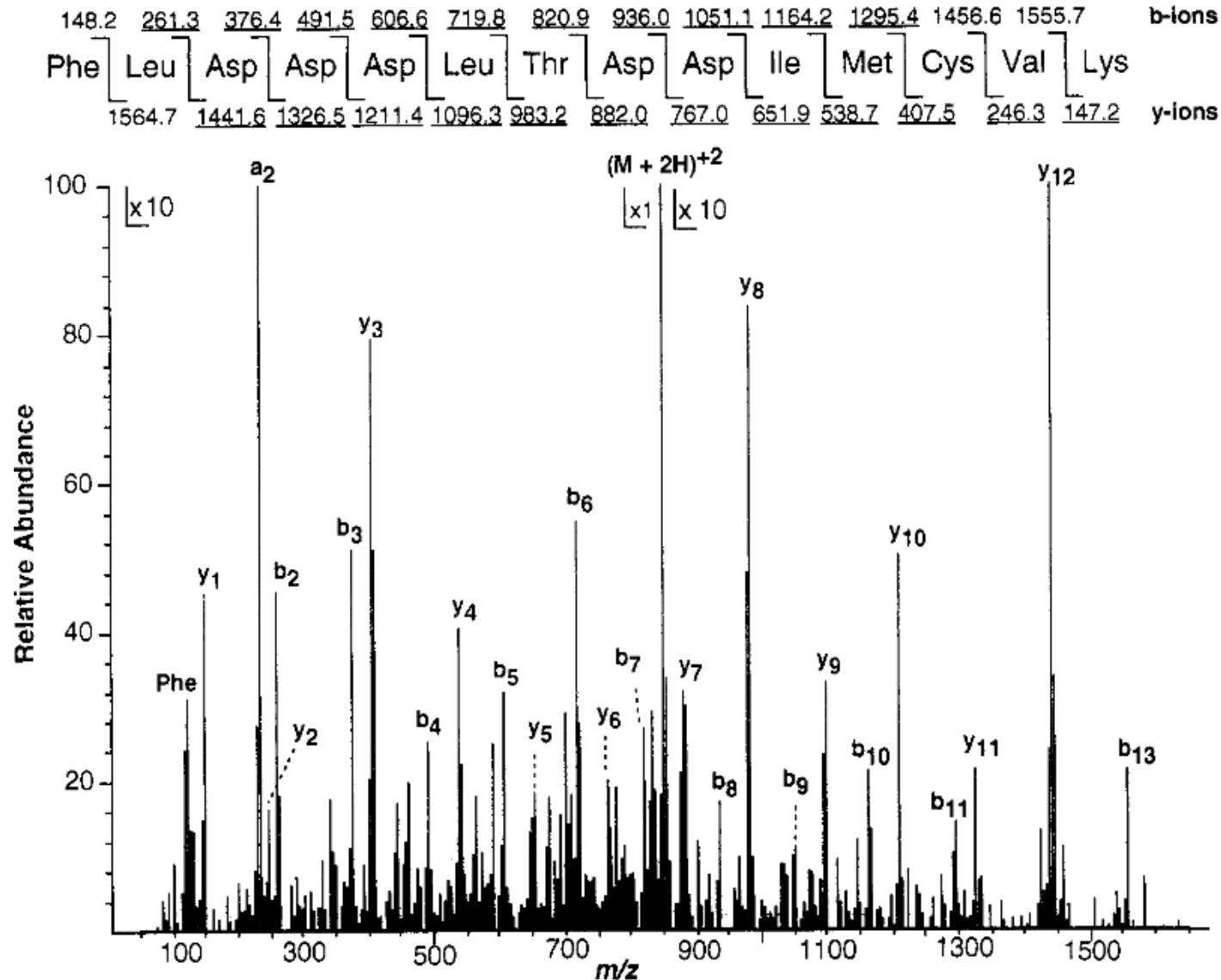
On-line Separation: MudPIT

Washburn et al. (2001) "Large-scale analysis of the yeast proteome by multidimensional protein identification technology" Nat Biotech Vol 19

MRM: MIDAS and QTRAP

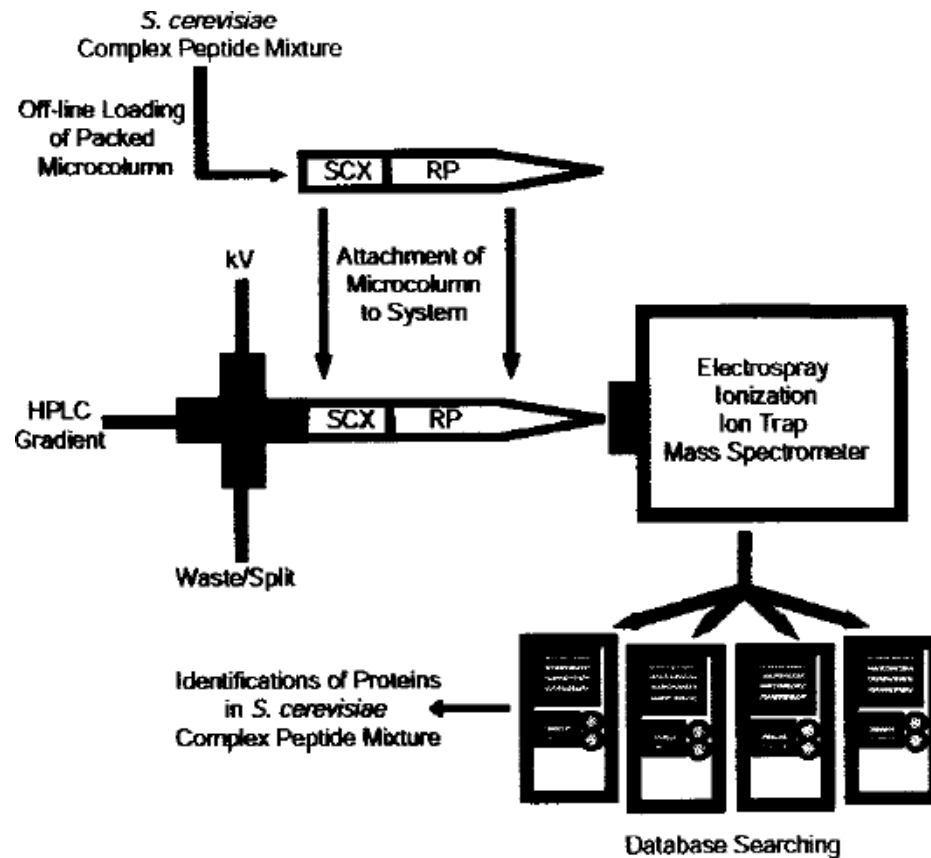
Unwin et al (2005) "Multiple Reaction Monitoring to Identify Sites of Protein Phosphorylation with High Sensitivity" MCP 4.8

MS/MS Peptide Identification



Eng et al. (1994). J Am Soc Mass Spectrom 5: 976–989.

MudPIT: More MS/MS Peptide Identifications



Washburn et al. Nat. Biotech (19) 2001

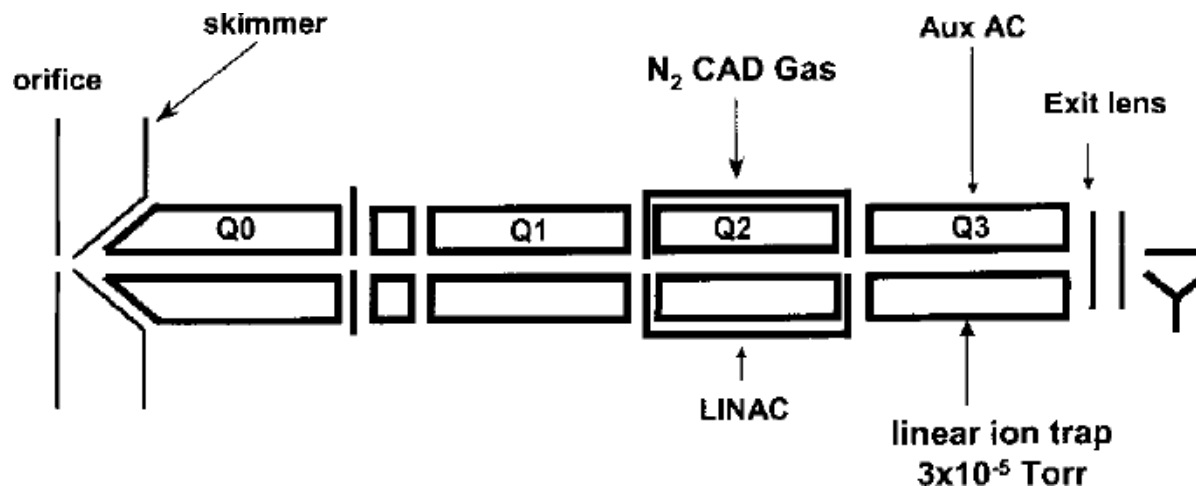
Challenges in MS/MS

- Speed and Sensitivity
 - Keshishian et al, MCP 2007
- Measuring Changes
 - ICAT: Gygi et al, Nat. Biotech 1999
 - SILAC: Ong et al, MCP 2002
 - ITRAQ: Ross et al, MCP 2004
 - AQUA: Kirkpatrick, Methods 2005
- Post-translational Modifications
 - Bonanza: Falkner et al, JPR 2008

The Promise of MRM

- Works only on known targets
- More sensitive and faster than MS/MS
 - Better lower level of detection
 - Can monitor low m/z ions
 - Minimal fractionation required (10 min gradients!?)
- 5-10% CV and absolute quant
 - Heavy labeled peptides required

MRM: Triple Quadrupoles



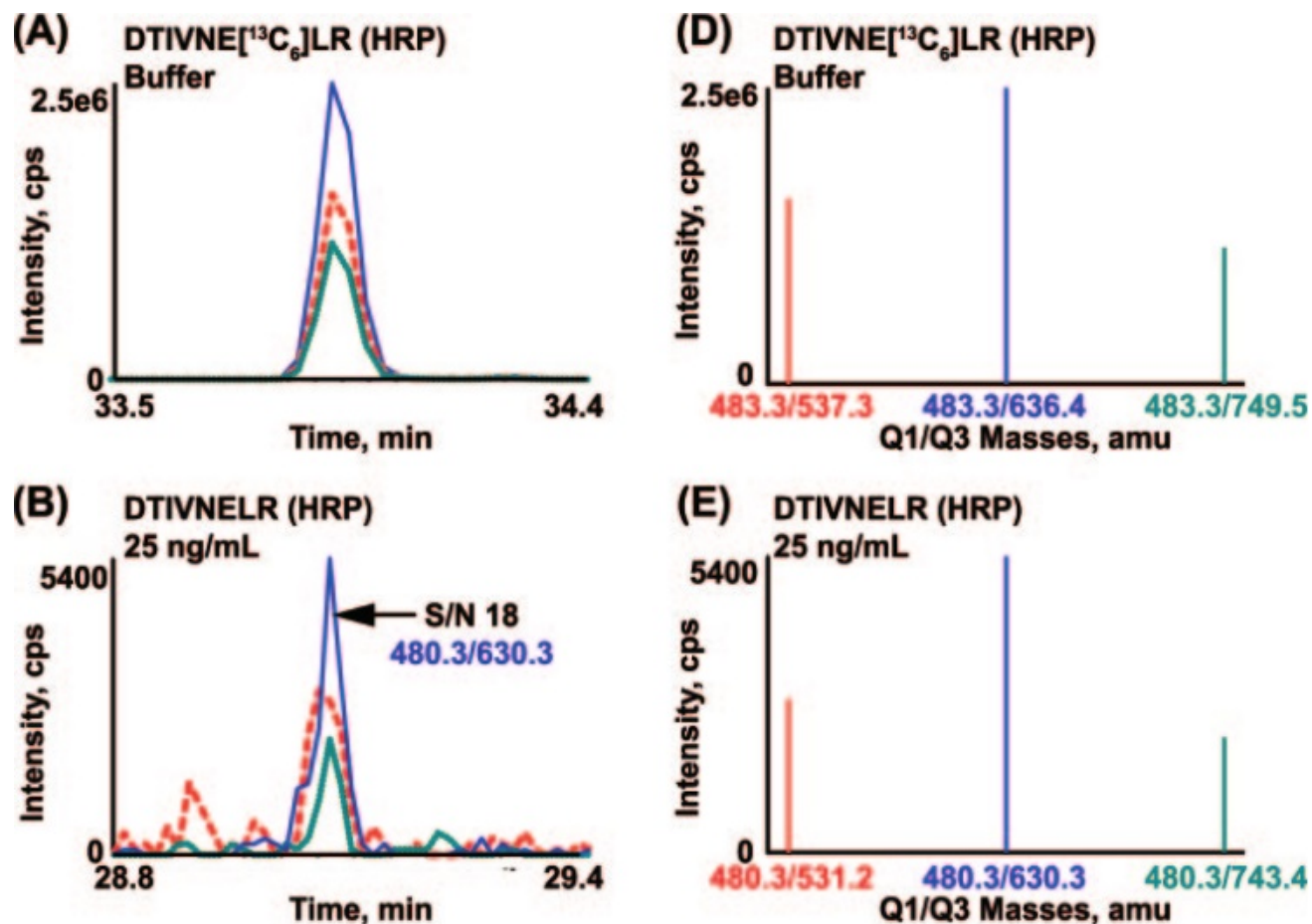
Hager *Rapid Comm. Mass Spec.* 2002; 16: 512-526

- Better by design vs 3D trap
 - Holds more ions, 2x injection, 8x ion ejection
 - No low mass loss (aka 1/3rd m/z issue)
- EPI mode filters only the ion of interest

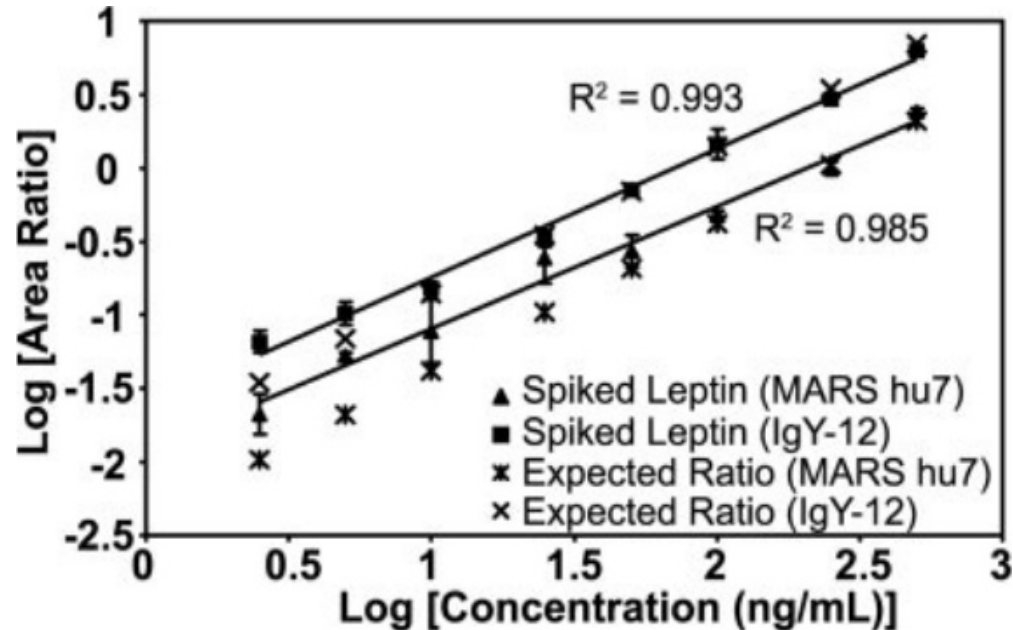
MRM: Known Targets

| Protein | Source | MW (kDa) | Signature Peptide | MH+ (mono) | z (Q1) | MRM Transitions ^b | |
|---------------------------|--------------|-------------|--|---------------|-----------|------------------------------|------------------------------|
| | | | | | | Q1 | Q3 |
| Aprotinin | bovine lung | 6.4 | AGLC _{amc} QTFVYGGC _{amc} R ^a | 1488.7 | 2 | 744.8 | 858.3, 959.4 , 1087.5 |
| | | | AGLC _{amc} QTF[¹³ C ₅]VYGGC _{amc} R ^a | 1493.7 | 2 | 747.3 | 863.4, 964.4 , 1092.5 |
| Leptin | mouse | 10 | INDISHTQSVSAK | 1399.7 | 3 | 467.2 | 543.2, 586.8, 643.8 |
| | | | INDISHTQS[¹³ C ₅]VSAK | 1404.7 | 3 | 468.9 | 543.2, 589.3, 646.3 |
| Myoglobin | Horse heart | 17 | LFTGHPETLEK | 1271.7 | 3 | 424.6 | 506.2, 579.7 , 716.3 |
| | | | LFTGHPET[¹³ C ₆]LEK | 1277.7 | 3 | 426.6 | 509.2, 582.8 , 722.4 |
| Myelin Basic Protein | bovine | 18 | HGFLPR | 726.4 | 2 | 363.7 | 385.2, 532.3, 589.3 |
| | | | HGF[¹³ C ₆]LPR | 732.4 | 2 | 366.7 | 391.2, 538.3, 595.3 |
| | | | YLAASASTMDHAR | 1322.6 | 3 | 441.5 | 488.2, 523.7 , 817.3 |
| | | | Y[¹³ C ₆]LAASASTMDHAR | 1328.6 | 3 | 443.5 | 488.2, 523.7 , 817.3 |
| Prostate Specific Antigen | human | 30 | IVGGWEC _{amc} EK ^a | 1077.5 | 2 | 539.3 | 808.3, 865.3 , 964.4 |
| | | | I[¹³ C ₅]VGGWEC _{amc} EK ^a | 1082.5 | 2 | 541.7 | 808.3, 865.3 , 969.4 |
| | | | LSEPAELTDAVK | 1272.7 | 2 | 636.7 | 775.4, 846.4, 943.4 |
| | | | LSEPAE[¹³ C ₆]LTDVAK | 1278.7 | 2 | 639.7 | 781.3, 852.5, 949.4 |
| Peroxidase | Horse radish | 48 | DTIVNELR | 959.5 | 2 | 480.3 | 531.2, 630.3 , 743.4 |
| | | | DTIVNE[¹³ C ₆]LR | 965.5 | 2 | 483.3 | 537.3, 636.3 , 749.4 |
| | | | SSDLVALSGGHTFGK | 1475.7 | 3 | 492.6 | 703.3, 790.3 , 974.5 |
| | | | SSDLVA[¹³ C ₆]LSGGHTFGK | 1481.8 | 3 | 494.6 | 703.3, 790.3 , 980.5 |

MRM: Known Targets



MRM: Absolute Quant



Keshishian et al MCP 6.12 2007

- Plasma = most complex background
 - 2.5ng/ml at least for quant
 - CVs less than 15% and ~5% on average
- 1000-fold LOQ increase from IgY-12

Human Lens Proteome

Cataract development in lens may be caused by destabilizing post-translational modifications of crystallins. This live repository analyzes datasets and summarizes such modifications in healthy and cataractous human lens proteins.

About

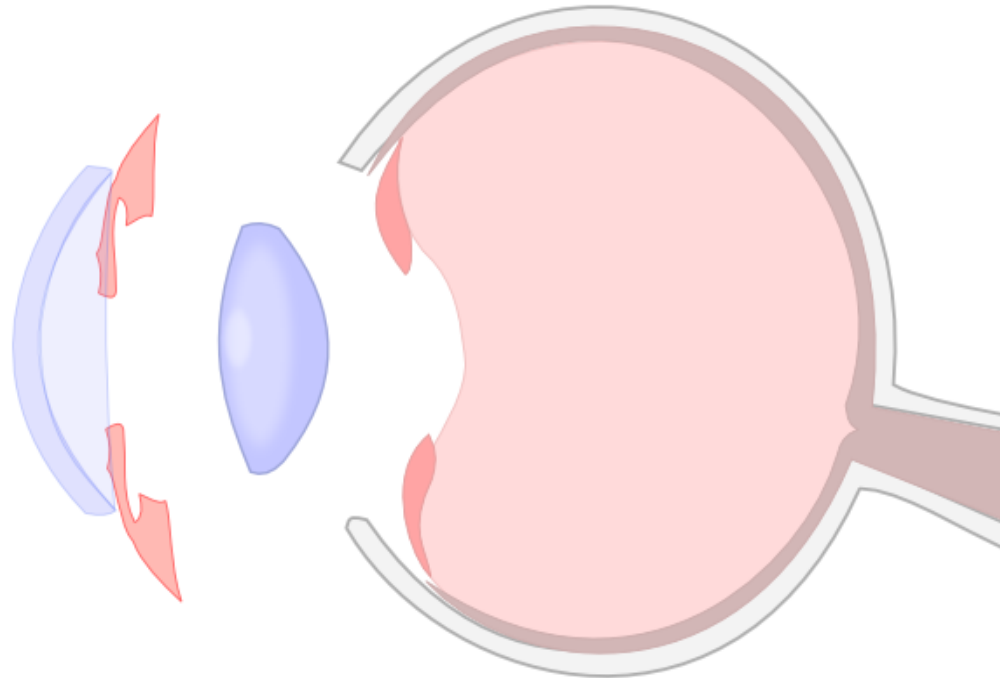
- [NEI Grant# 5R01EY007755-16](#)
- [SOSI / Cluster / Mr. M](#)

Publications

- [Wilmarth et al. 2006](#)

Reference

- [Protein List](#)
- [Peptide/Modification List](#)



Key Figures ([Supporting Data](#))



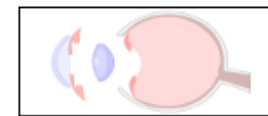
Protein Abundance: CRBA1

Show Image



Mod: CRYAB 0 Acetylation

Show Image



[Lens Cartoon](#)

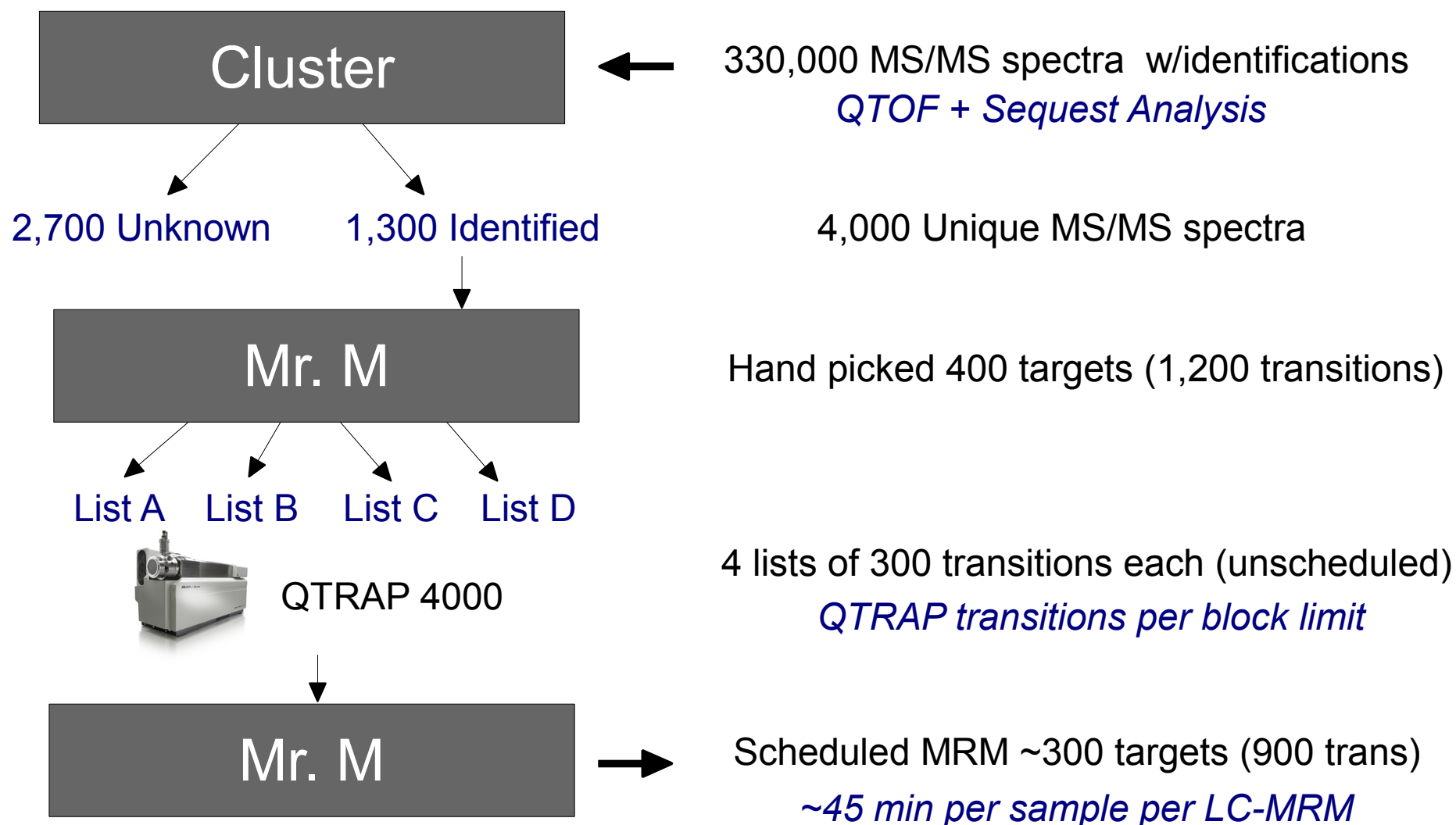
Revising Analysis Strategies

- Initial Plan
 - MS/MS MudPIT of data
 - ~~MS/MS quantification (O18 labeling)~~
 - Spectral count to compare proteins and mods
- Revised Plan
 - Reanalyze MS/MS survey data
 - MRM relative quant w/exogenous standard
 - Compare proteins and mods

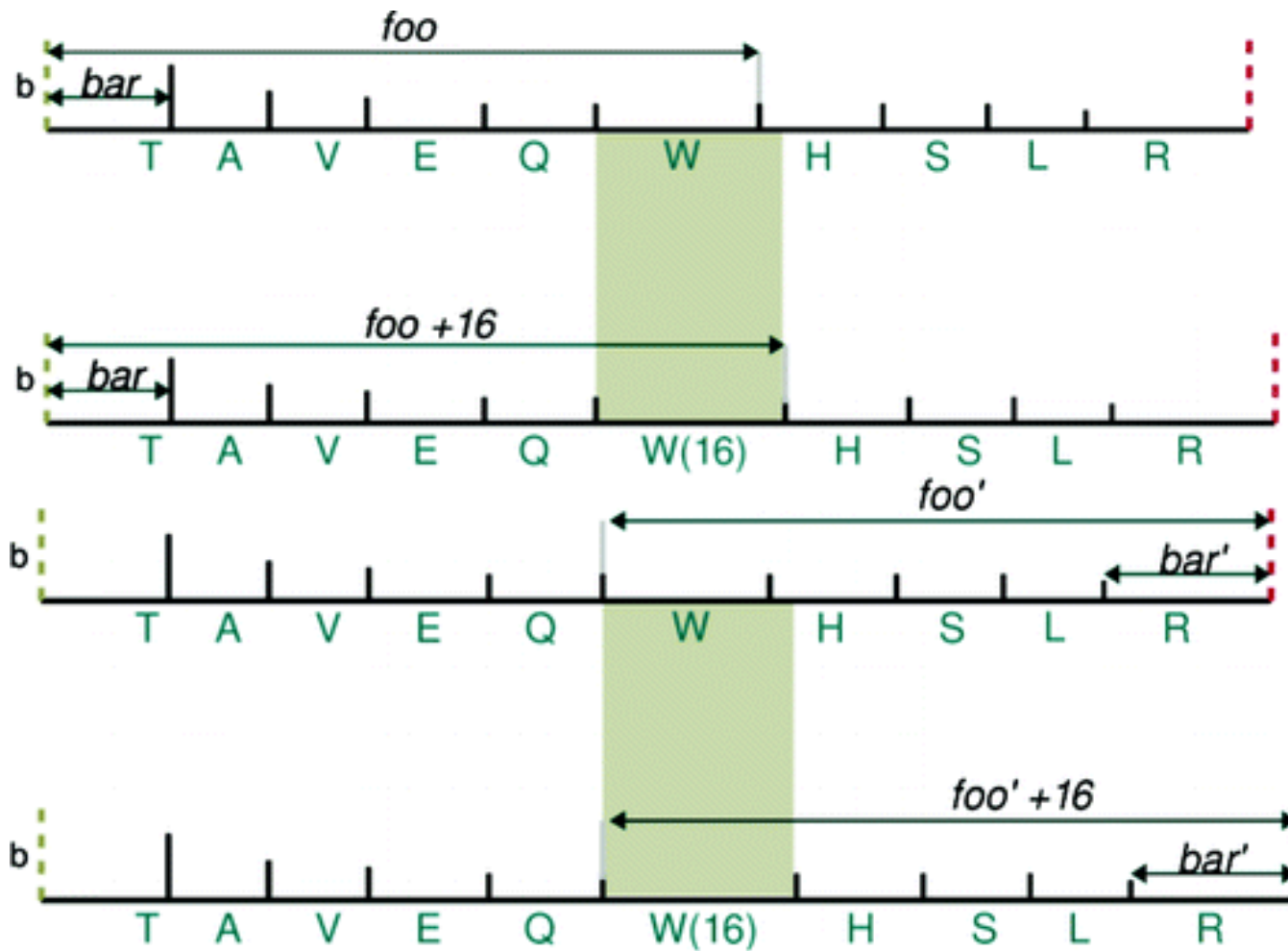
MS/MS Data to MRM

- 28 samples of ~40 fractions = 1,000+ LC-MS/MS runs
- 330,000 spectra for cluster/p-mod analysis
 - ~4,000 unique peak lists
 - ~1,300 identified unique peak lists
- 3x transition per peptide; i.e. ~12,000 trans
 - Picked 400 targets to “scout” (1,200 trans)
 - 4 MRM methods w/100 targets each (300 trans per run)
 - Final MRM method with 300 targets (900 trans)

MS/MS Data to MRM

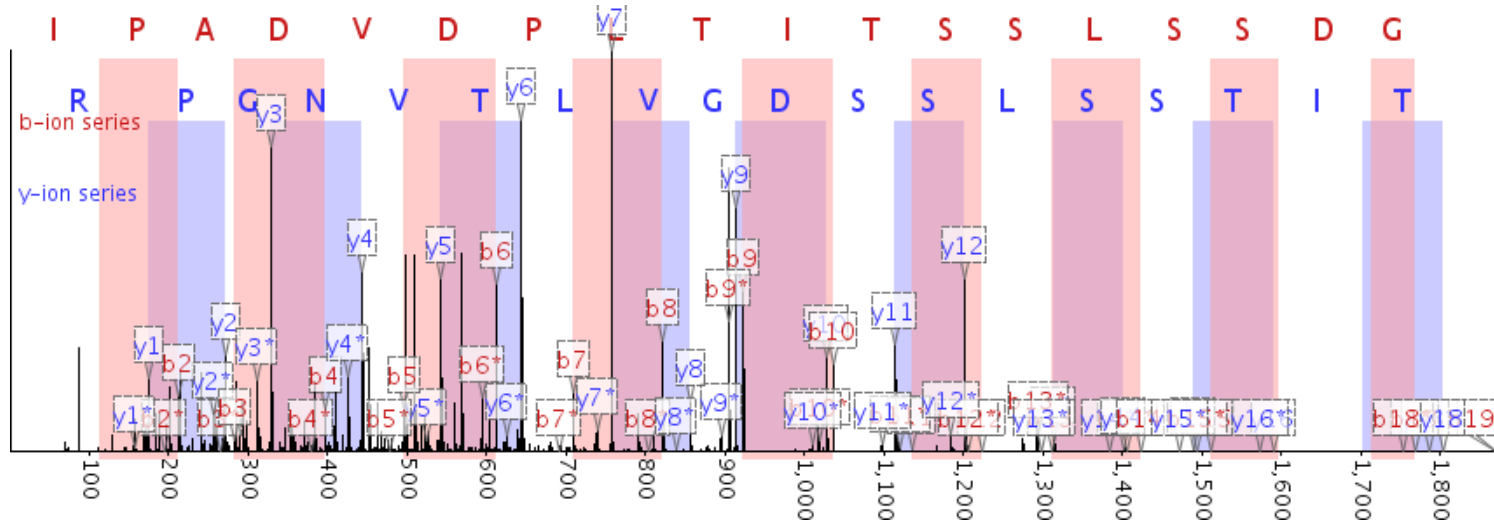


MS/MS Similarity Grouping



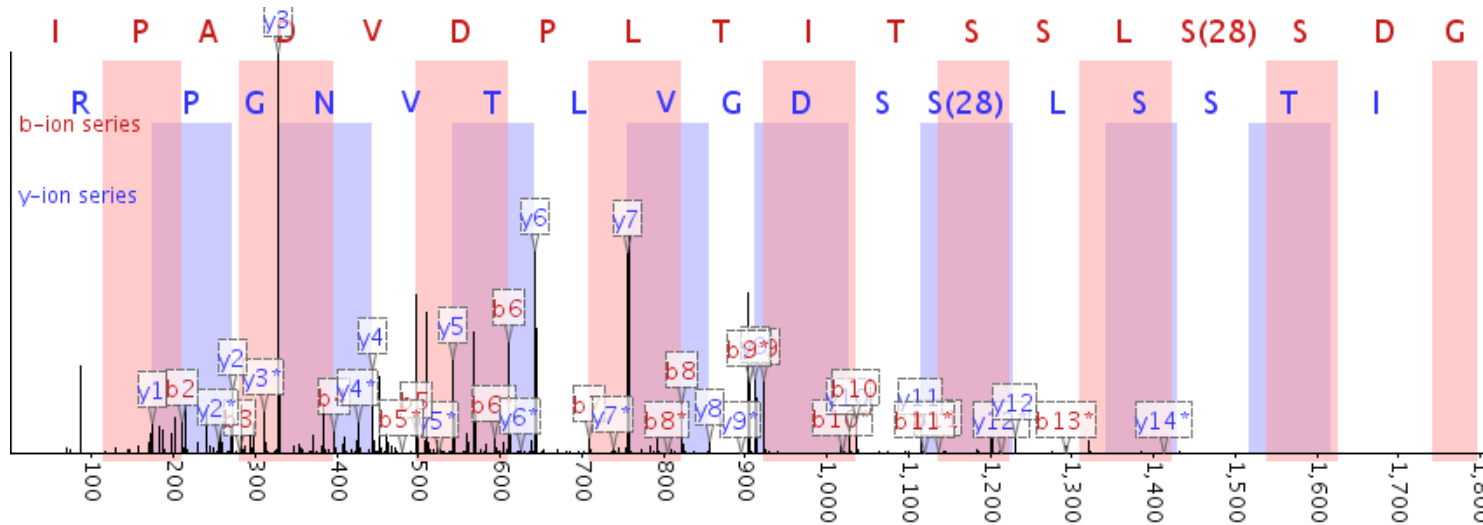
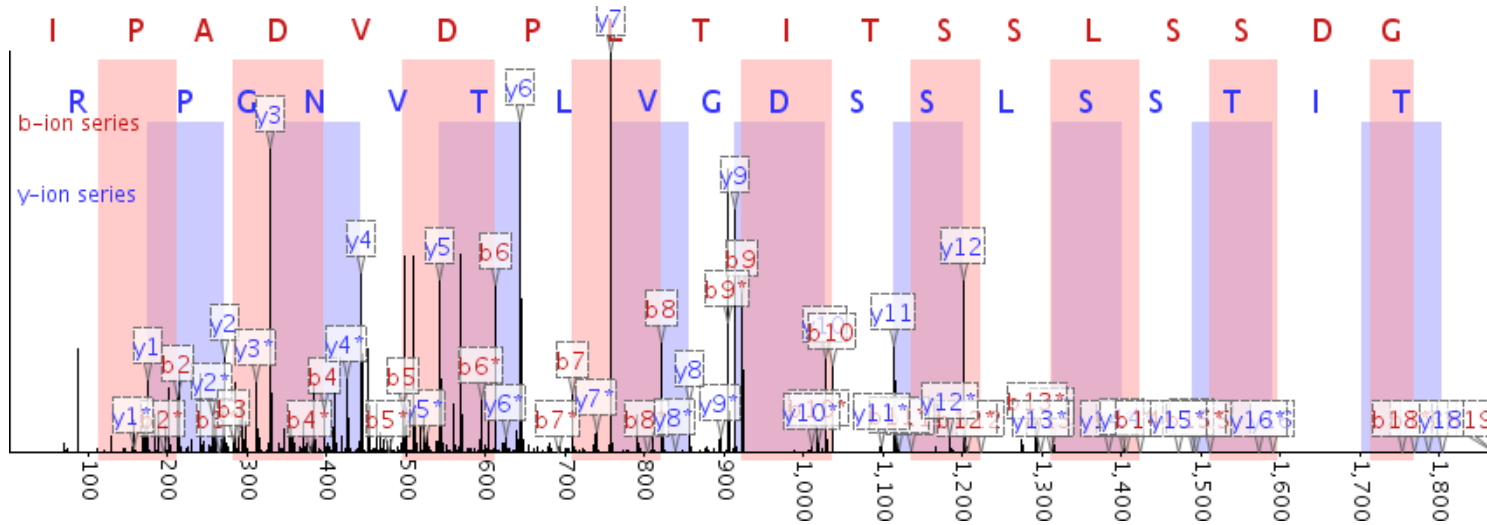
Falkner et al, J. Proteome Res., 2008, 7 (11)

Example “cluster”

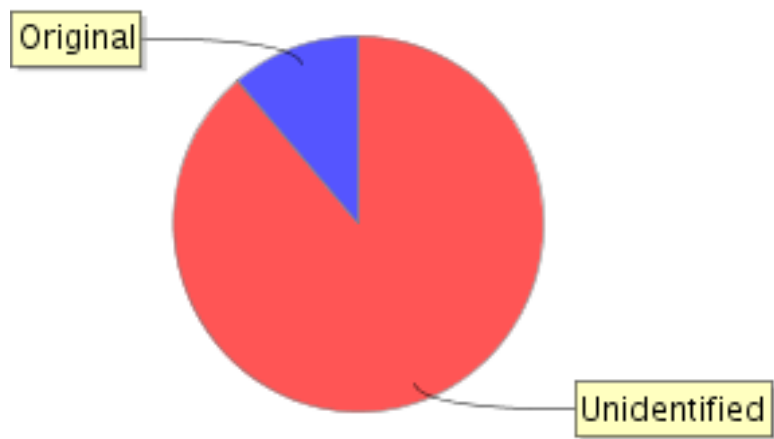


| ID | Peptide | Score | m/z | m/z Shift | # m/z Shifts ▼ | RT |
|-----------------------|--------------------------------|-------|--------|-----------|----------------|--------|
| 35115 | IPADVDPITITSSLSSDGLTVNGPR | 1.00 | 875.45 | 0.00 | 867 | 99.84 |
| 35218 | YRIPADVDPITITSSLSSDGLTVNGPR | 0.52 | 981.84 | 106.39 | 748 | 93.73 |
| 35437 | IPADVDPITITSSLSSDGLTVNGPR | 0.25 | 882.78 | 7.33 | 196 | 96.15 |
| 34420 | IPADVDPITITSSLSSDGLTVN(1)GPR | 0.55 | 875.79 | 0.33 | 146 | 25.75 |
| 35867 | IPADVDPITITSSLSS(28)DGLTVNGPR | 0.71 | 884.79 | 9.34 | 97 | 104.60 |
| 34188 | IPADVDPITITSSLSSDGLTVN(1)GPRK | 0.24 | 918.52 | 43.06 | 77 | 40.73 |
| 35583 | IPADVDPITITSSLSSDGLT(-17)VNGPR | 0.36 | 869.81 | -5.64 | 36 | 30.00 |

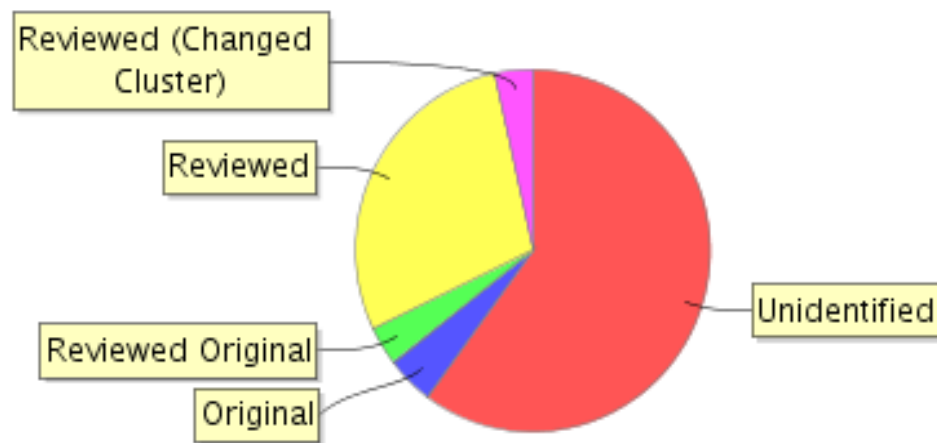
Example “cluster”



Identified Peak Lists Before and After Cluster Analysis

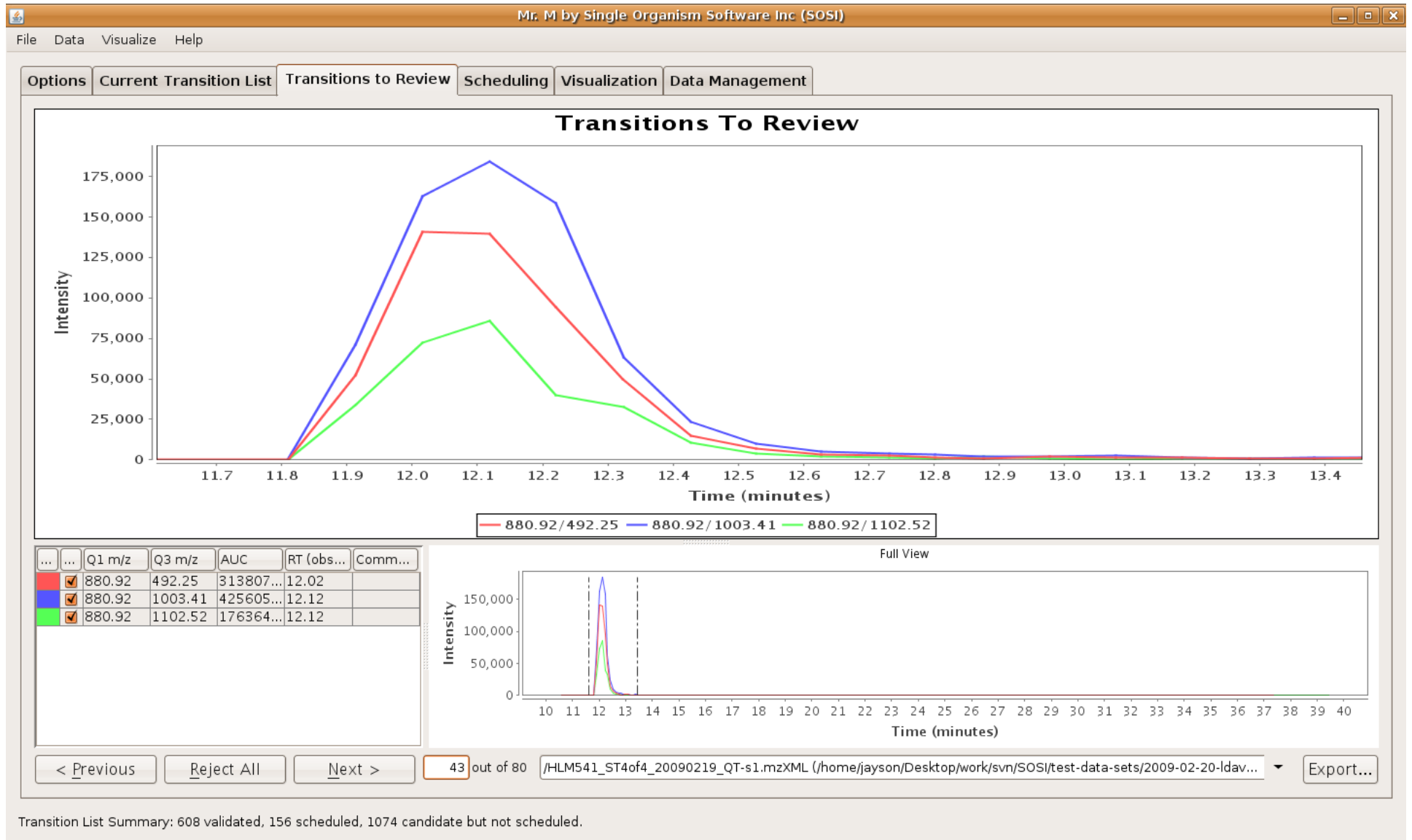


Sequest + Decoy

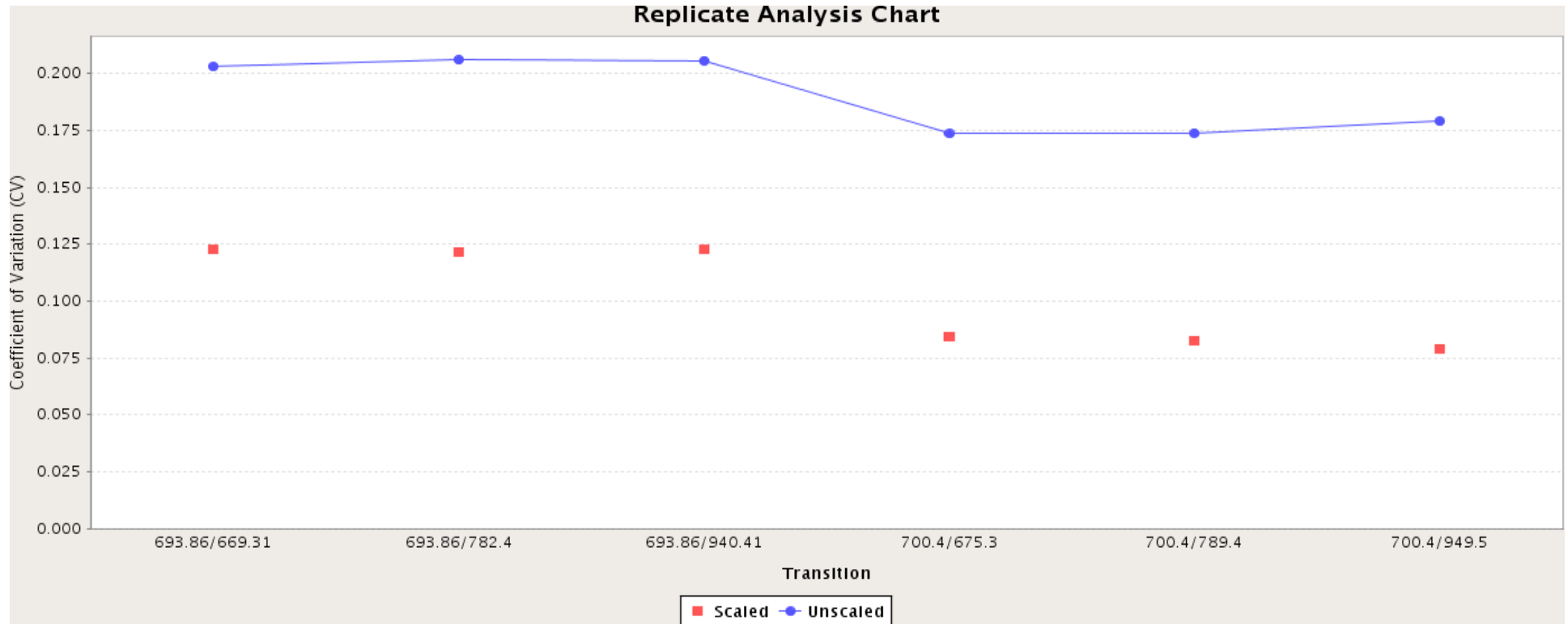


Sequest + Decoy + Cluster

MRM AUC Sensitivity

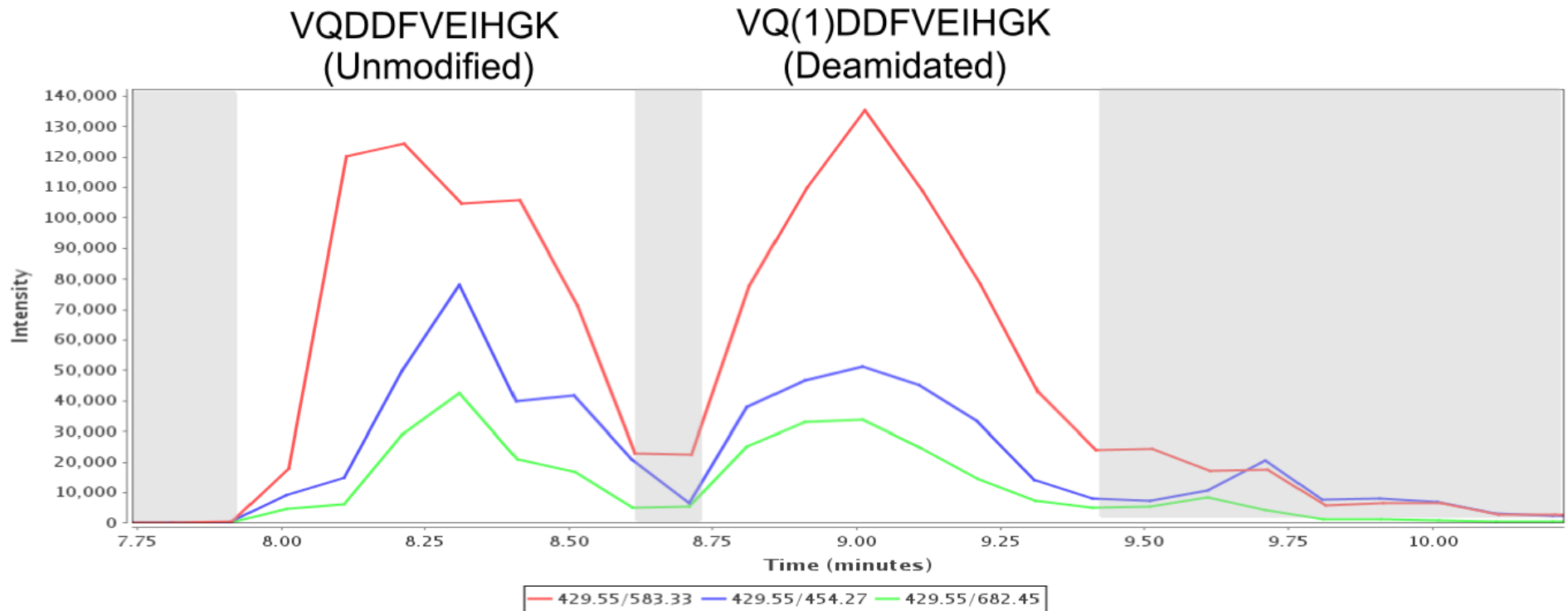


MRM Quant Variance



- 10-30% CV prior to correction
- 5-15% CV w/exogenous protein standard

MRM of Tricky P-mods



- +1 Da; too small to target
- Software can target using RP shift

Conclusion

- MS/MS works well for discovery
 - MudPIT takes a long time (weeks)
 - Build a library for MRM use
- MRM works well for monitoring
 - Monitor ~300 targets in <1 hour
 - Great sensitivity
 - Minimal sample variance
 - Minimal sample handling