



Webinar #27

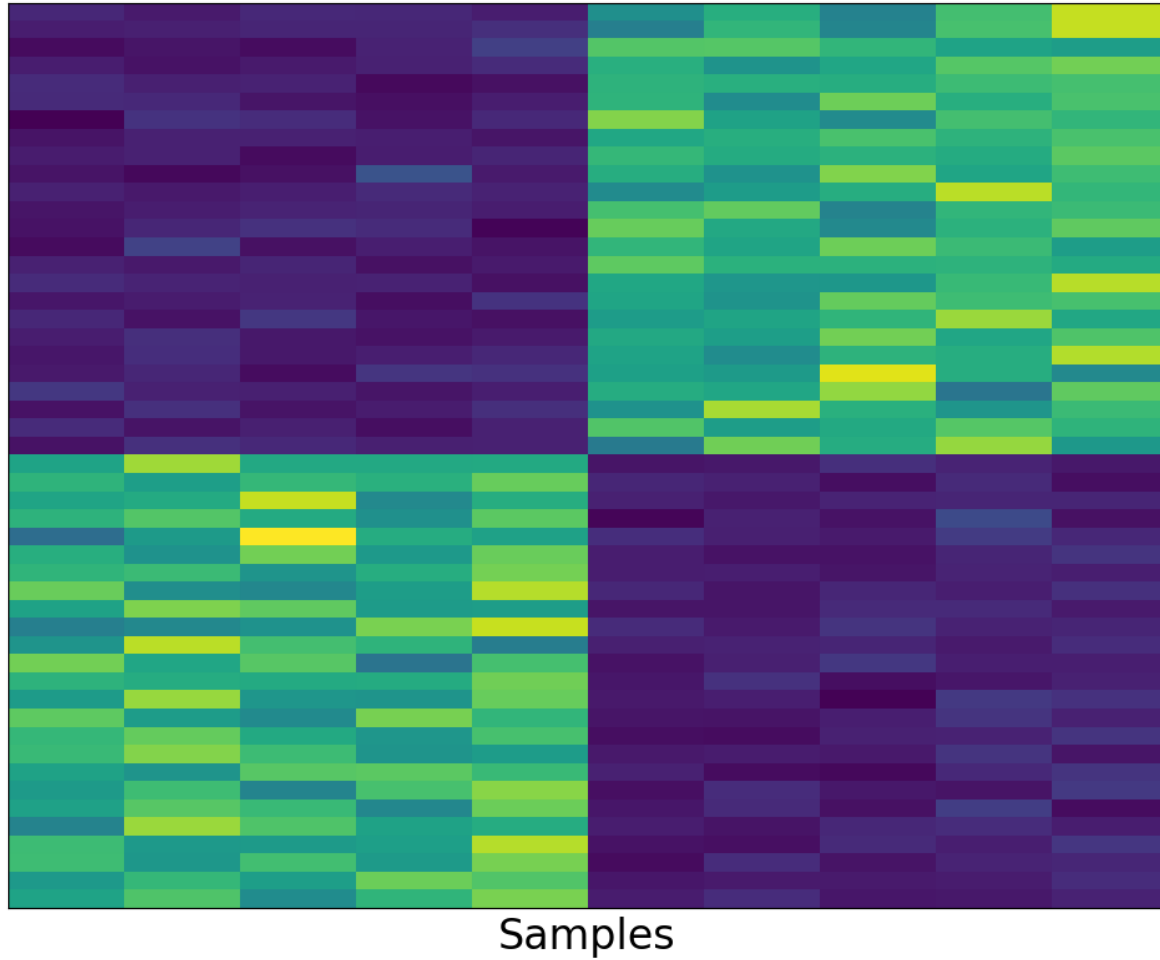
Skyline Peak Boundary Imputation for Differential Proteomics

Michael MacCoss and Nick Shulman

Challenge with Missing Data

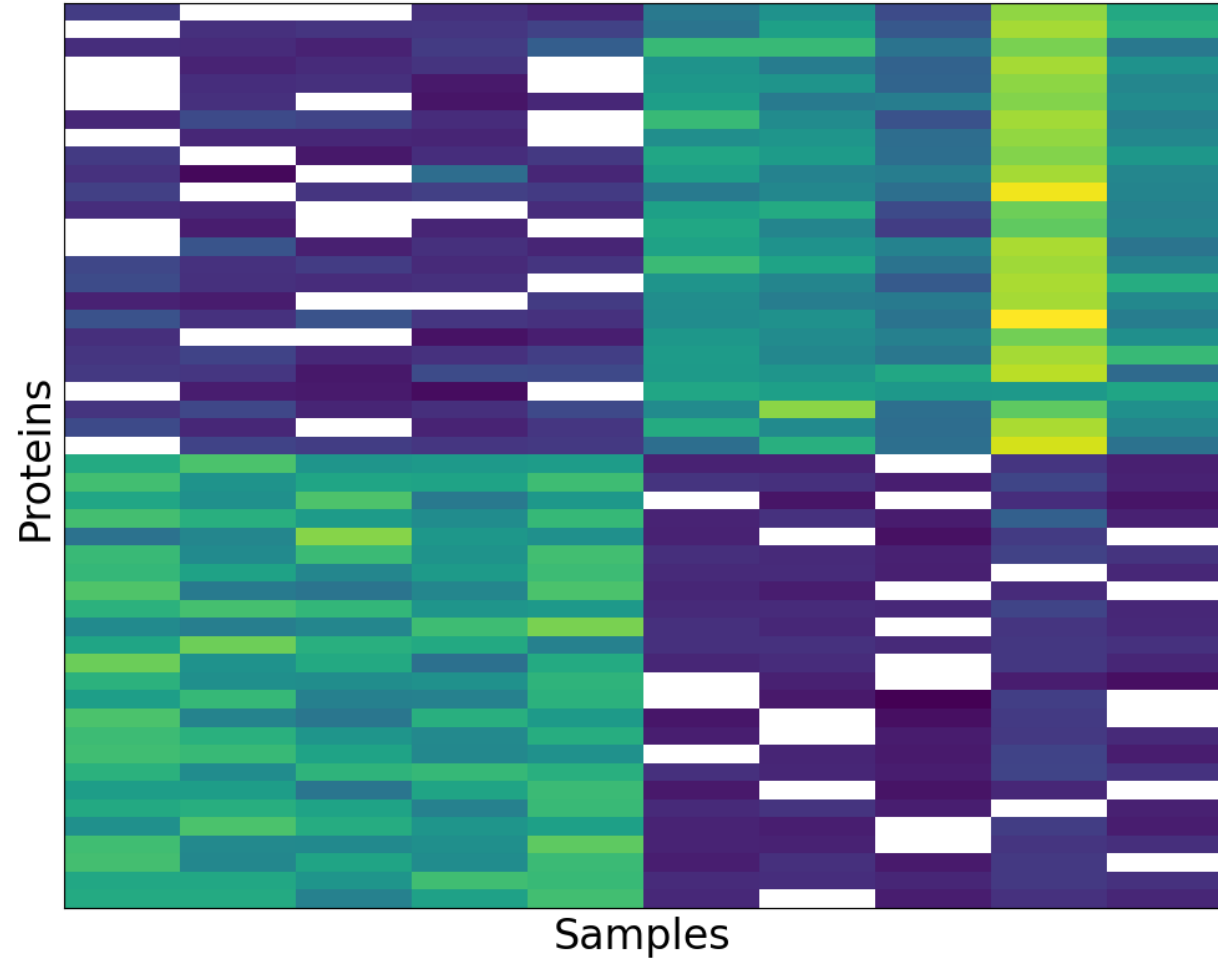
Group A

Group B

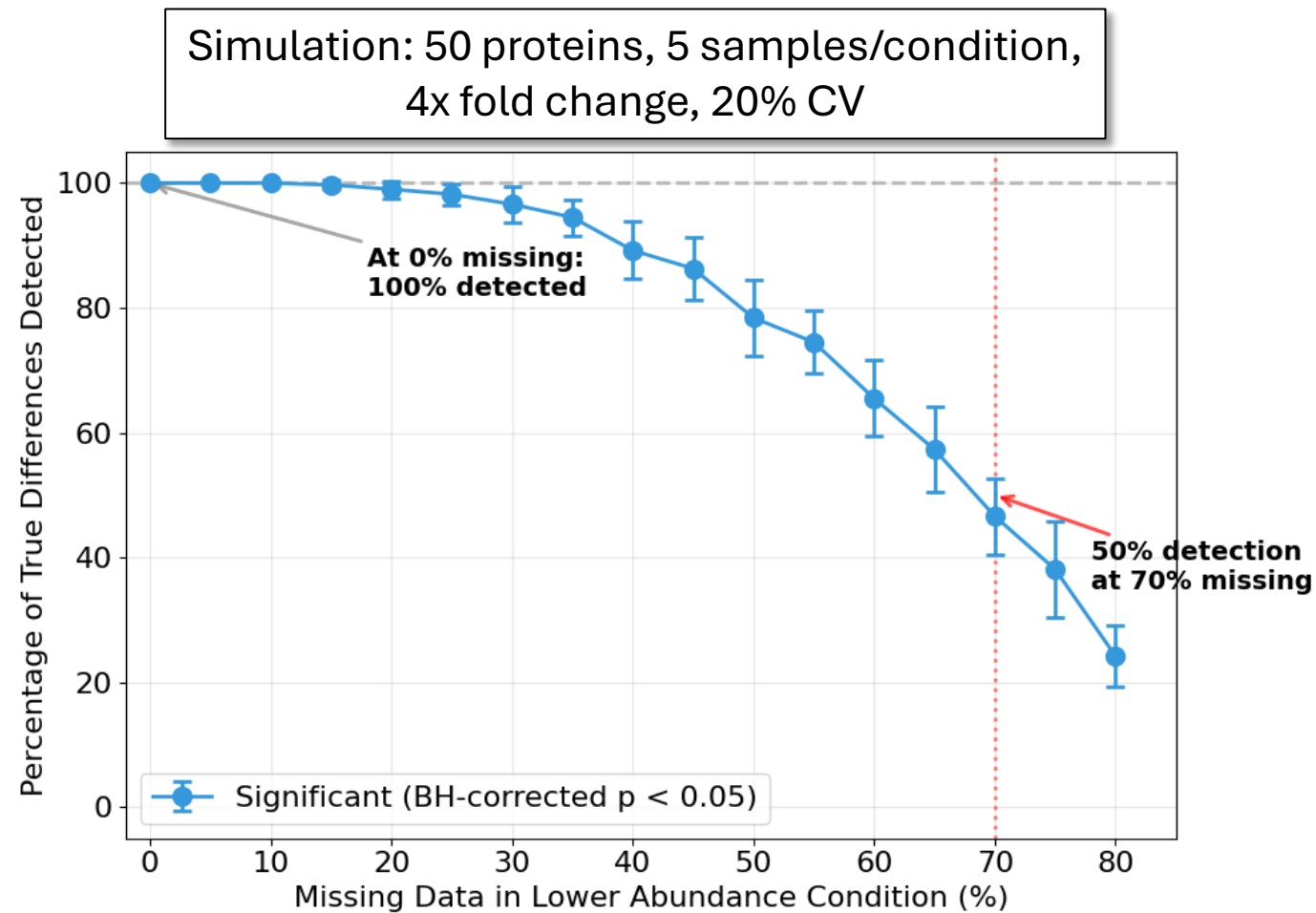
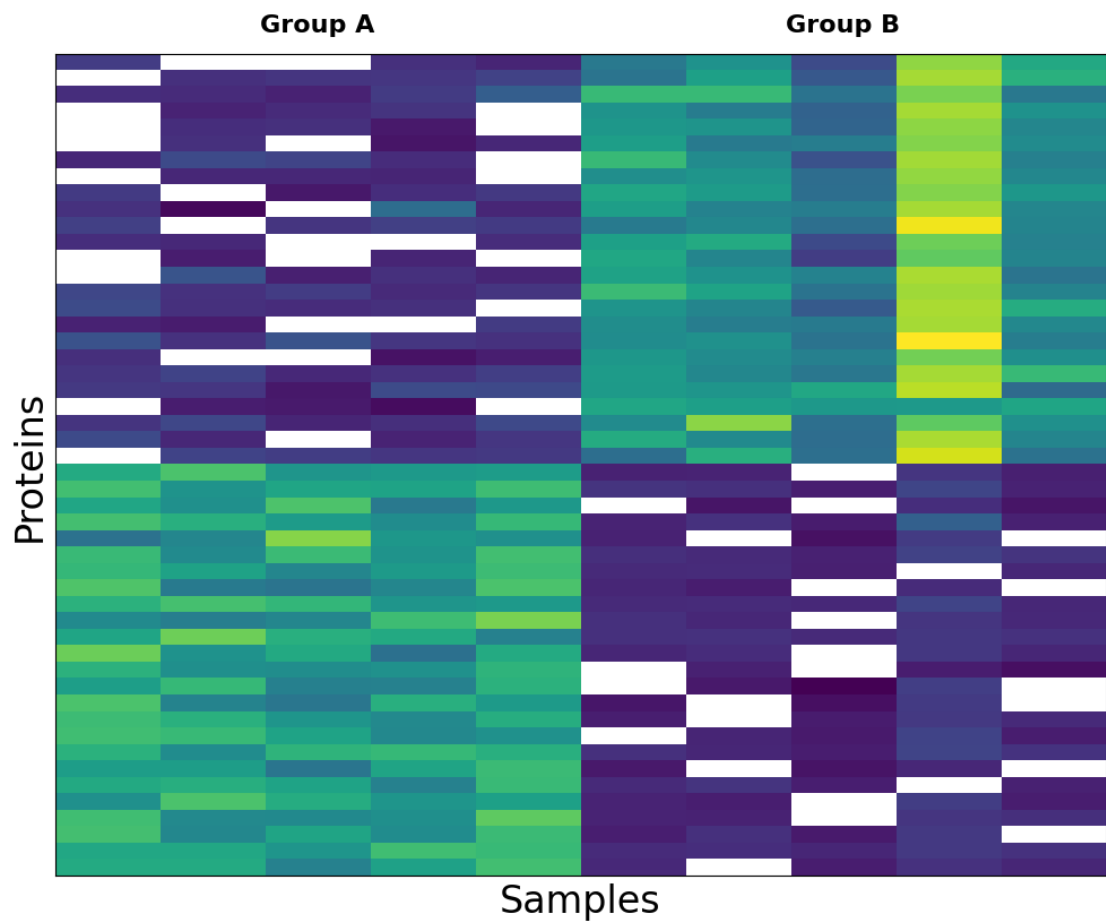


Group A

Group B



Challenge with Missing Data

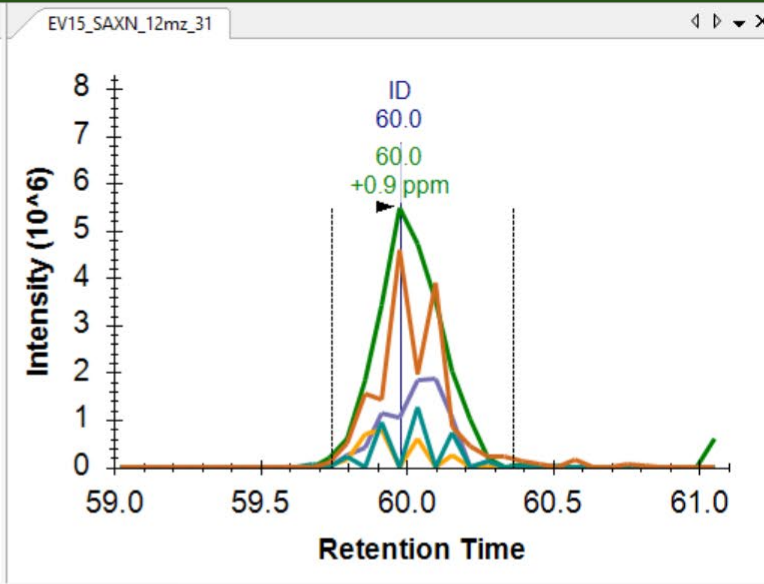
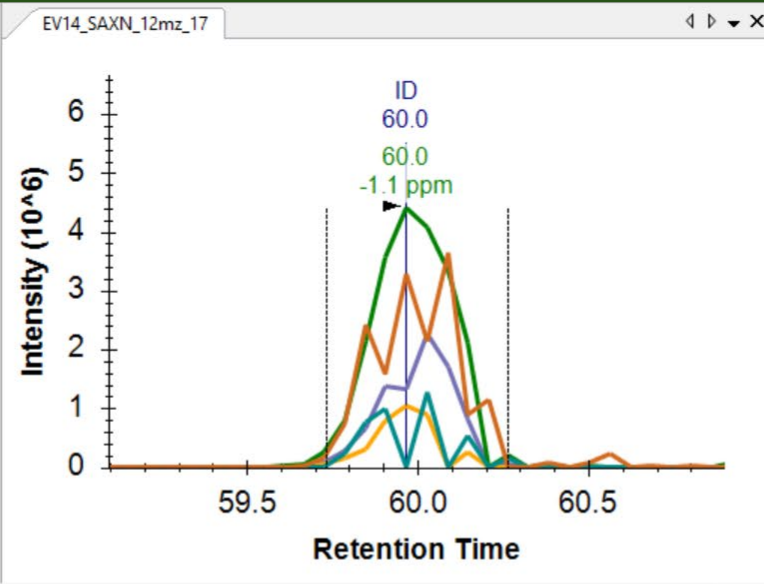
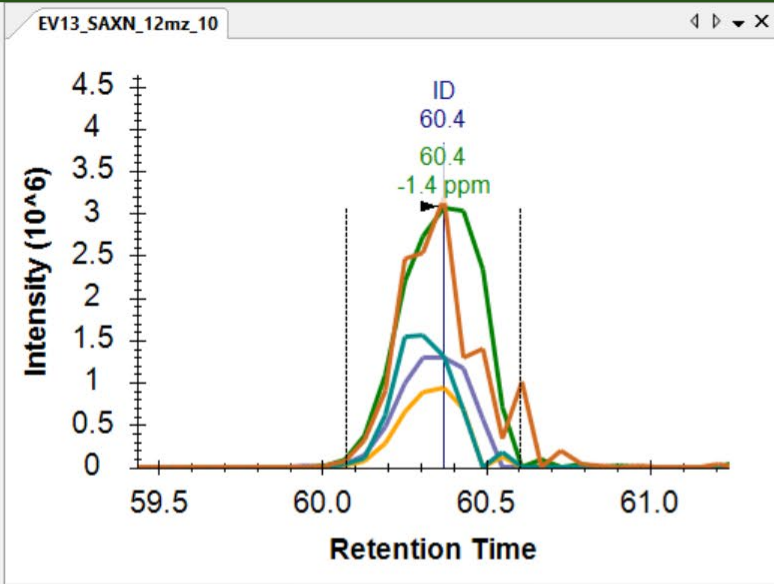


Imputation? Two options ...

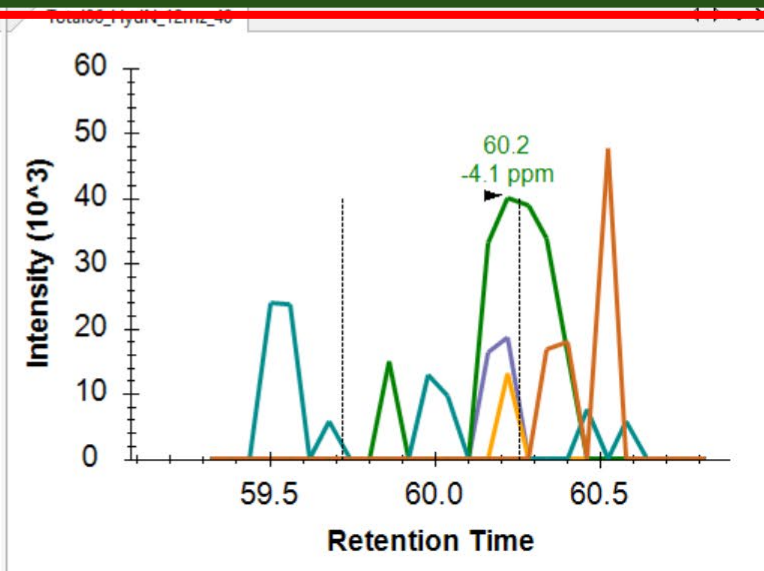
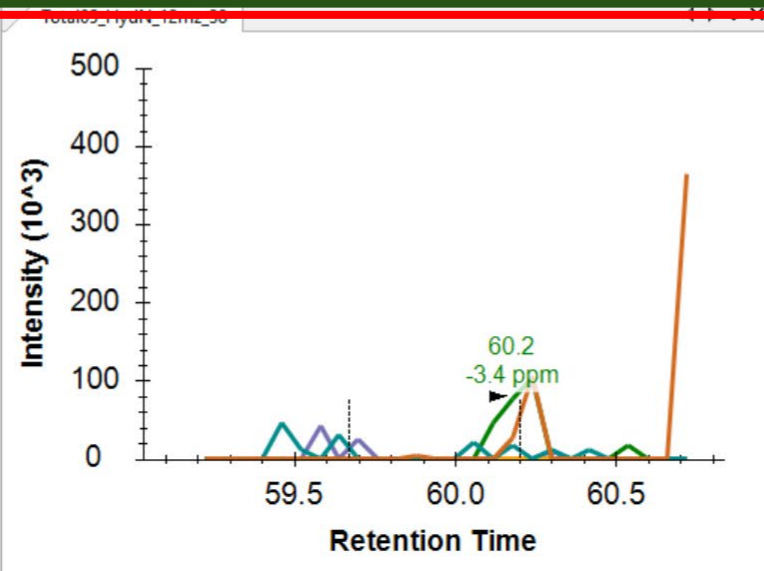
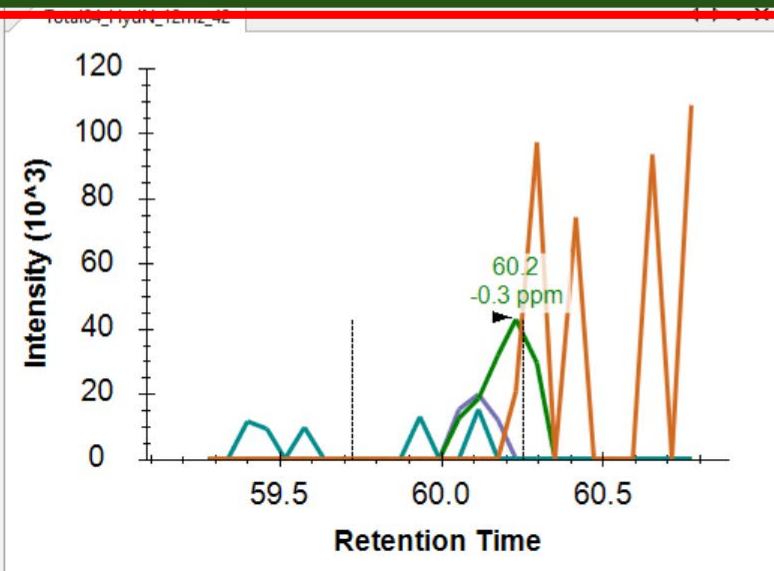
- Do we make up low abundance quantities?
- Do we use the background signal at the retention time and m/z values we know it exists?

Peak Boundary Imputation Provides a Lower Boundary for the Signal

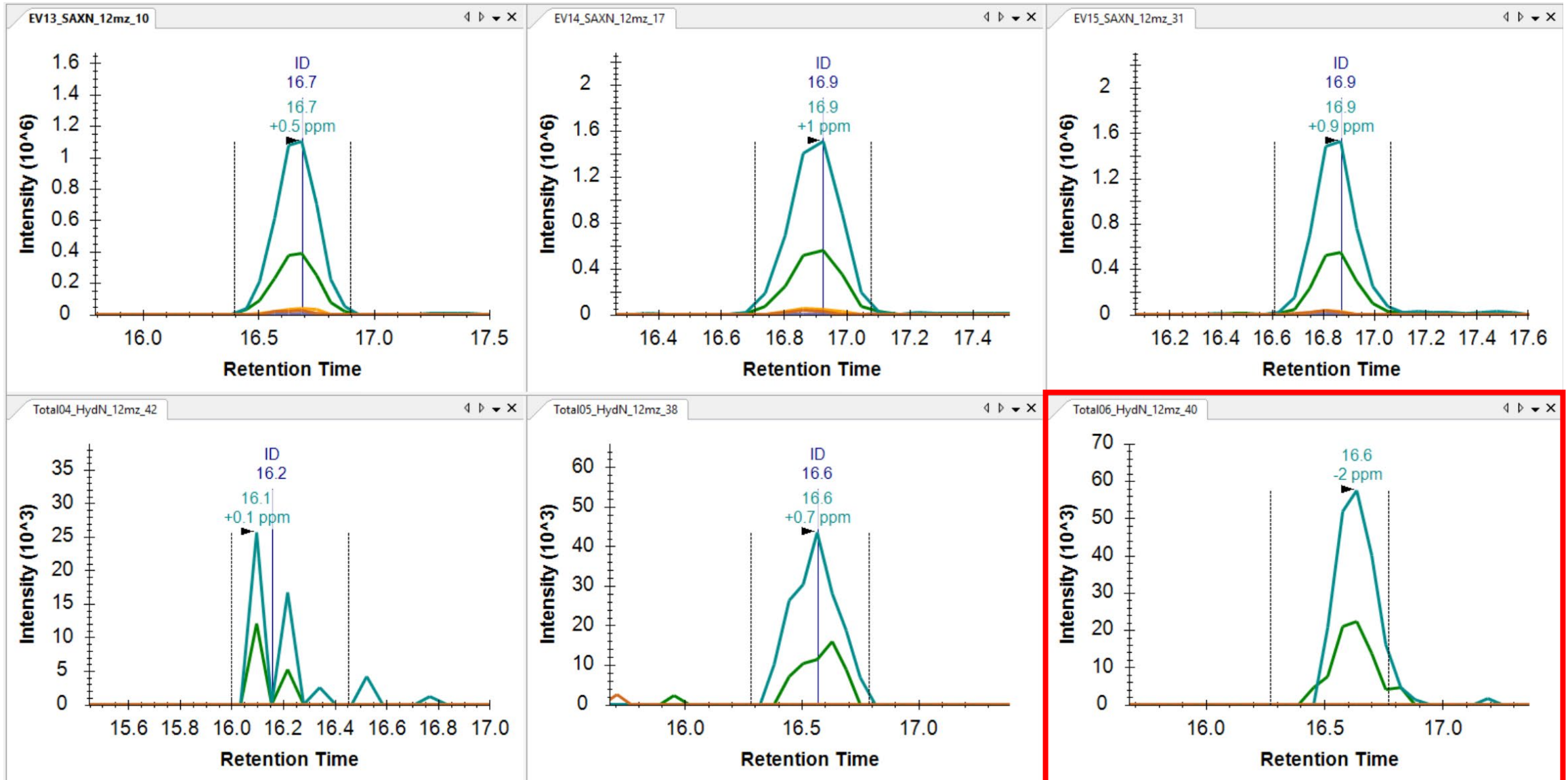
Detected By DIANN



Not Detected By DIANN



Can recover a signal when search tools miss a peak



Introduce the EV Dataset

nature communications



Article


<https://doi.org/10.1038/s41467-025-60595-7>












Enrichment of extracellular vesicles using Mag-Net for the analysis of the plasma proteome

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 Check for updates

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Dataset – Panorama Public

https://panoramaweb.org/public.url

The screenshot shows the PanoramaWeb public search interface. At the top, there is a navigation bar with the PanoramaWeb logo, search, settings, and user profile icons, and the name 'Mike MacCoss'. Below the navigation bar are tabs for 'Panorama Public', 'Panorama Public Datasets', 'Panorama Dashboard', 'Datasets Summary', and 'Help'. The main content area is titled 'Panorama Public Search' and contains a search form with tabs for 'Experiment Search', 'Protein Search', 'Peptide Search', and 'Small Molecule Search'. The search form includes input fields for 'Author:', 'Title:', 'Organism:', and 'Instrument:', a 'Clear All' link, and a 'Search' button. Below the search form is a section titled 'Panorama Public Experiments' which displays a table of search results.

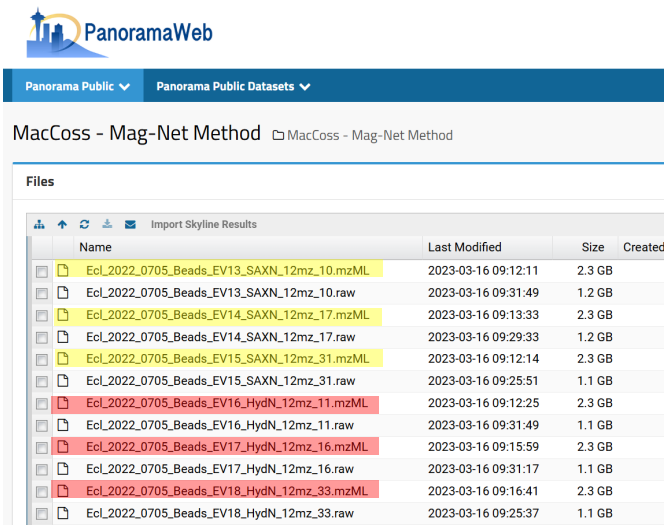
Created	Share	Title	Organism	Instrument	Publication	Spike In	Skyline Docs	PX ID
2025-11-28	Share	ATI in NILs with different Rht genes	Triticum aestivum	Q Exactive Plus		Yes	12	
2025-11-12	Share	Increasing PFAS Concentrations in Human Serum Correlate with Elevated Blood Lipid Levels	Homo sapiens	6560 Q-TOF LC/MS	To be submitted	No	4	
2025-11-03	Share	CeD-active Peptides in Gluten-Free Beer	Hordeum vulgare, Triticum	Orbitrap Exploris 480	Tissen E, Geisslitz S, Scherf KA. Absolute quan...	Yes	3	PXD070271
2025-10-31	Share	LFQ Benchmark Dataset - Generation Beta: Assessing Modern Proteomics Instruments and Acquisition Workflows with High-Throughput LC Gradients	Homo sapiens, Saccharomyces cerevisiae, Escherichia coli	ZenoTOF 7600+, ZenoTOF 8600, timsTOF Ultra, timsTOF Ultra 2, Orbitrap Astral		No	9	PXD070173

https://panoramaweb.org/Mag-Net.url

The screenshot shows the PanoramaWeb Mag-Net method page. At the top, there is a navigation bar with the PanoramaWeb logo, search, settings, and user profile icons, and the name 'Mike MacCoss'. Below the navigation bar are tabs for 'Panorama Public', 'Panorama Public Datasets', 'Panorama Dashboard', 'Raw Data', and 'Supplementary Files'. The main content area is titled 'MacCoss - Mag-Net Method' and contains a section titled 'Targeted MS Experiment'. The section includes the title 'Mag-Net: Rapid enrichment of membrane-bound particles enables high coverage quantitative analysis of the plasma proteome', a '[More Details...]' link, a 'Permanent Link' to the dataset, and a 'Share' button. Below this is the citation information: 'Wu CC, Tsantilas KA, Park J, Plubell D, Sanders JA, Naicker P, Govender I, Buthelezi S, Stoychev S, Jordaan J, Merrihew G, Huang E, Parker ED, Riffle M, Hoofnagle AN, Noble WS, Poston KL, Montine TJ, MacCoss M.J. Enrichment of extracellular vesicles using Mag-Net for the analysis of the plasma proteome. Nat Commun. 2025 Jul 1;16(1):5447. doi: 10.1038/s41467-025-60595-7. PMID: 40595564. [Publication]'. The citation also includes 'Data License: CC BY 4.0', 'ProteomeXchange: PXD042947', and 'doi: https://doi.org/10.6069/jskg-0809'. Below the citation are the 'Organism: Homo sapiens', 'Instrument: Orbitrap Eclipse', 'Spikeln: No', 'Keywords: extracellular vesicles, DIA mass spectrometry, plasma proteomics, quantitative proteomics, neurodegenerative disease', and 'Lab head: Michael MacCoss', 'Submitter: Michael MacCoss'. At the bottom of the section is an 'Abstract' box containing the text: 'Membrane-bound particles in plasma are composed of exosomes, microvesicles, and apoptotic bodies and represent ~1-2% of the total protein composition. Proteomic interrogation of this subset of plasma proteins augments the representation of tissue-specific proteins, representing a "liquid biopsy," while enabling the detection of proteins that would otherwise be beyond the dynamic range of liquid chromatography-tandem mass spectrometry in unfractionated plasma. We have developed a one-step enrichment...' and a '[Show more]' link.

Workflow

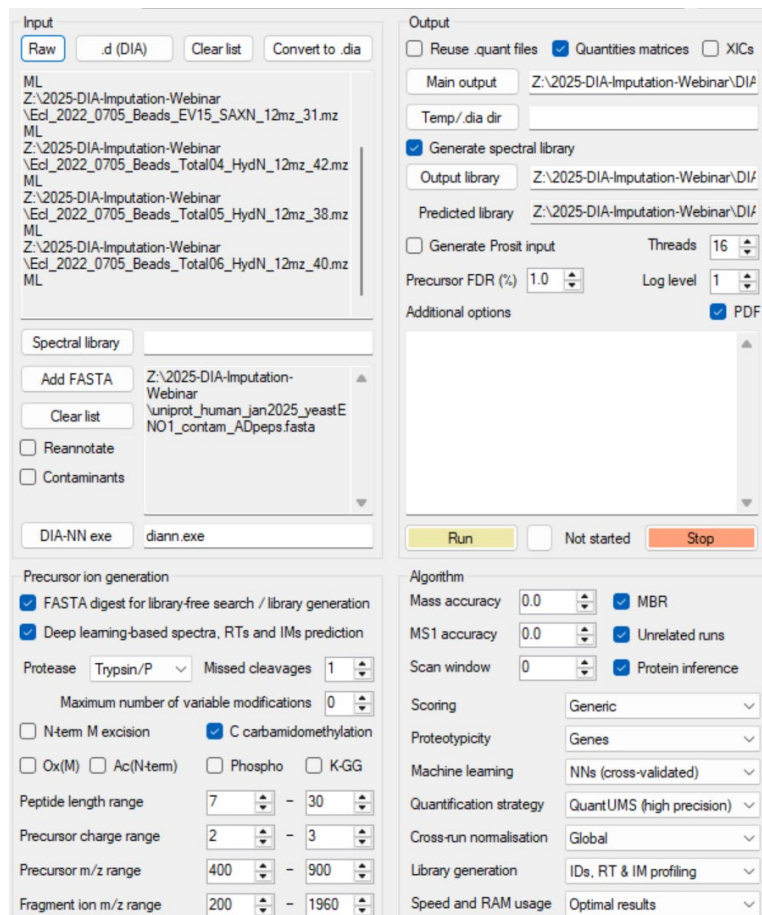
Download demux mzML Files



MacCoss - Mag-Net Method

Name	Last Modified	Size	Created
Ecl_2022_0705_Beads_EV13_SAXN_12mz_10.mzML	2023-03-16 09:12:11	2.3 GB	
Ecl_2022_0705_Beads_EV13_SAXN_12mz_10.raw	2023-03-16 09:31:49	1.2 GB	
Ecl_2022_0705_Beads_EV14_SAXN_12mz_17.mzML	2023-03-16 09:13:33	2.3 GB	
Ecl_2022_0705_Beads_EV14_SAXN_12mz_17.raw	2023-03-16 09:29:33	1.2 GB	
Ecl_2022_0705_Beads_EV15_SAXN_12mz_31.mzML	2023-03-16 09:12:14	2.3 GB	
Ecl_2022_0705_Beads_EV15_SAXN_12mz_31.raw	2023-03-16 09:25:51	1.1 GB	
Ecl_2022_0705_Beads_EV16_HydN_12mz_11.mzML	2023-03-16 09:12:25	2.3 GB	
Ecl_2022_0705_Beads_EV16_HydN_12mz_11.raw	2023-03-16 09:31:49	1.1 GB	
Ecl_2022_0705_Beads_EV17_HydN_12mz_16.mzML	2023-03-16 09:15:59	2.3 GB	
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Ecl_2022_0705_Beads_EV18_HydN_12mz_33.mzML	2023-03-16 09:16:41	2.3 GB	
Ecl_2022_0705_Beads_EV18_HydN_12mz_33.raw	2023-03-16 09:25:37	1.1 GB	

Search with DIA-NN v2.2



Input: Raw, .d (DIA), Clear list, Convert to .dia

Output: Reuse .quant files, Quantities matrices, XICs

Main output: Z:\2025-DIA-Imputation-Webinar\DI

Temp/.dia dir:

Generate spectral library:

Output library: Z:\2025-DIA-Imputation-Webinar\DI

Predicted library: Z:\2025-DIA-Imputation-Webinar\DI

Generate Prosit input:

Threads: 16

Precursor FDR (%): 1.0

Log level: 1

Additional options: PDF

Spectral library:

Add FASTA: Z:\2025-DIA-Imputation-Webinar\uniprot_human_jan2025_yeastE_NO1_contam_ADpeps.fasta

Clear list

Reannotate:

Contaminants:

DIA-NN exe: diann.exe

Run:

Precursor ion generation: FASTA digest for library-free search / library generation, Deep learning-based spectra, RTs and IMs prediction

Protease: Trypsin/P, Missed cleavages: 1

Maximum number of variable modifications: 0

N-term M excision: Ox(M): Ac(N-term): Phospho: K-GG: C carbamidomethylation:

Peptide length range: 7 - 30

Precursor charge range: 2 - 3

Precursor m/z range: 400 - 900

Fragment ion m/z range: 200 - 1960

Algorithm: Mass accuracy: 0.0, MS1 accuracy: 0.0, Scan window: 0

MBR: Unrelated runs: Protein inference:

Scoring: Generic

Proteotypicity: Genes

Machine learning: NNs (cross-validated)

Quantification strategy: QuantUMS (high precision)

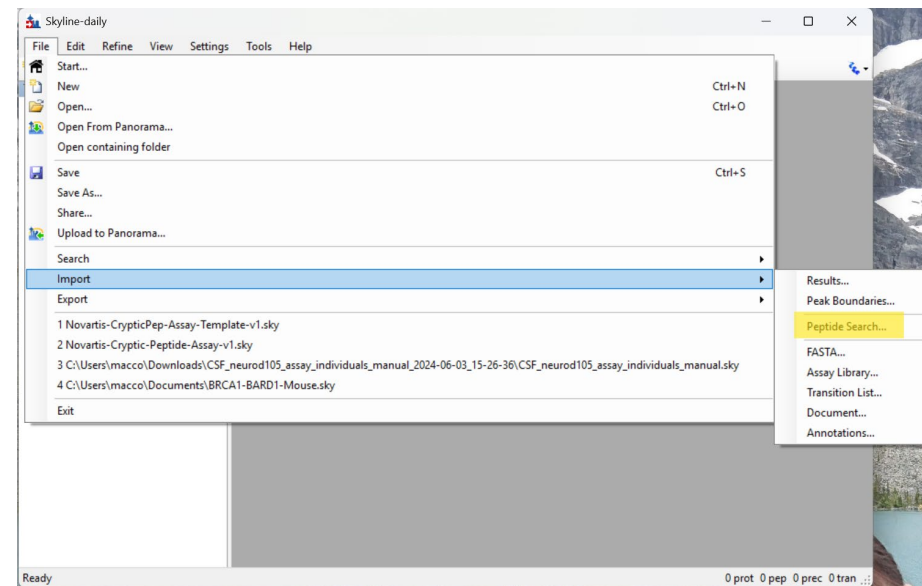
Cross-run normalisation: Global

Library generation: IDs, RT & IM profiling

Speed and RAM usage: Optimal results

Import into Skyline-Daily

File > Import > Peptide Search ...



Skyline-daily

File > Import > Peptide Search ...

1 Novartis-CrypticPep-Assay-Template-v1.sky

2 Novartis-Cryptic-Peptide-Assay-v1.sky

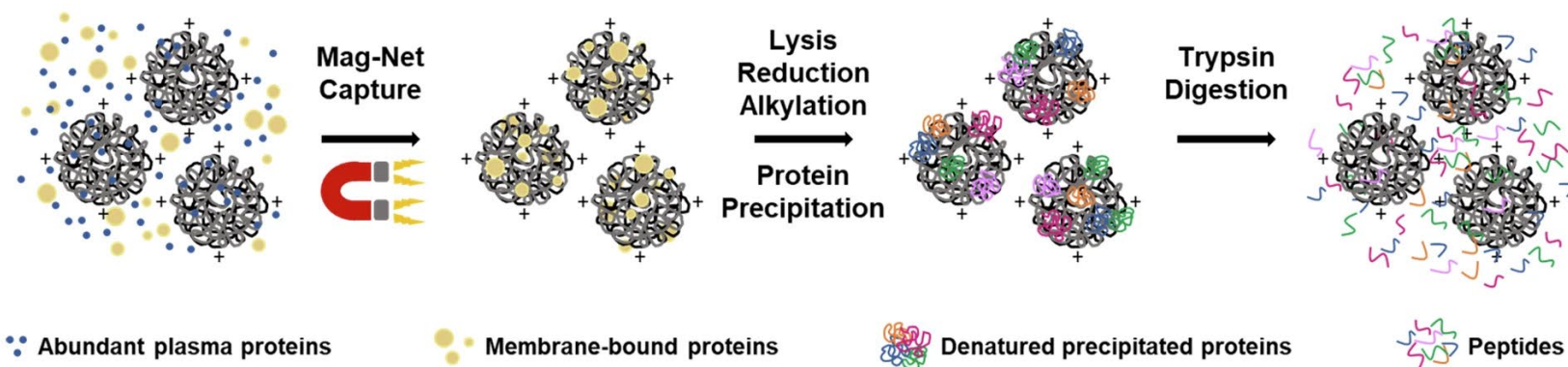
3 C:\Users\macco\Downloads\CSF_neurod105_assay_individuals_manual_2024-06-03_15-26-36\CSF_neurod105_assay_individuals_manual.sky

4 C:\Users\macco\Documents\BRCA1-BARD1-Mouse.sky

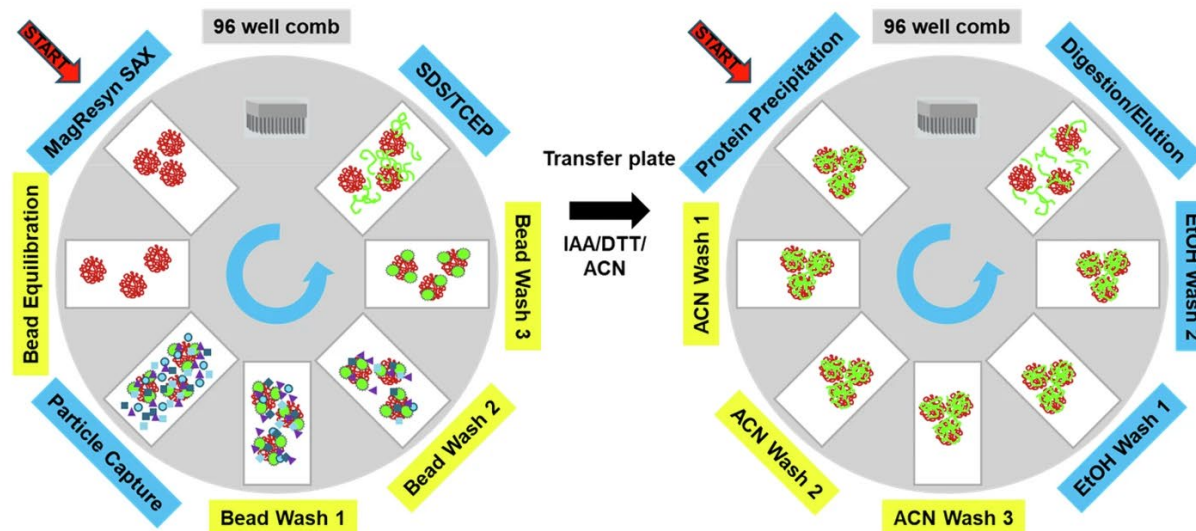
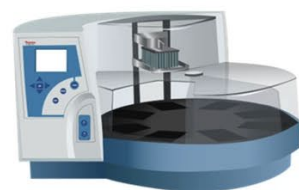
Results...
Peak Boundaries...
Peptide Search...
FASTA...
Assay Library...
Transition List...
Document...
Annotations...

Mag-Net Enriches for Proteins in Membrane-bound Vesicles

a



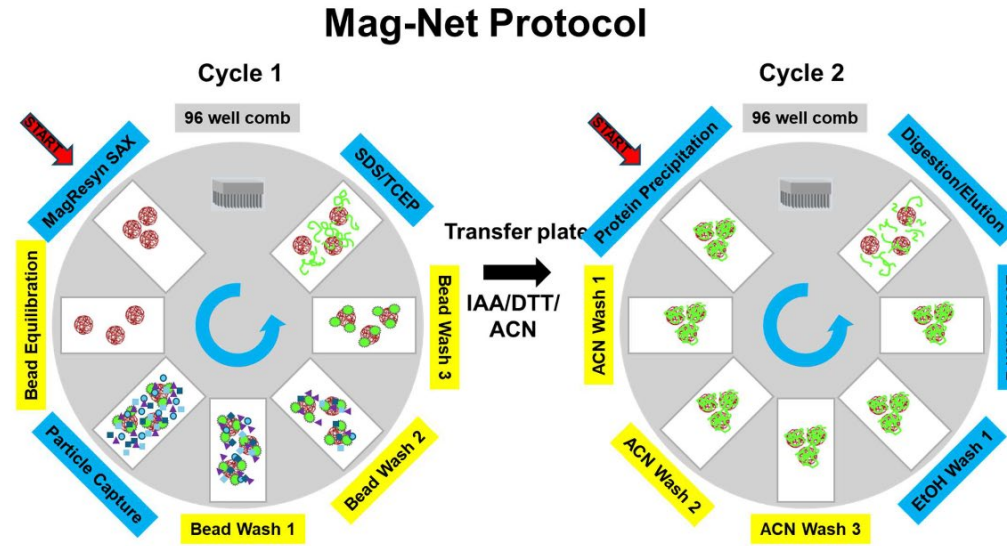
b



Comparison

Mag-Net Protocol:

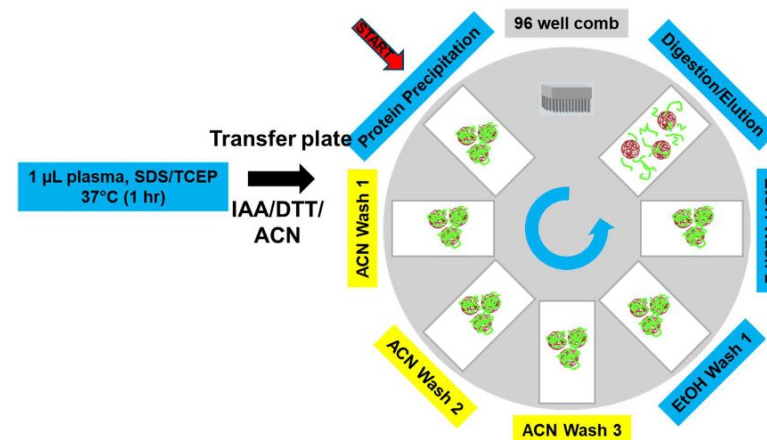
- **Cycle 1:** Enrich Vesicles
- **Cycle 2:** Lyse Vesicles and Digest



Total Plasma PAC Protocol

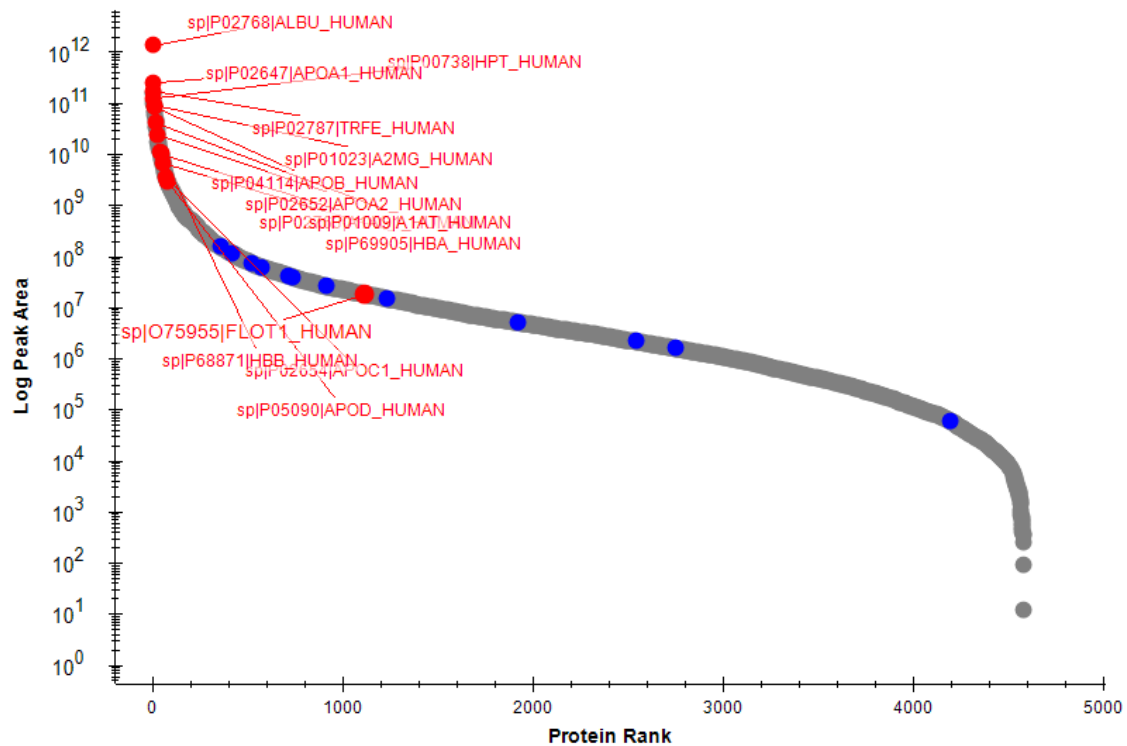
Plasma PAC Digest (aka SP3)

- Lyse Vesicles and Digest

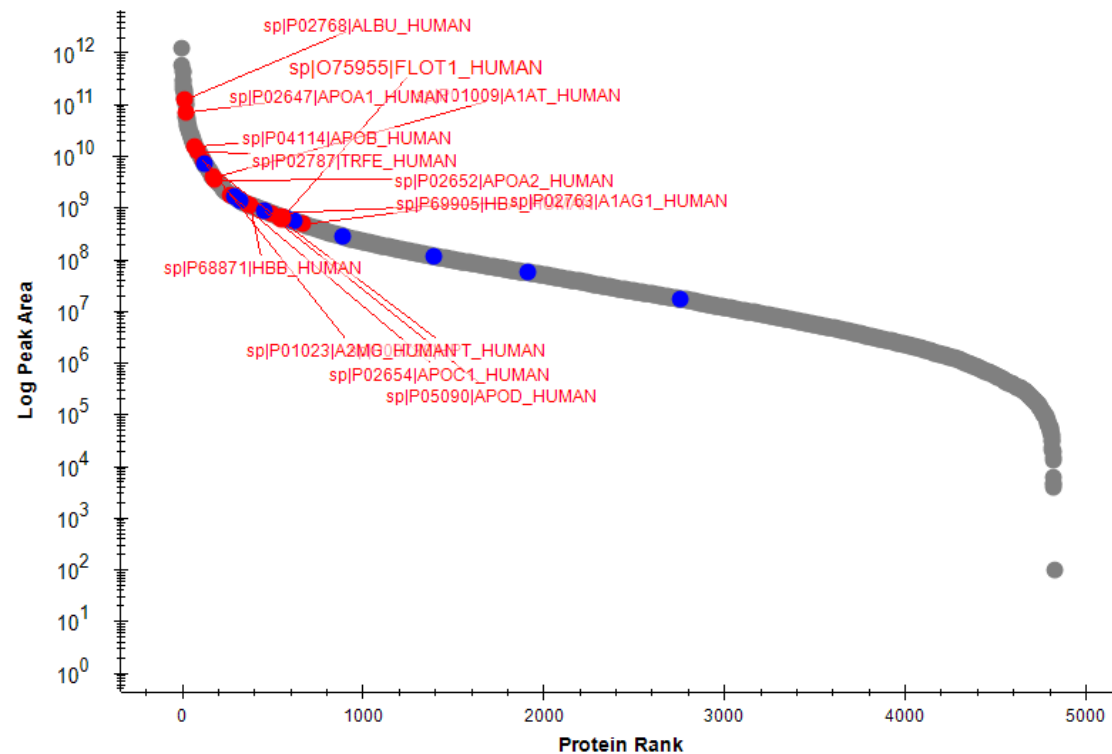


Relative Abundance Plot

In the HydN files: Total Plasma

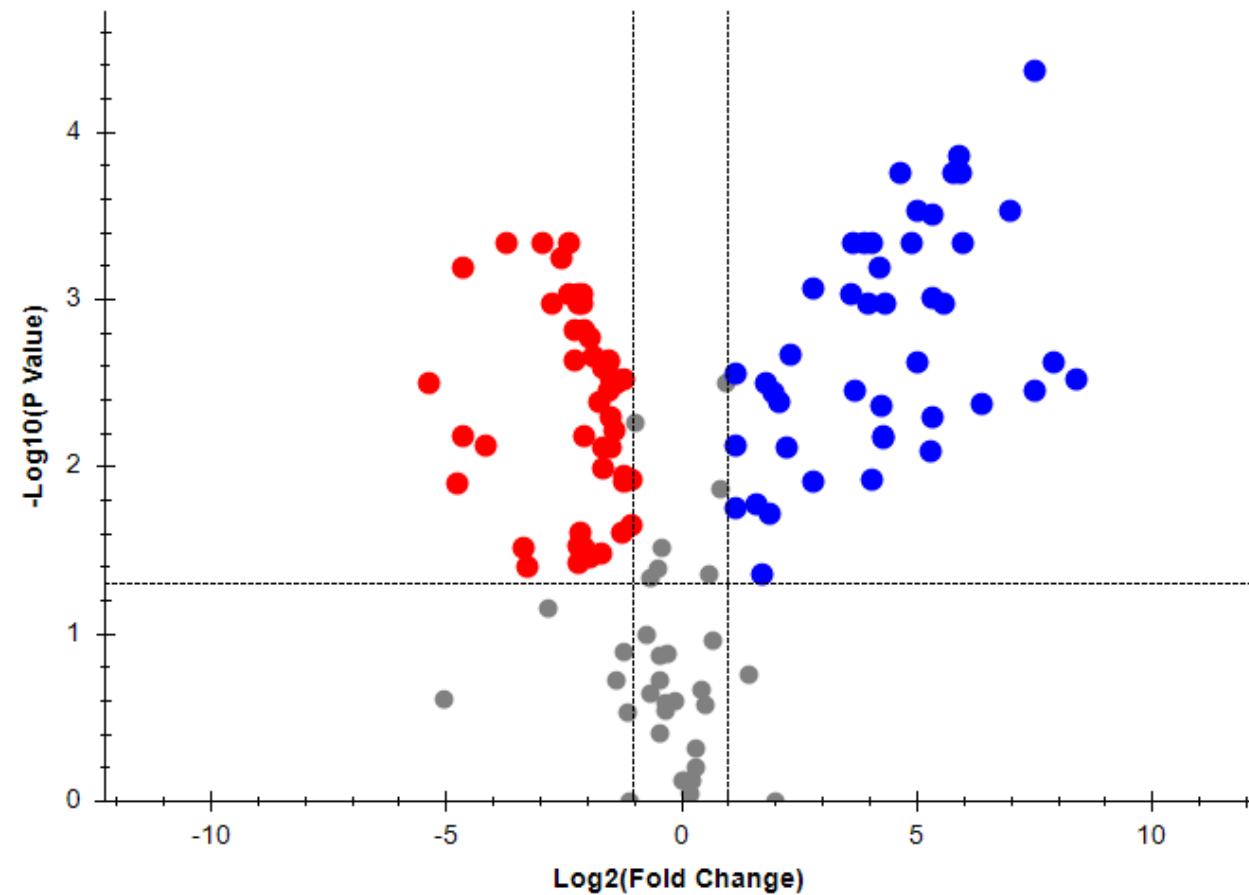


In the SAXN files: Extracellular Vesicles

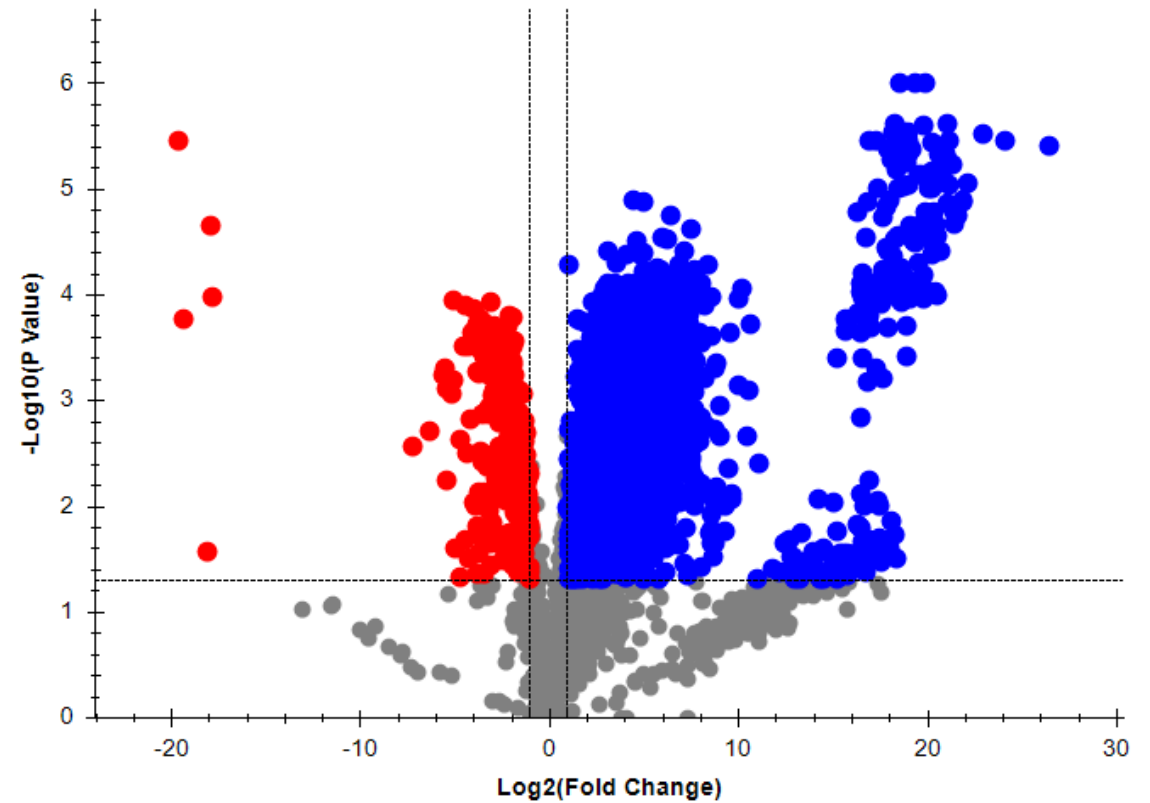


Differentially Abundant Proteins

Without Peak Boundary Imputation



With Peak Boundary Imputation



What are these extreme values?

