Standing in the Way of Proteome Profiling Depth

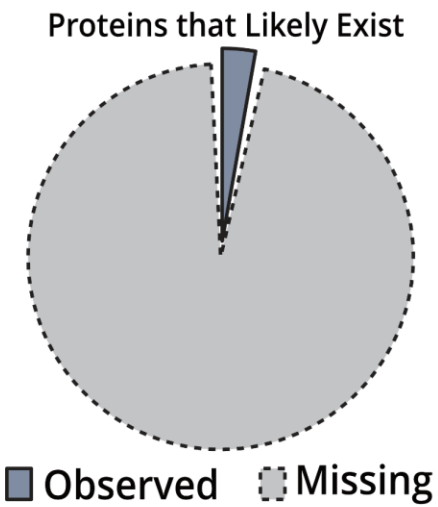
How the Selection of Chromatography and Mass Spectrometry Data Acquisition Limit Our View of the Proteome

Graham Delafield

Ph.D. Thesis Defense

3.24.23

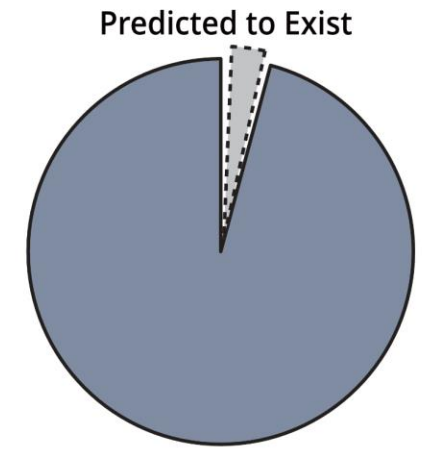
How Proteins Hide

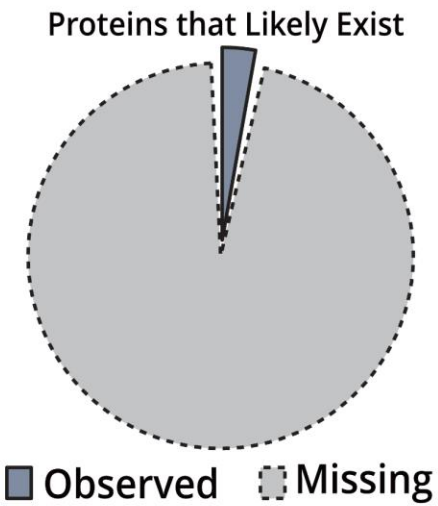


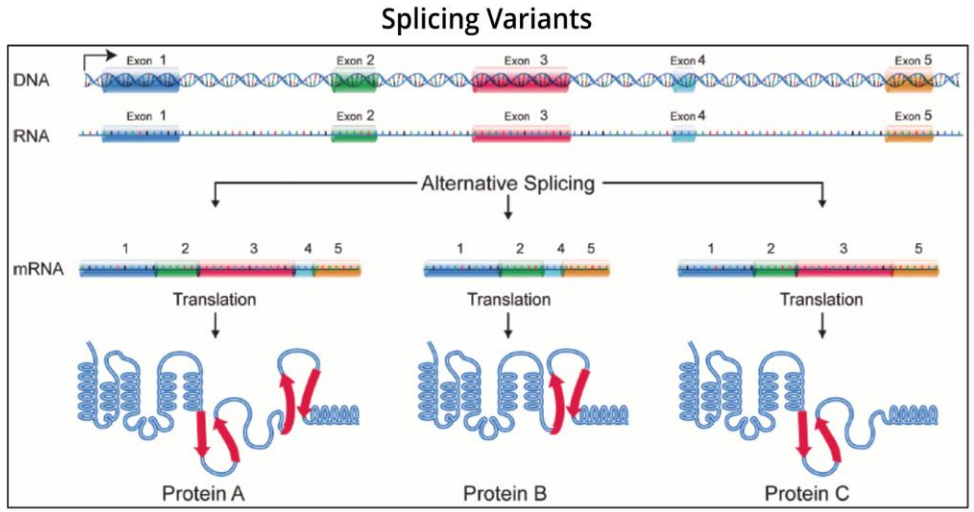
Observed Missing

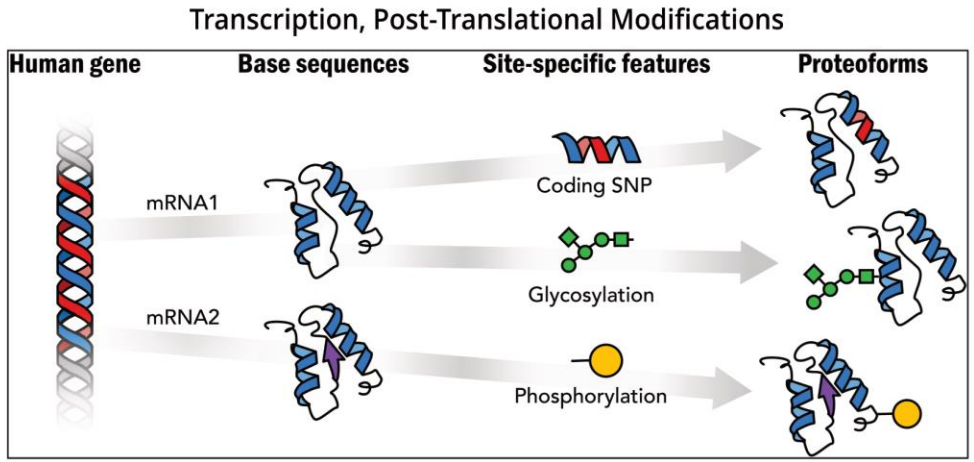
Smith, L. M., et al. (2021). *Science Advances.* **7**(46).
Sutto, L., et al. (2015). *Proceedings of the National Academy of Sciences.* **112**(44): 13567-13572.

How Proteins Hide









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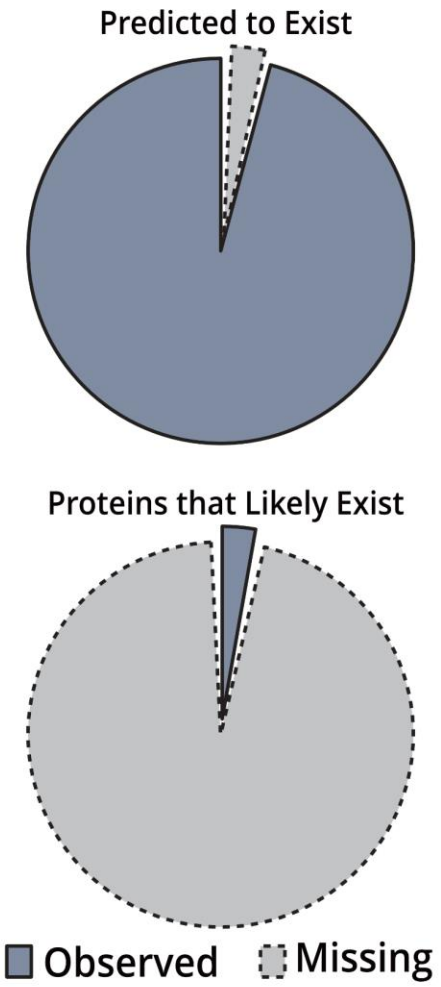

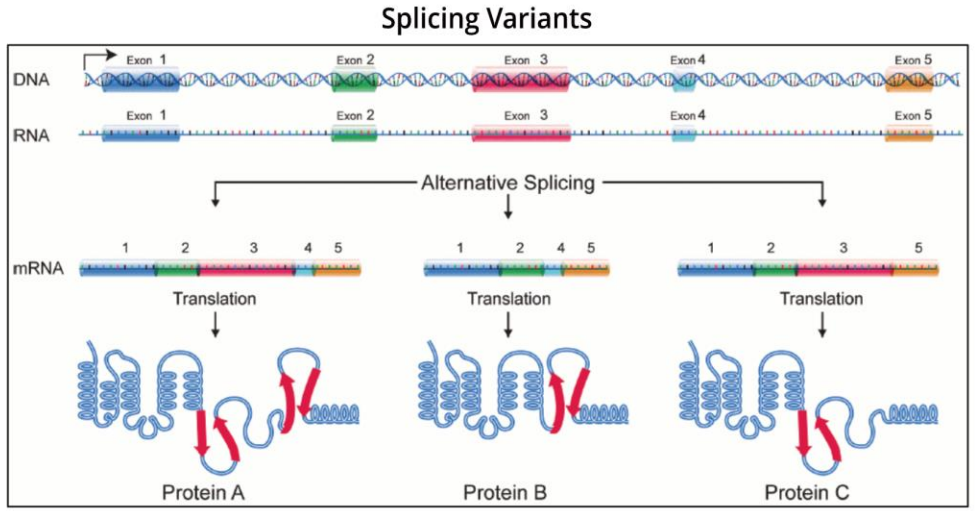

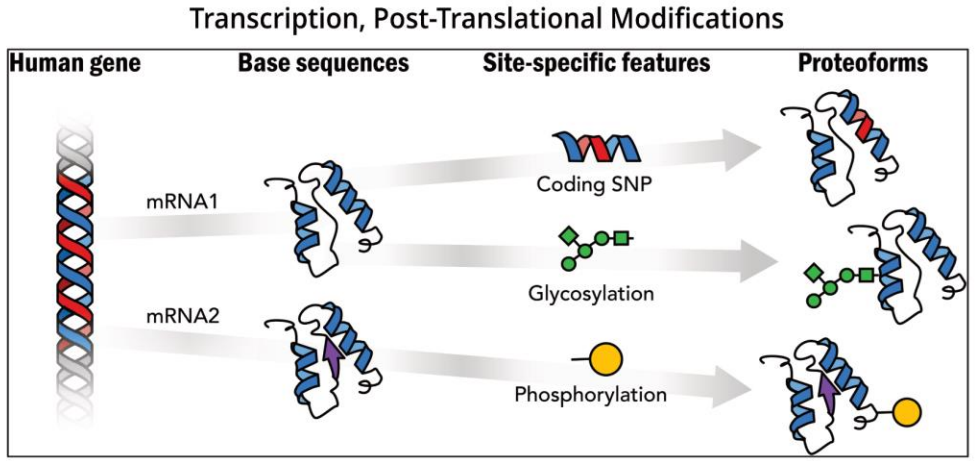

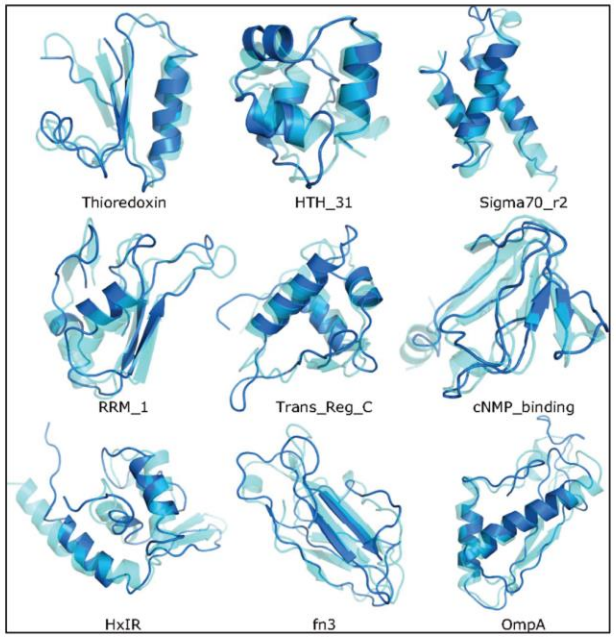

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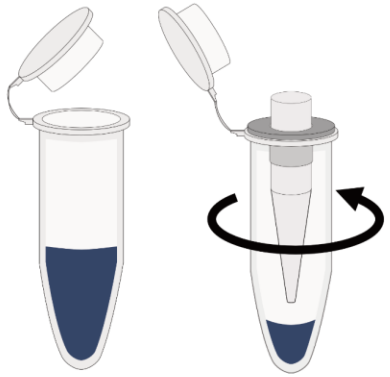
Mass Spectrometry-Based Proteomics

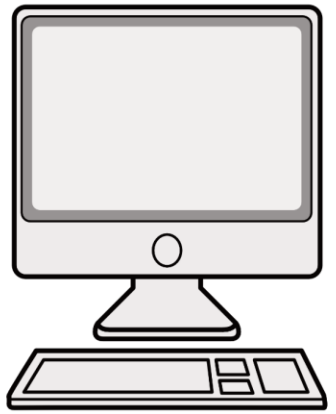
Preparation



Separation

Detection

Analysis



Mass Spectrometry-Based Proteomics

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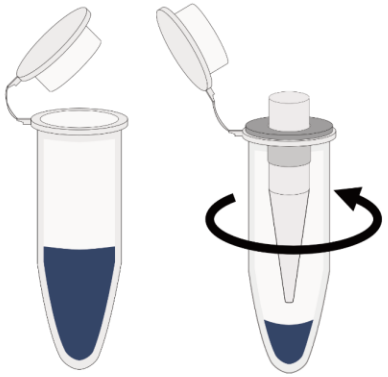

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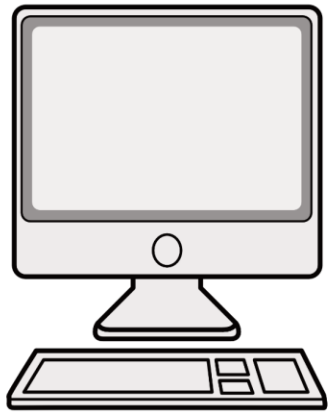
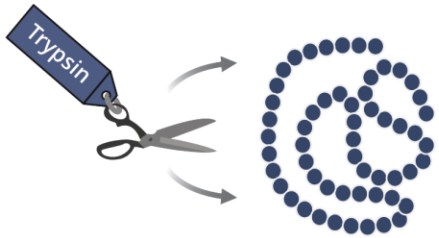
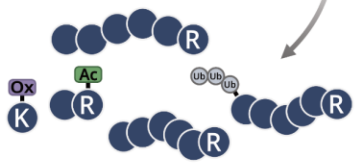


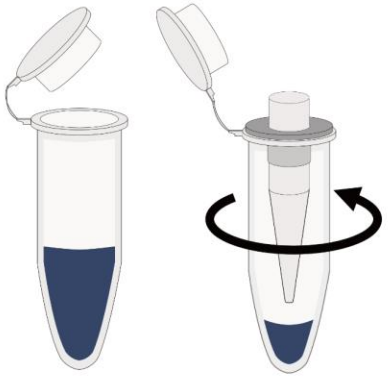




Peptides

Mass Spectrometry-Based Proteomics

Preparation



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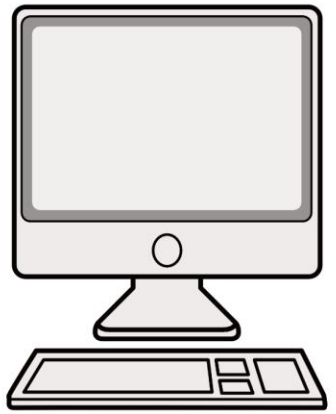

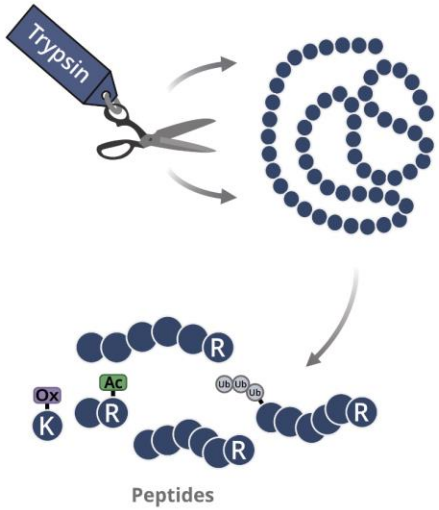

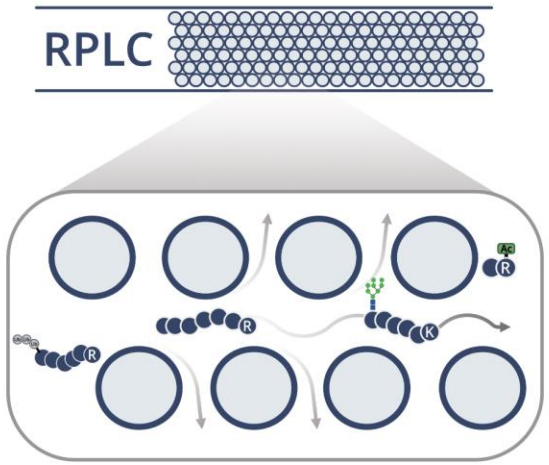

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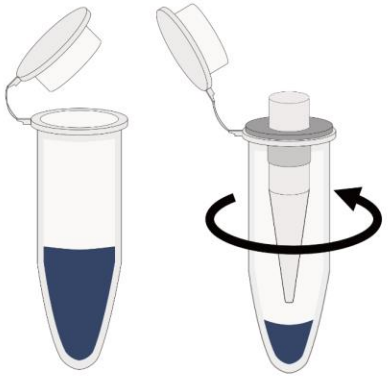

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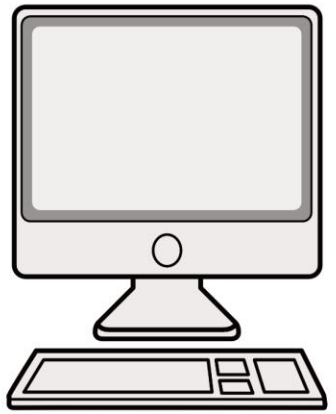

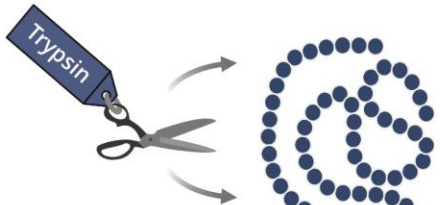

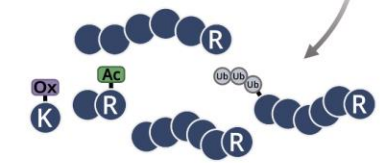

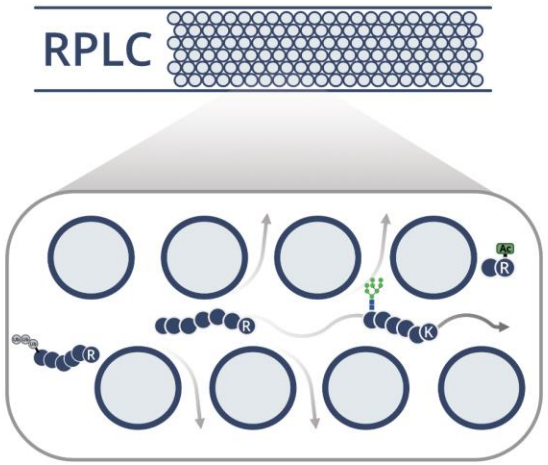

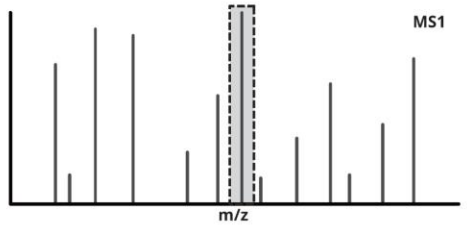

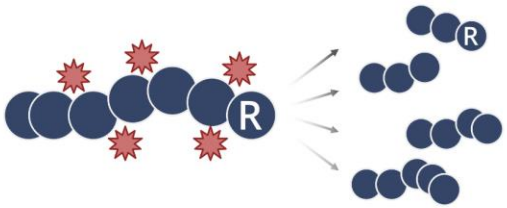

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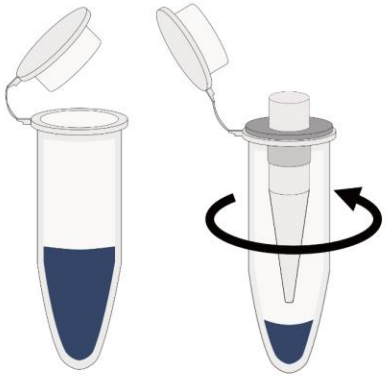

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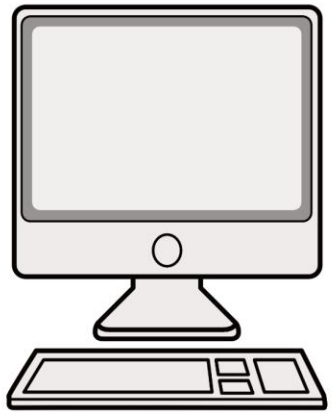

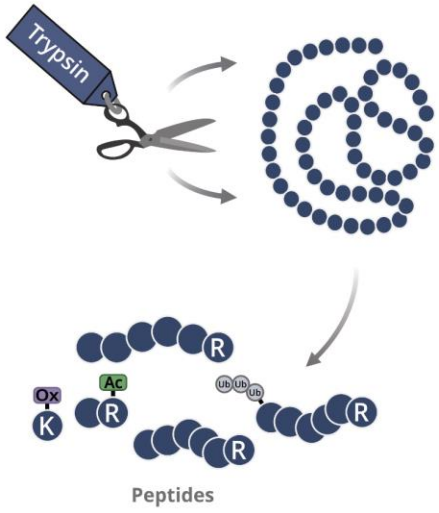

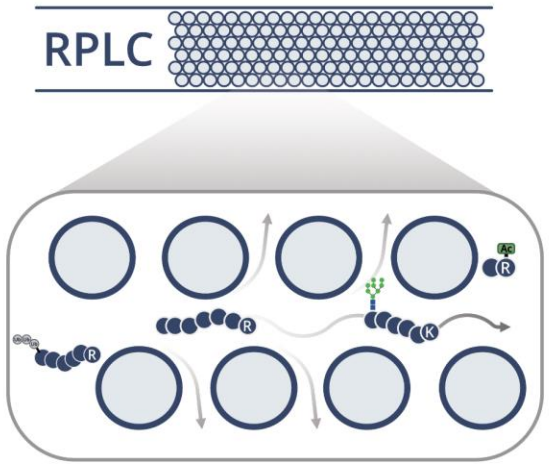

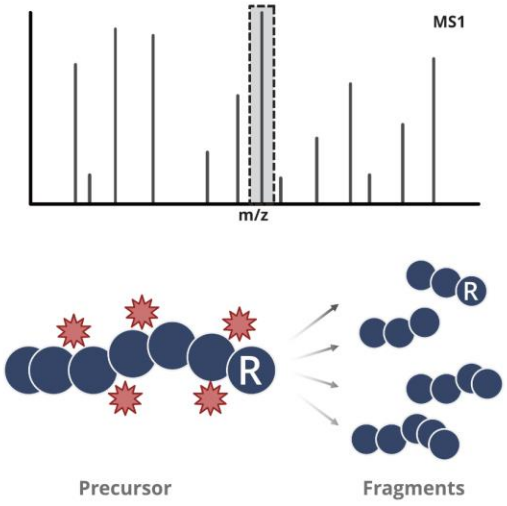

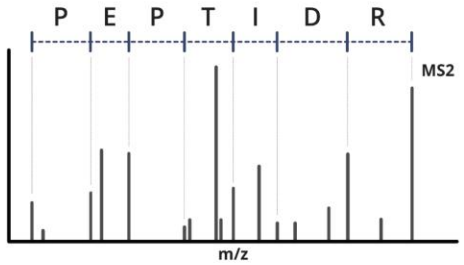

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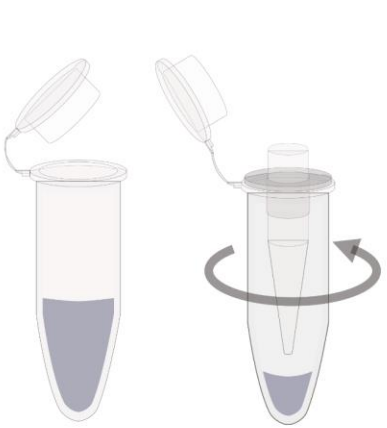
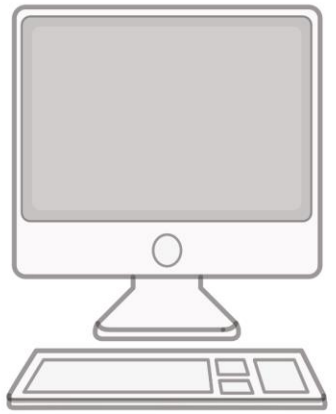
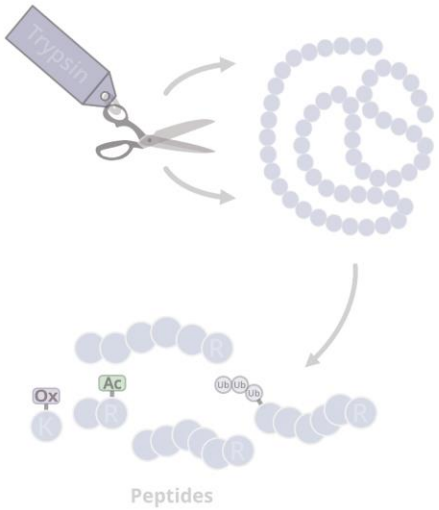
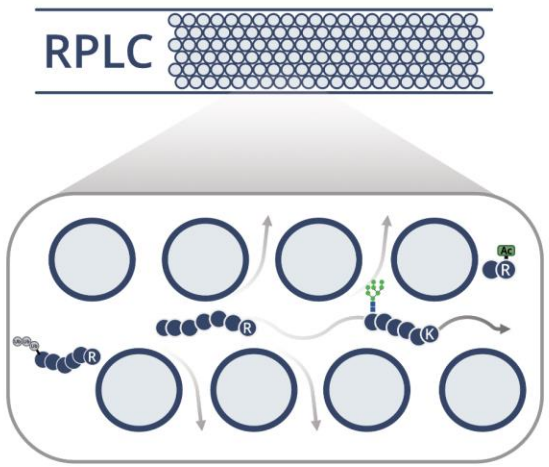
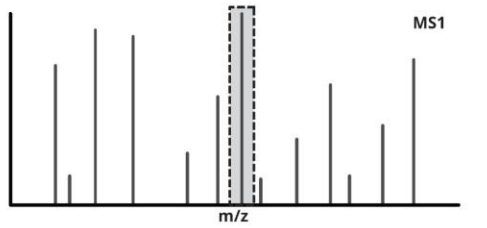
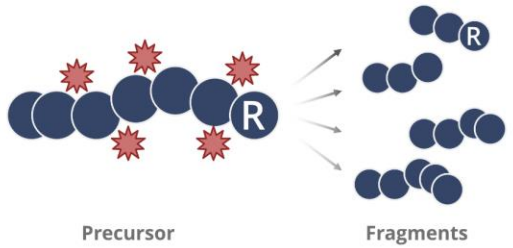


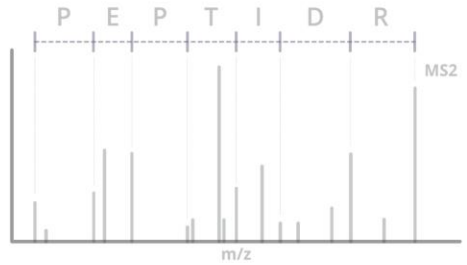




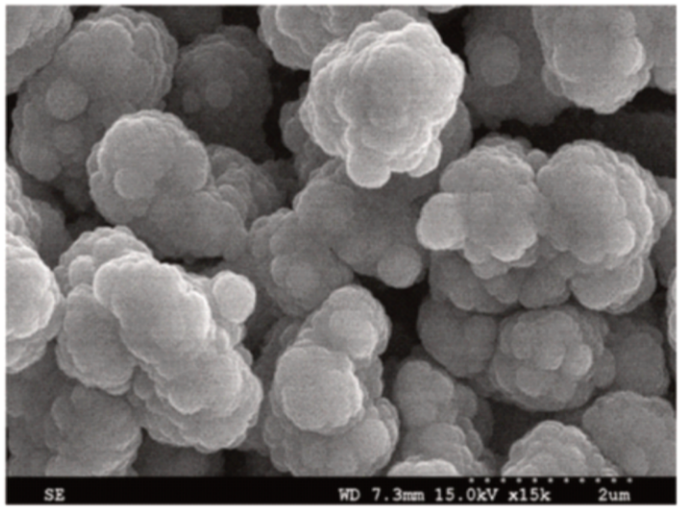








Utilizing Alternative Separations



RPLC C18

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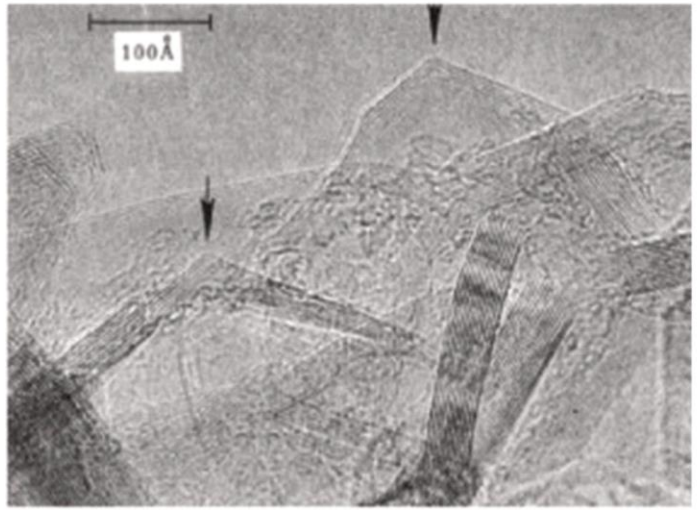

PGC

Porous Graphitic Carbon

- Retention based on polar and electrostatic interactions
- Solvent flexibility, amenable to traditional water-organic systems
- Retention orthogonal to RPLC without the need for phase changce
- Reproducible, available

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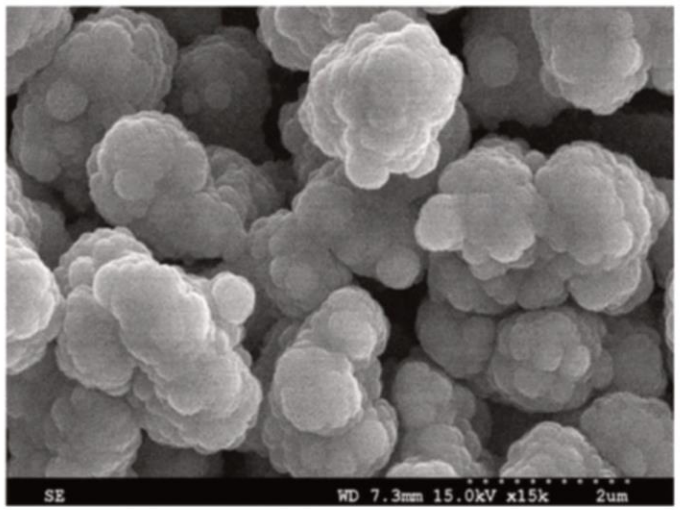

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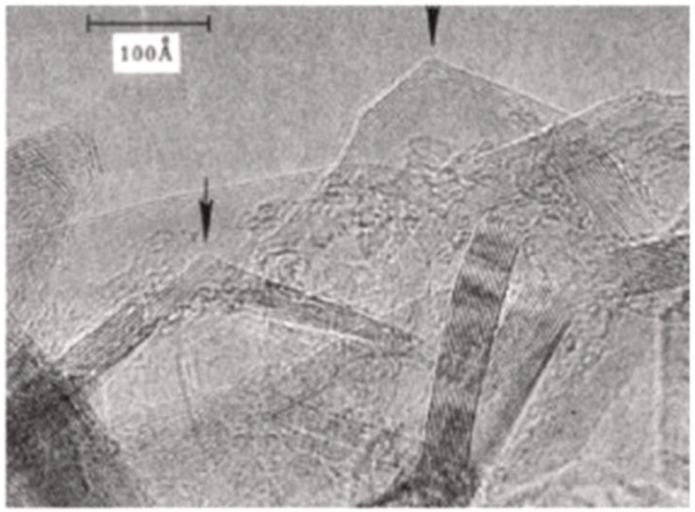

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Does PGC offer an amenable way to enhance proteome access?

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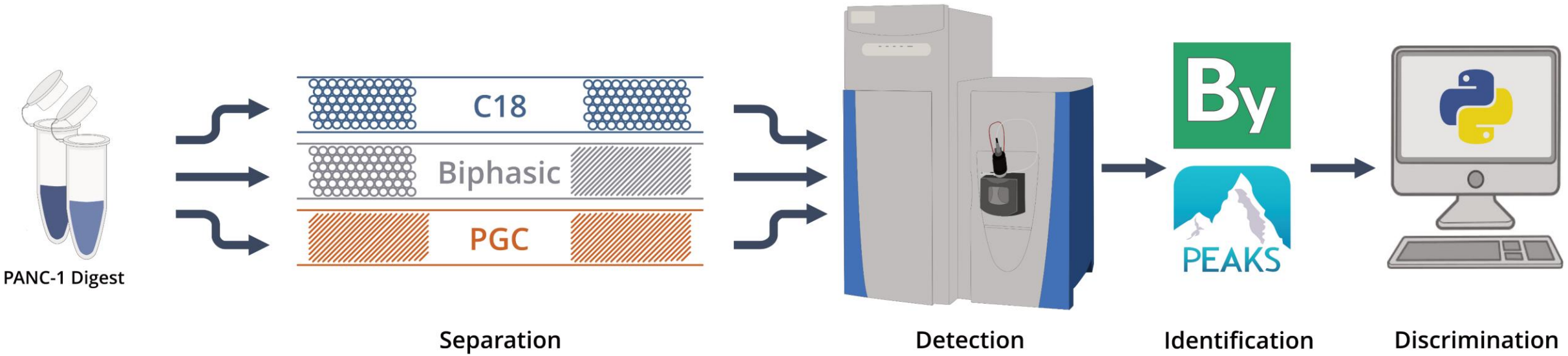
PGC-Based Shotgun Proteomics



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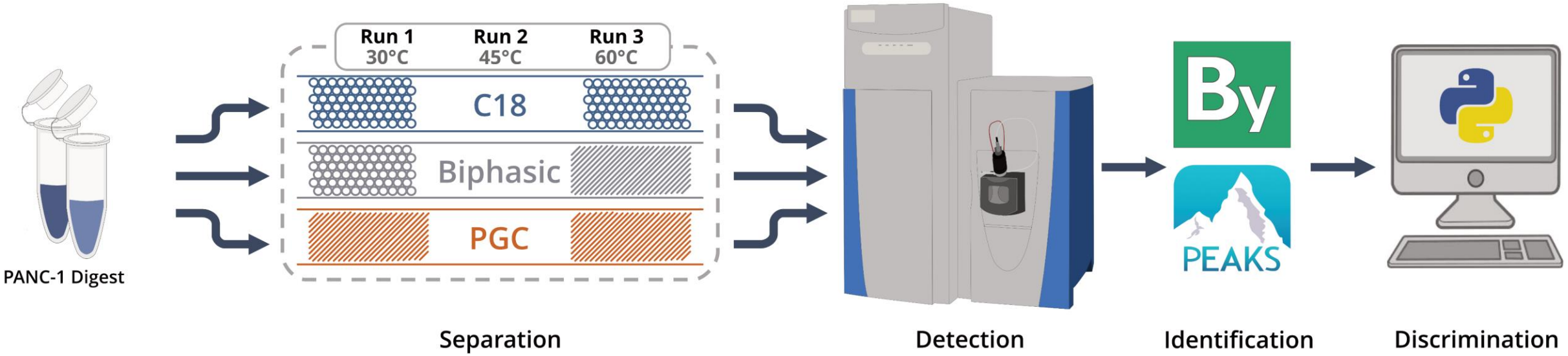

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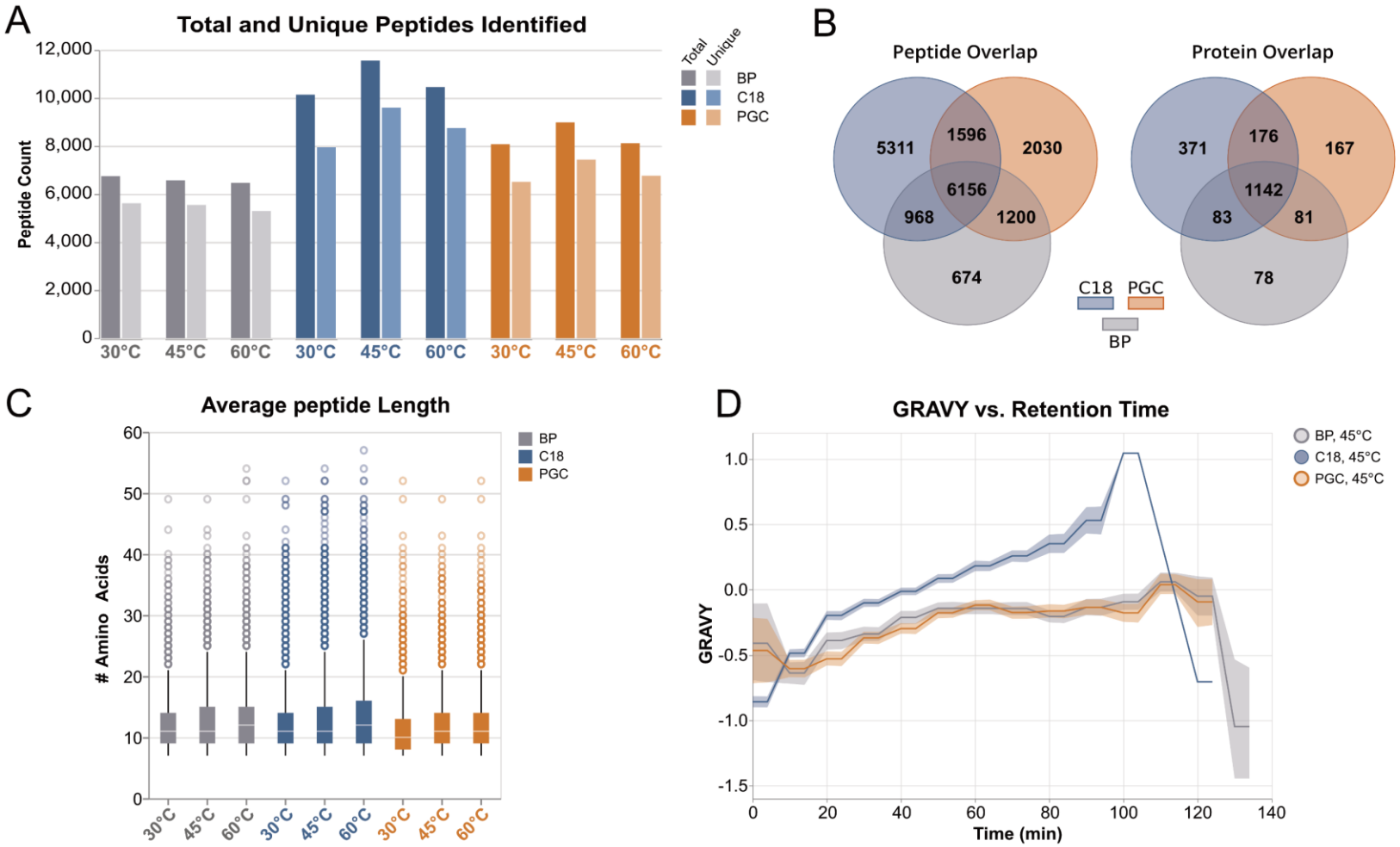
PGC Complements RPLC Analyses



Key Results

- PGC separations provide additional 15-20% peptide and protein identifications
- Preferential retention of smaller, hydrophilic peptides
- Complementary sample coverage though not orthogonal

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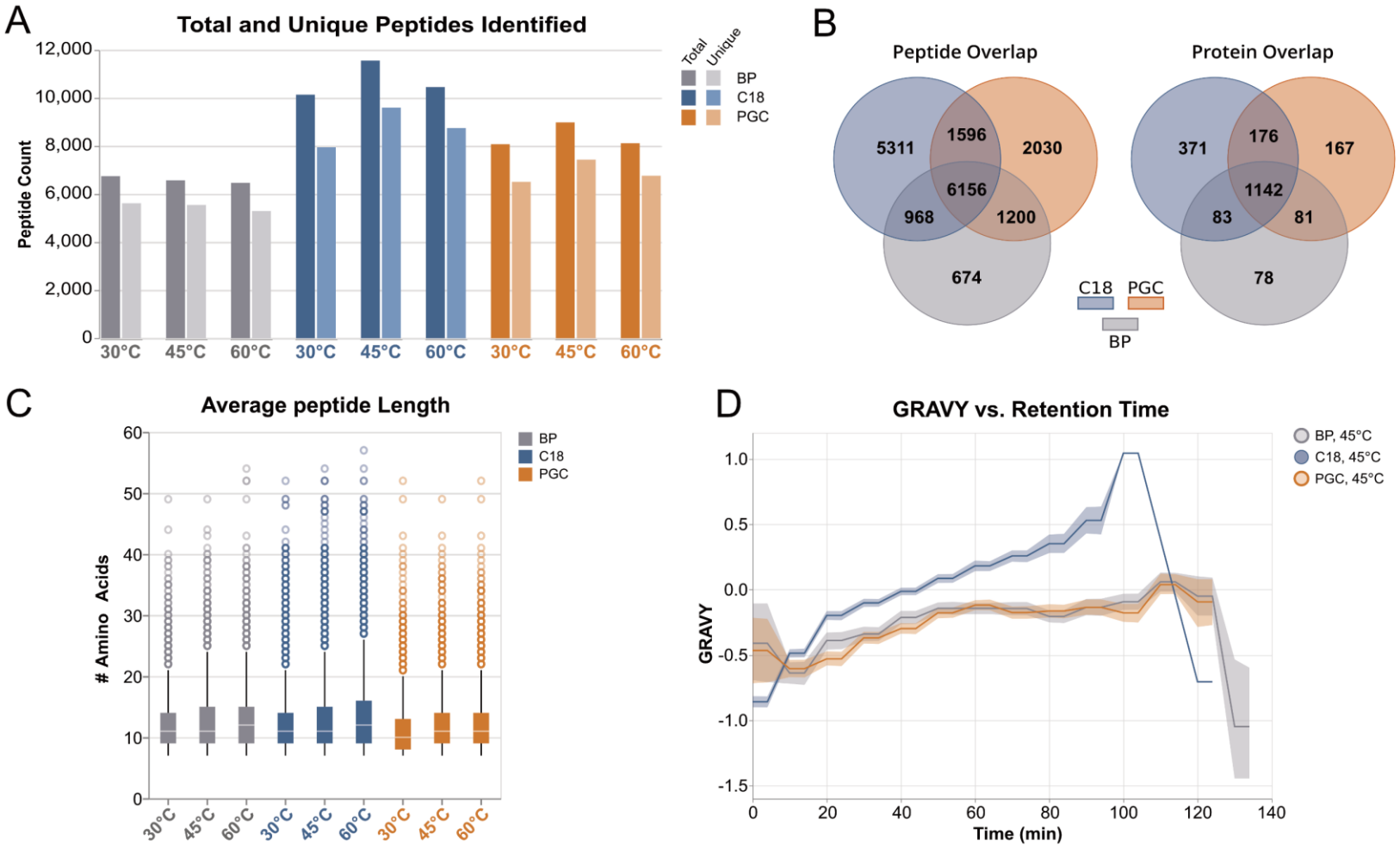

Key Results

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Knowing each separation provides access to different sample components, stepping away from shotgun proteomics will increase efficiency and decrease redundancy.

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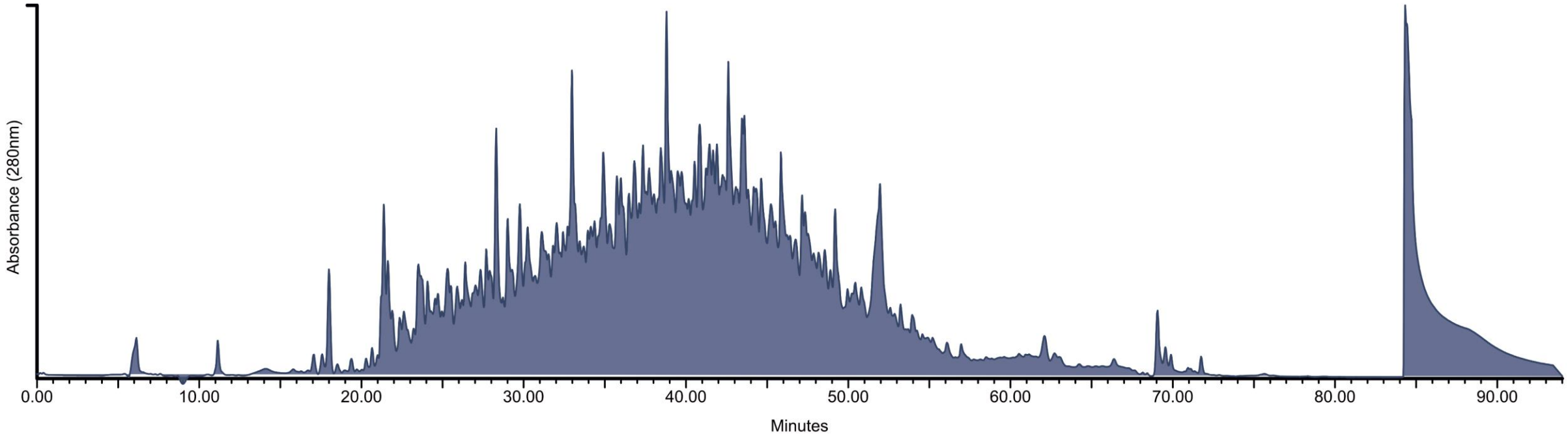
More Efficient Use of PGC-RPLC



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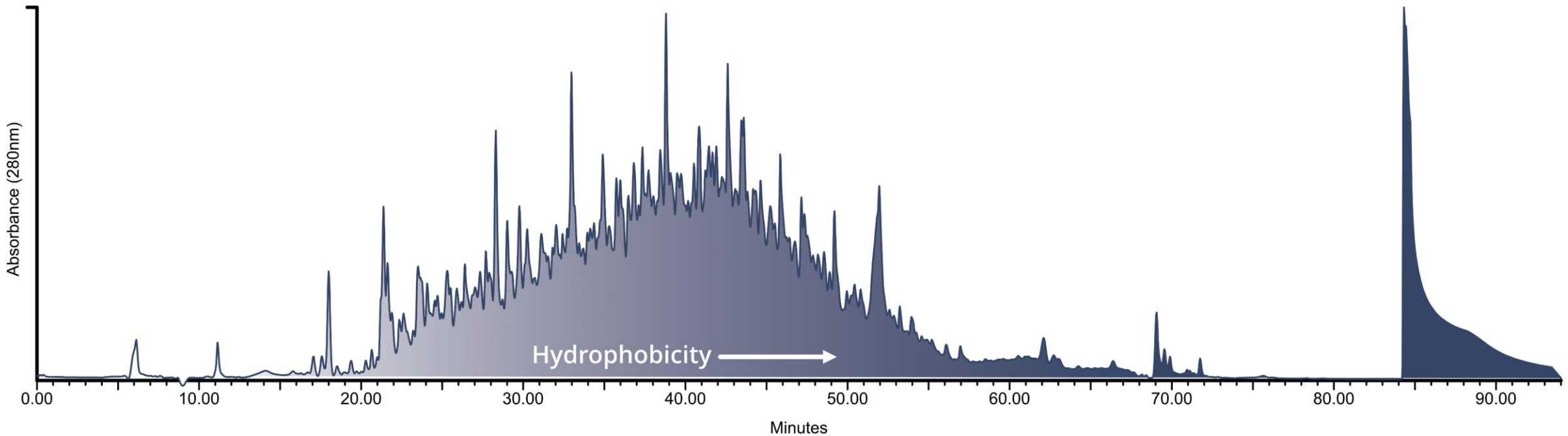

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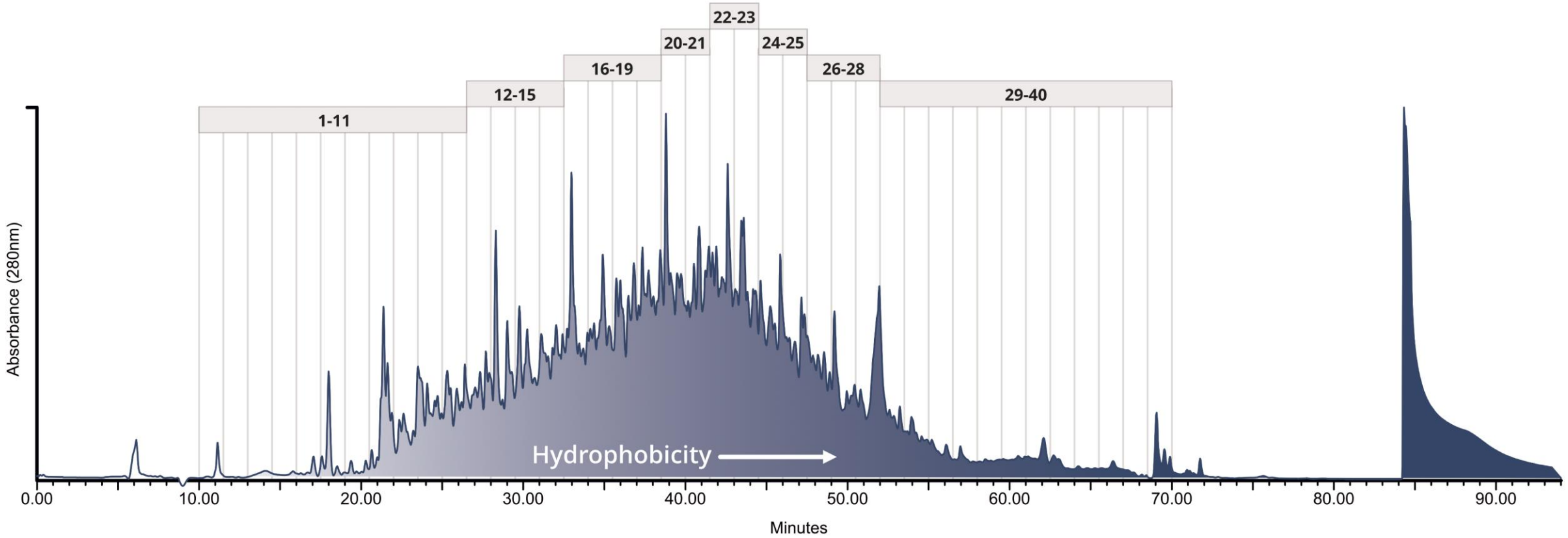

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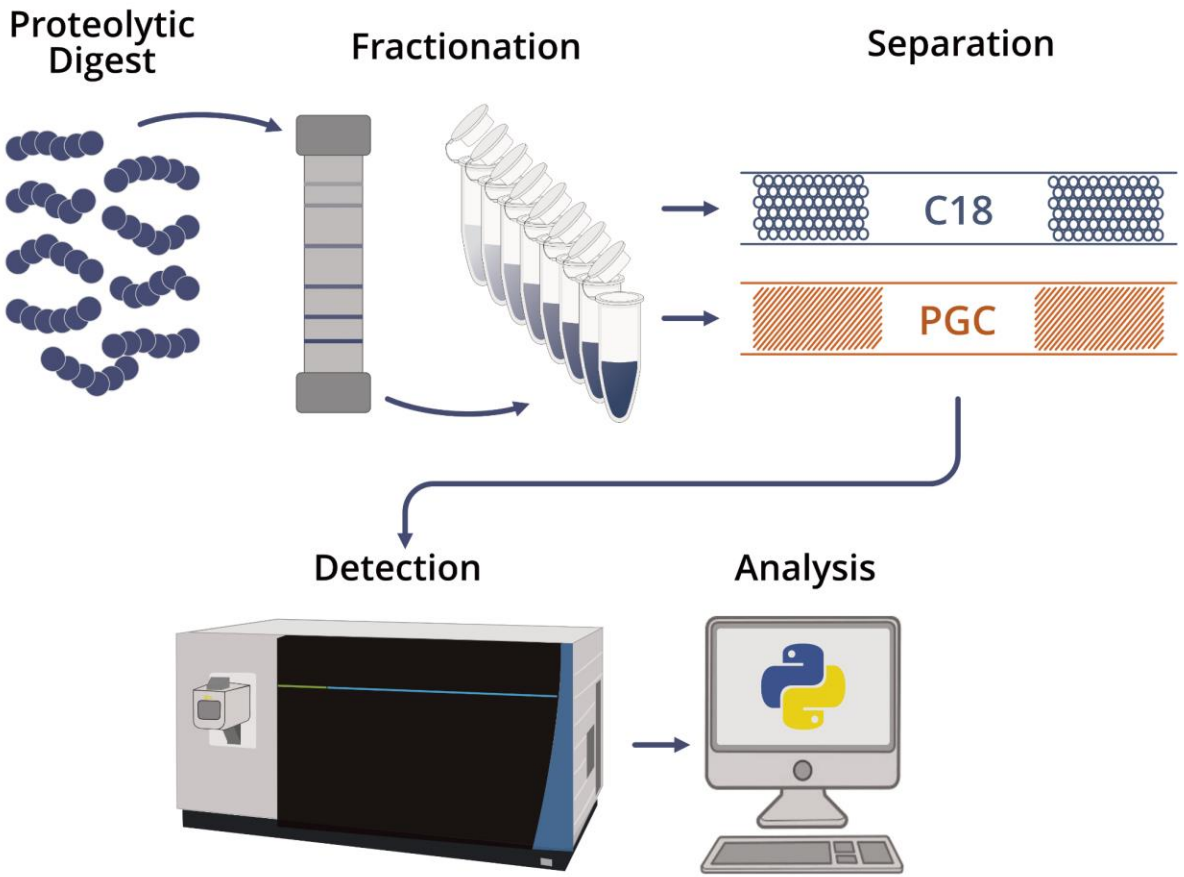
PGC is Competitive at Early Timepoints



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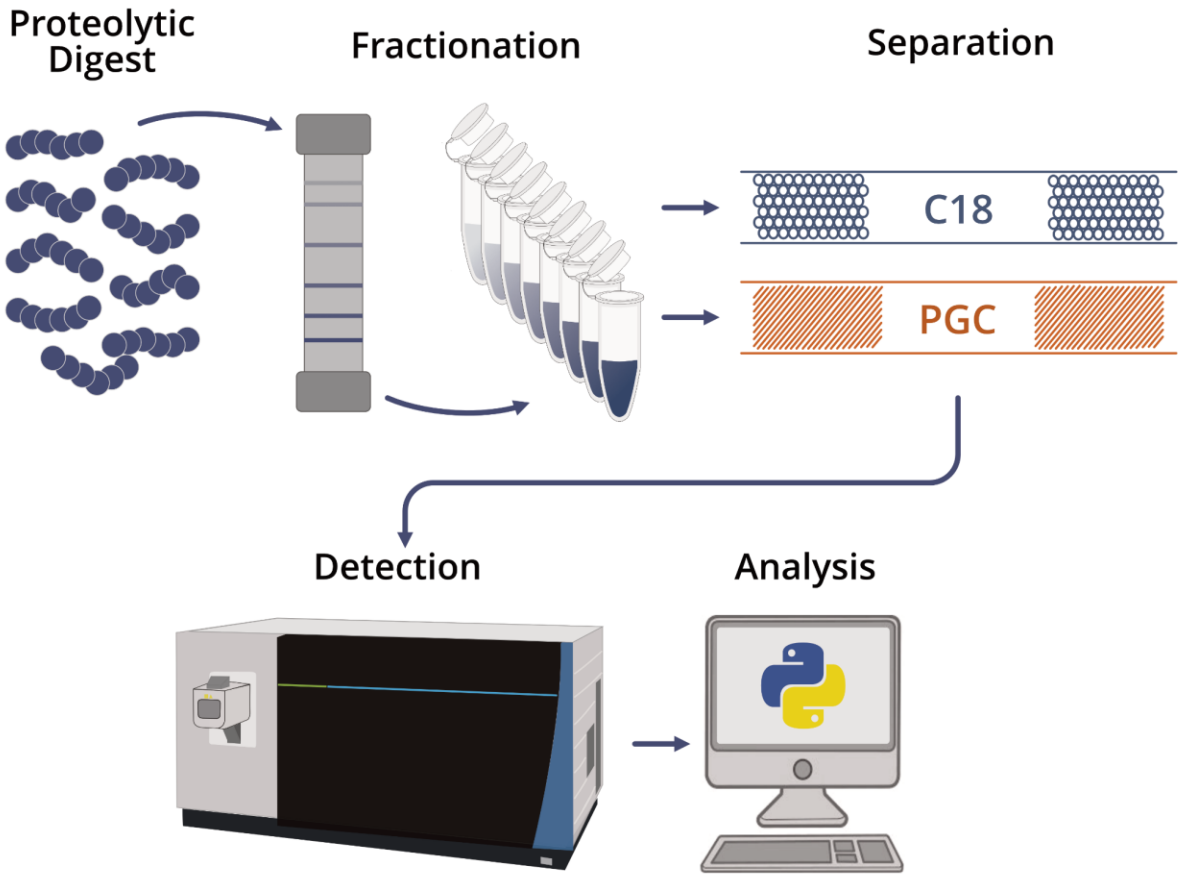
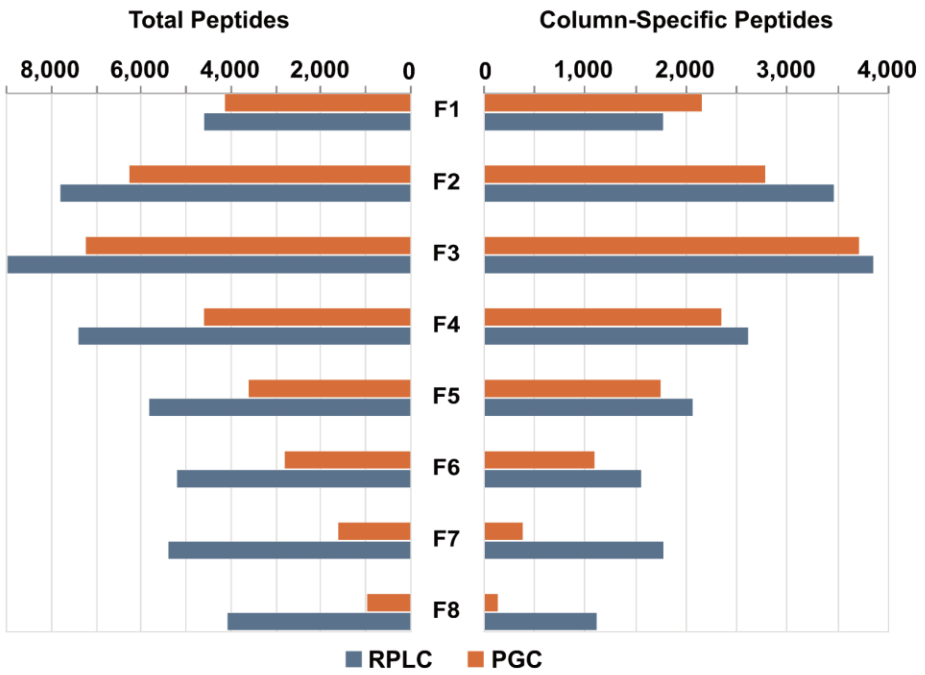
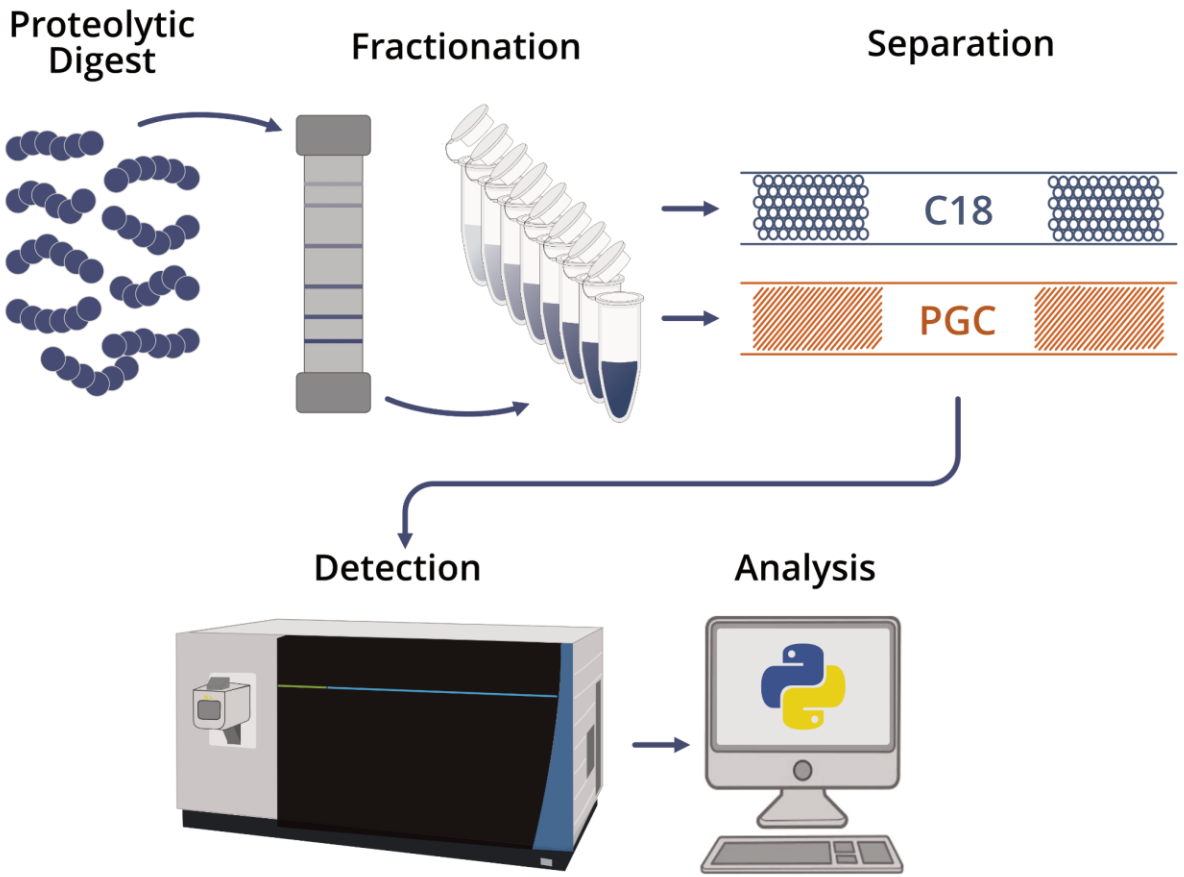
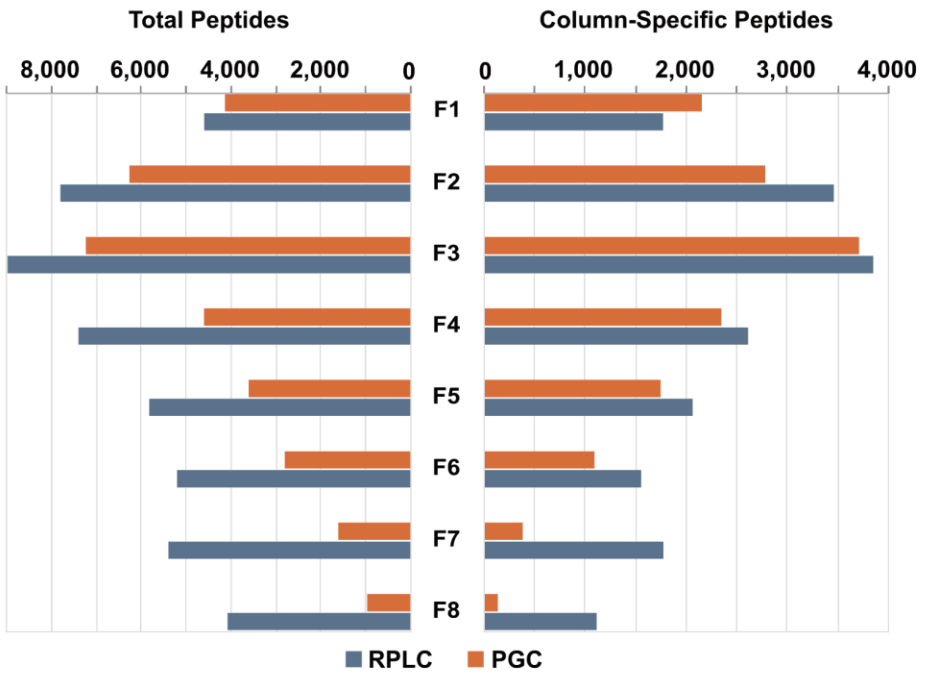


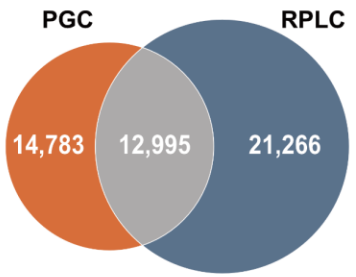


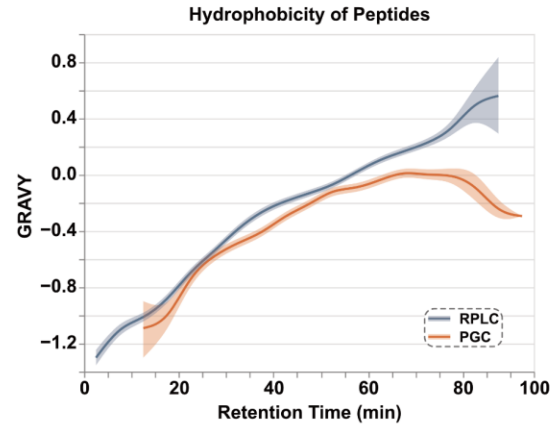
Delafield, D. G., et al. (2023). In Submission.

PGC is Competitive at Early Timepoints



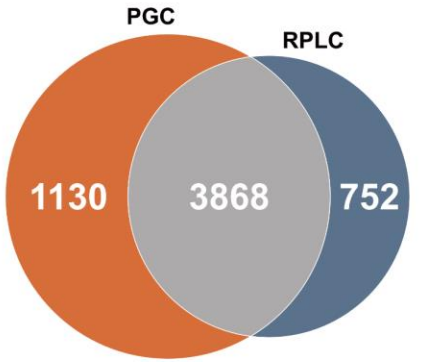


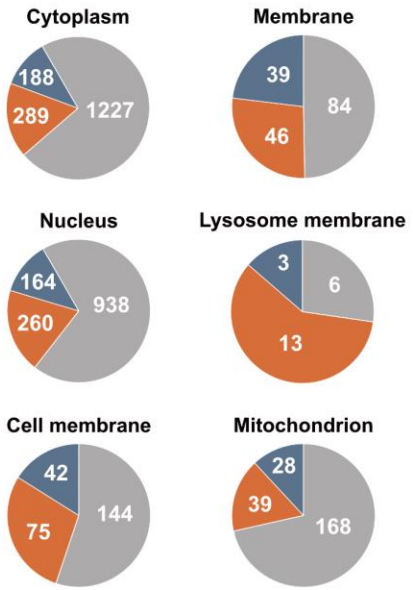




Delafield, D. G., et al. (2023). In Submission.

Greater Protein Identification, Regardless of Depth





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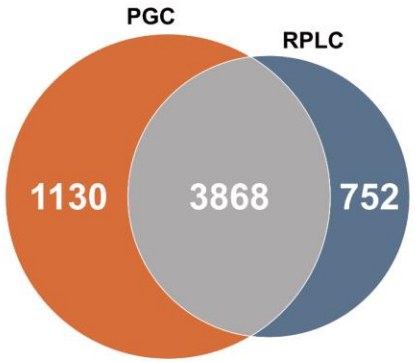

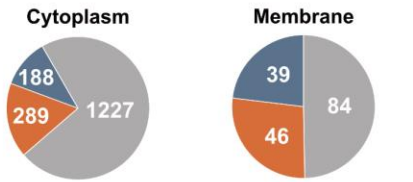

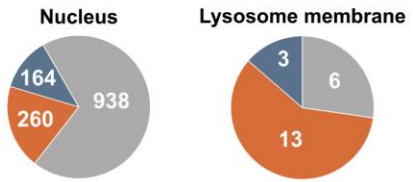

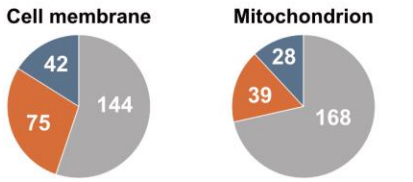

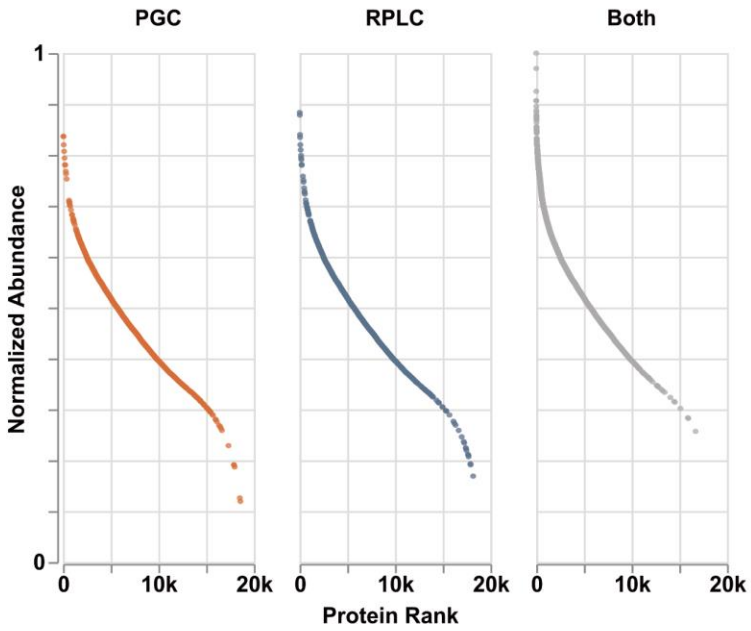

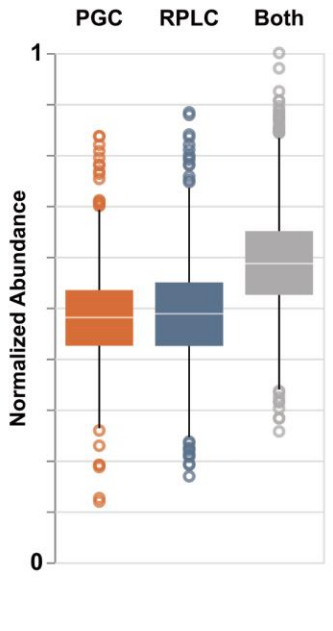

Delafield, D. G., et al. (2023). In Submission.

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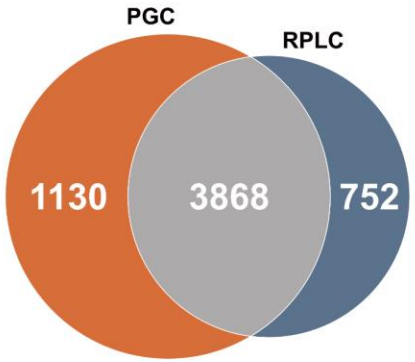
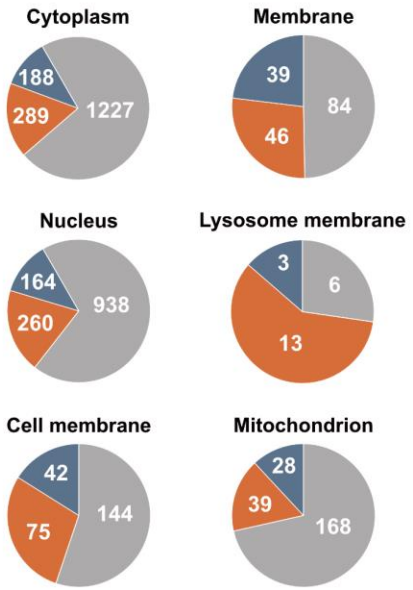
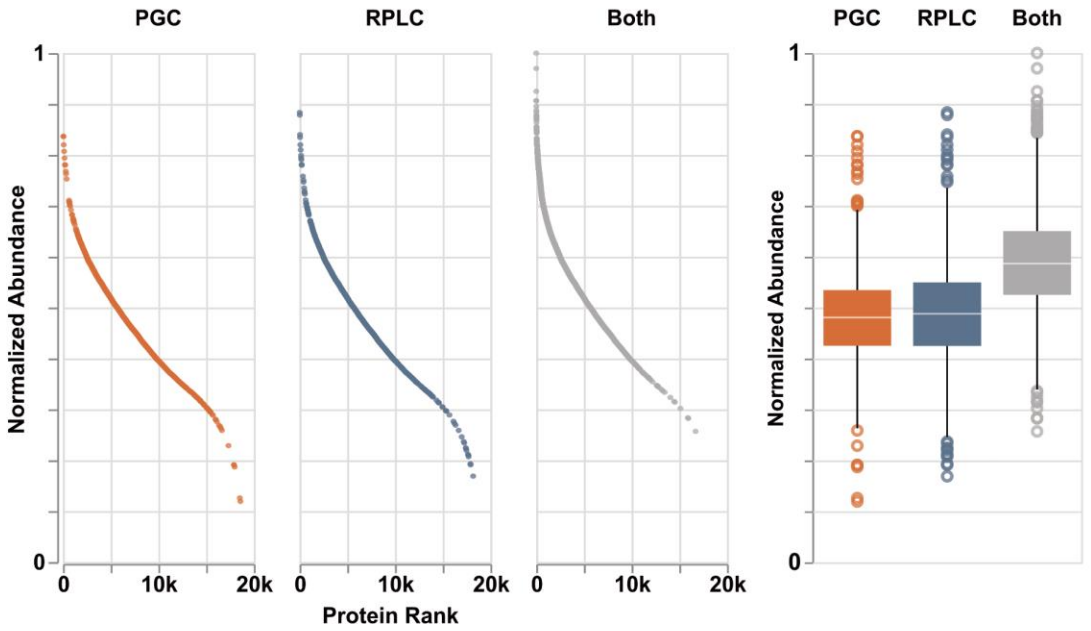
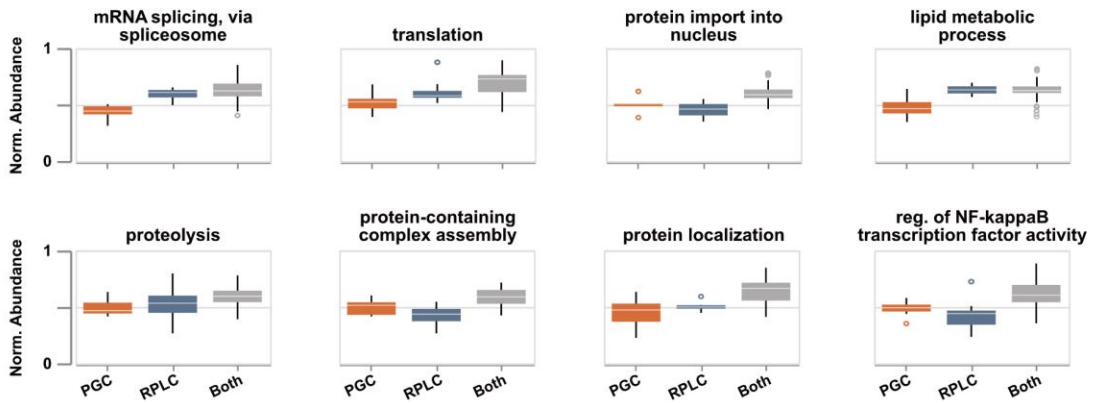


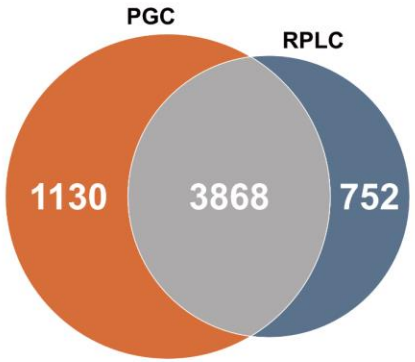


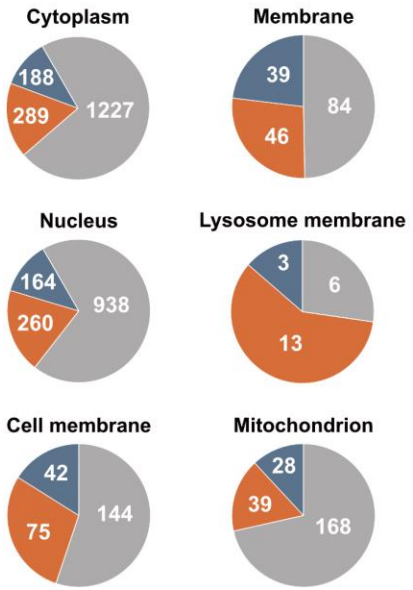


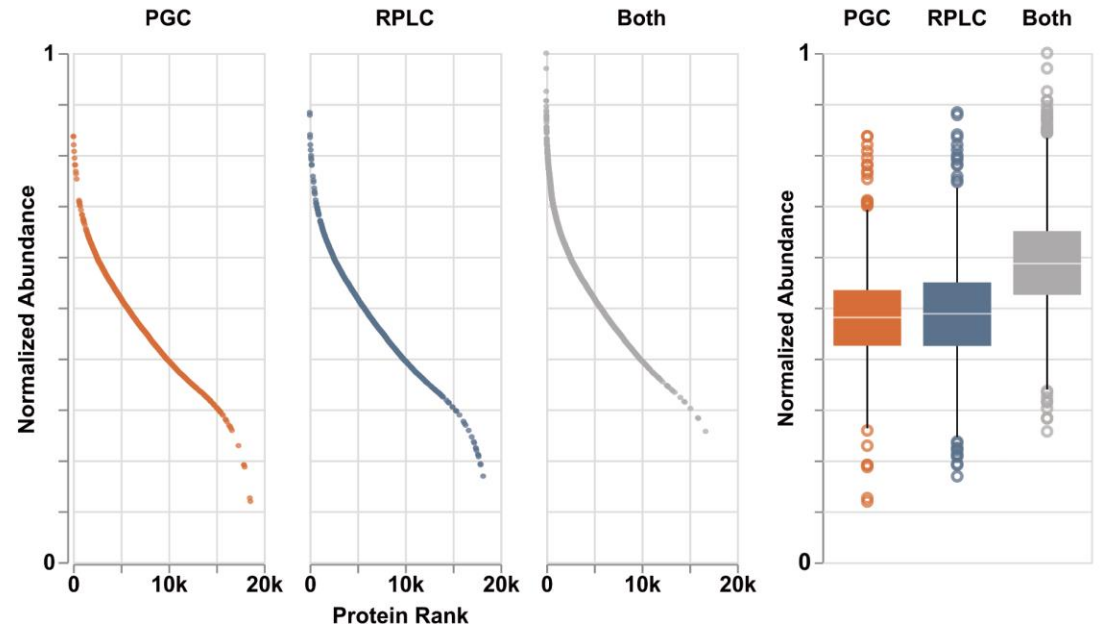


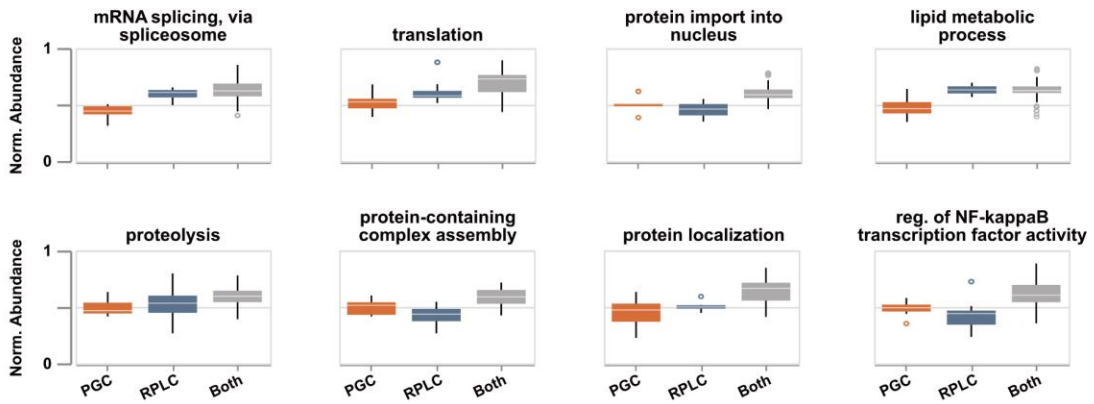
Delafield, D. G., et al. (2023). In Submission.

Greater Protein Identification, Regardless of Depth







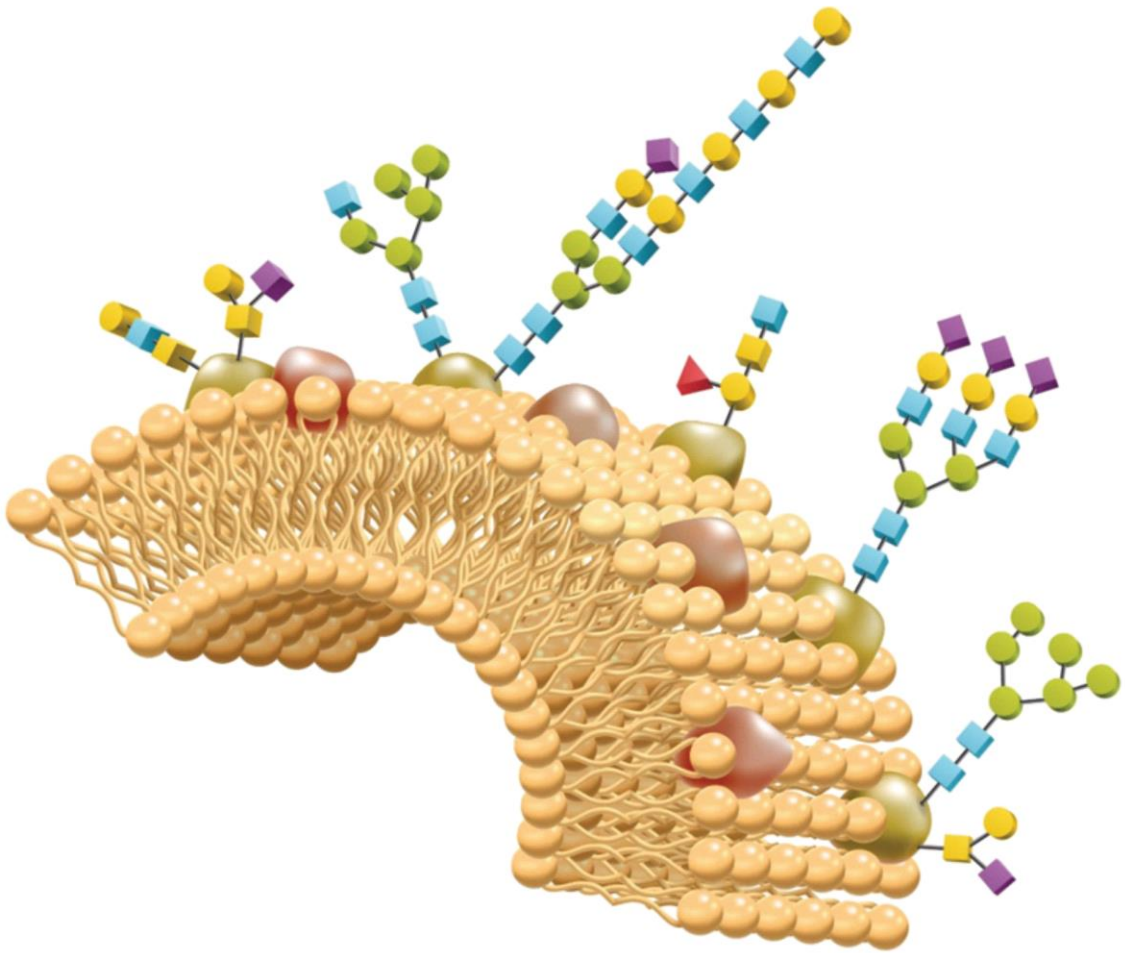


PGC for Proteomics

- With optimization, PGC improves peptide and protein identification by up to 40%
- Complementary retention regardless of sample complexity
- Profiling breadth readily achieved, depth is on the horizon

Delafield, D. G., et al. (2023). In Submission.

Glycoproteomics



Function

Cellular communication and immune response

Intrinsic/extrinsic signaling pathways

Protein folding and viability

Disease

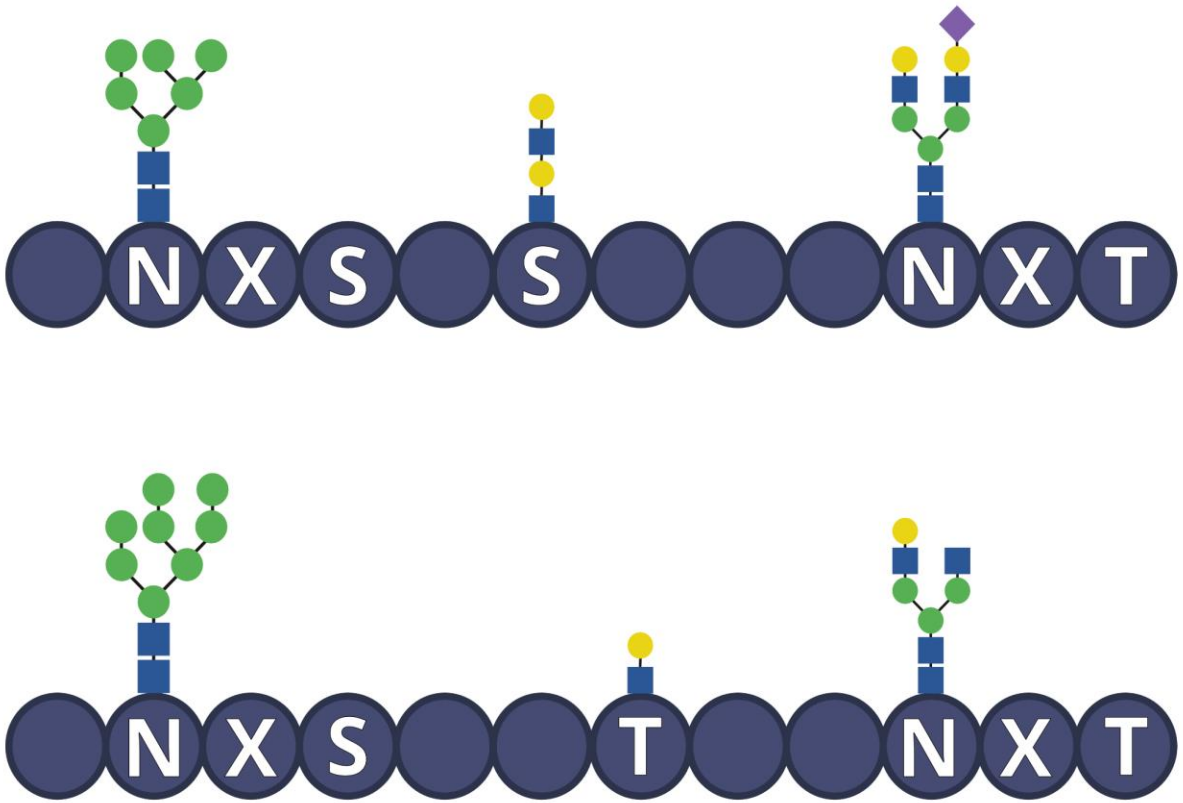
Target for pathogen invasion

Altered expression during disease propagation

Aberrant profiles across numerous disease

Varki, A., *Essentials of glycobiology*. 3rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y.
Arnold, J. N., et al. 2007. *Annu Rev Immunol.* 25, 21-50.
Lemmers, R. F. H., et al. 2017. *Biochimica et Biophysica Acta*. 1861 (9), 2240-2249.

Glycoproteomics



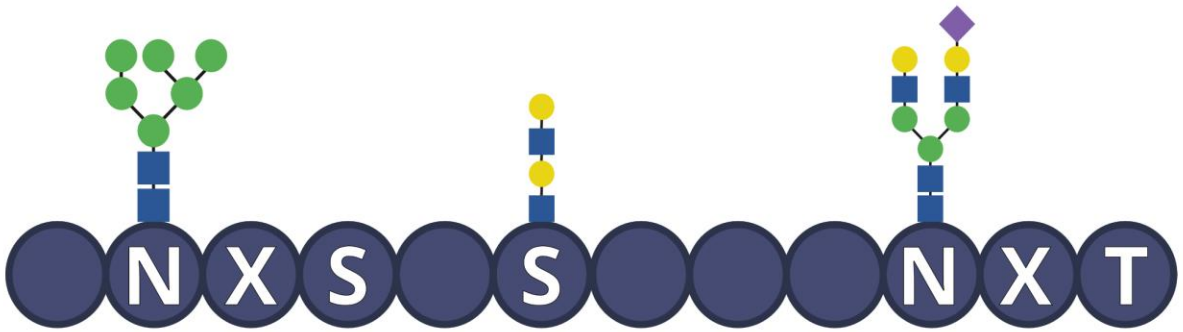
Challenges

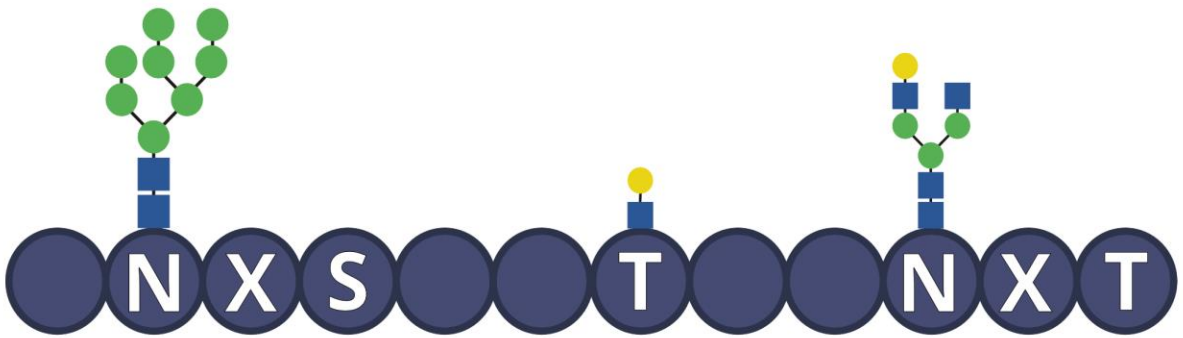
Biological

- **Class**
  - N-linked or O-linked
- **Composition**
  - 12 commonly occurring monosaccharides
- **Structure**
  - α/β orientation
  - Connectivity

Varki, A., *Essentials of glycobiology*. 3rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y.

Glycoproteomics





Challenges

Biological

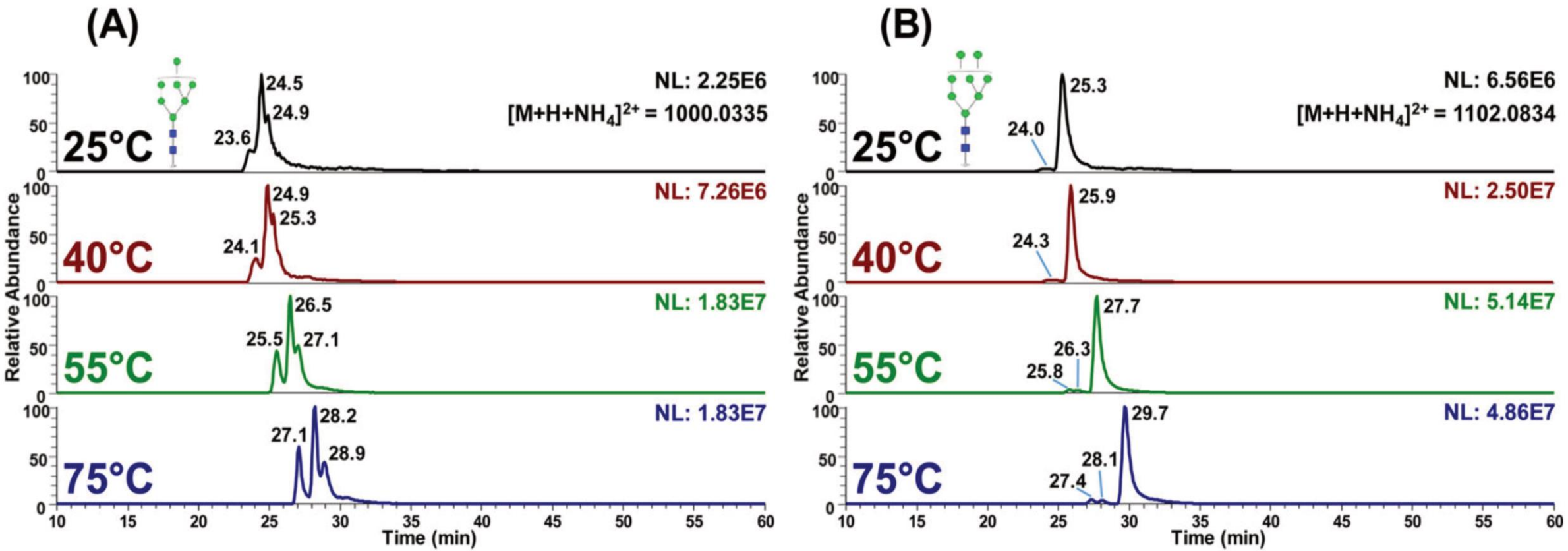
- **Class**
  - N-linked or O-linked
- **Composition**
  - 12 commonly occurring monosaccharides
- **Structure**
  - α/β orientation
  - Connectivity

Analytical

- **Retention**
  - Hydrophilic moieity
- **Charge suppression**
  - Outcompeted by hydrophobic, basic peptides
- **Heterogeneity**
  - Site occupation
  - Structure

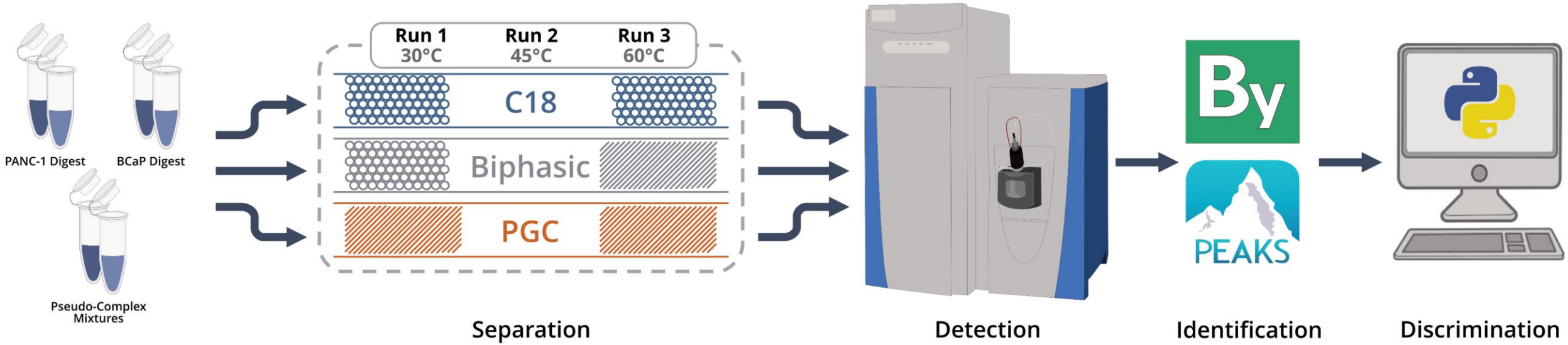
Varki, A., *Essentials of glycobiology*. 3rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y.

PGC for Glycoproteomics



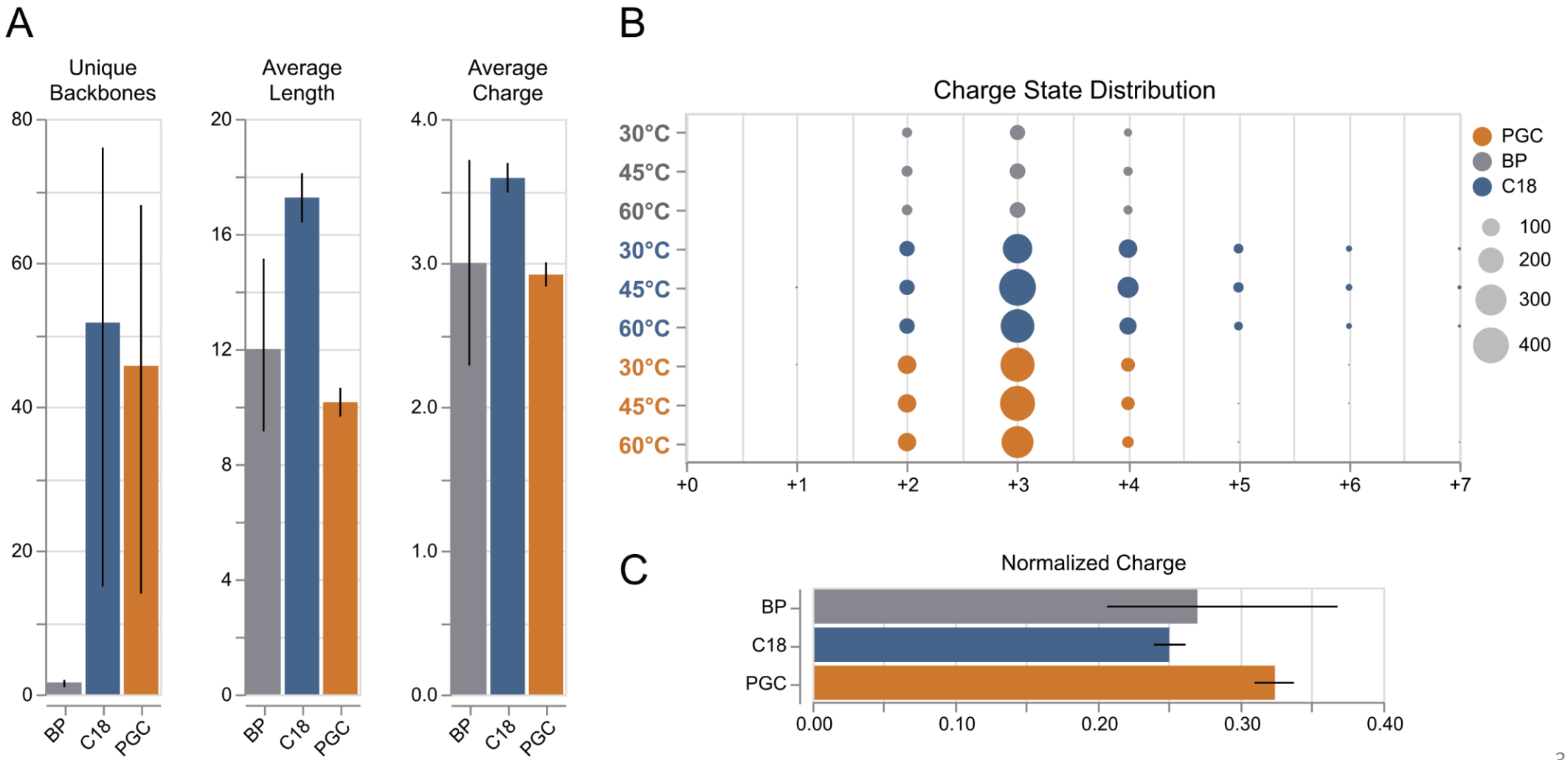
Zhu, R., et al. (2020) *Analytical Chemistry.* 92 (14), 9556-9565.
Zhou, S., et al. (2017) *Analytical Chemistry.* 2017, 89 (12), 6590-6597.

PGC-Based Shotgun Proteomics



Delafield, D. G., et al. (2022). *Anal Bioanal Chem*. 414(18):5461-5472.

Glycopeptide Character



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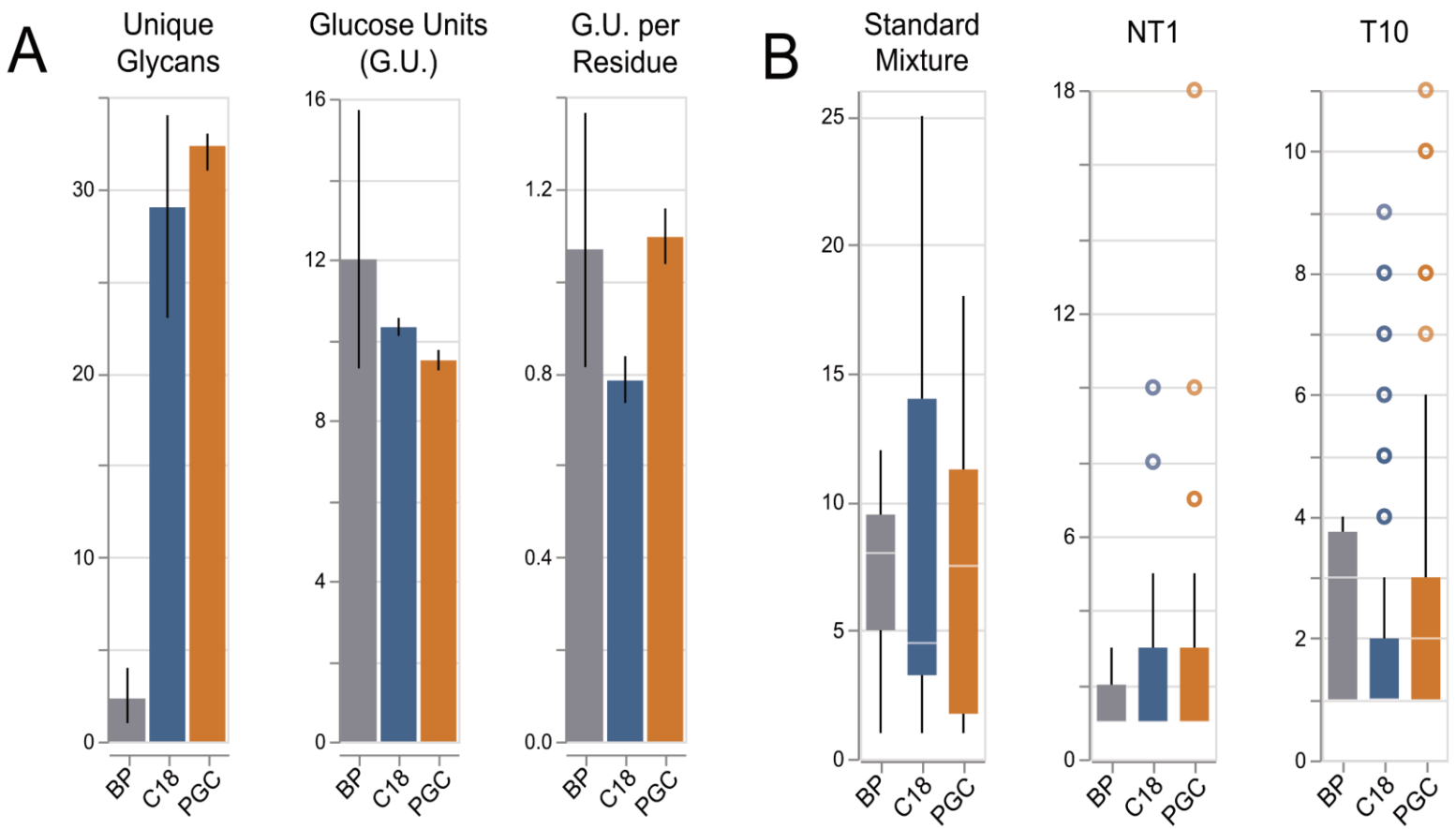
Glycopeptide Character

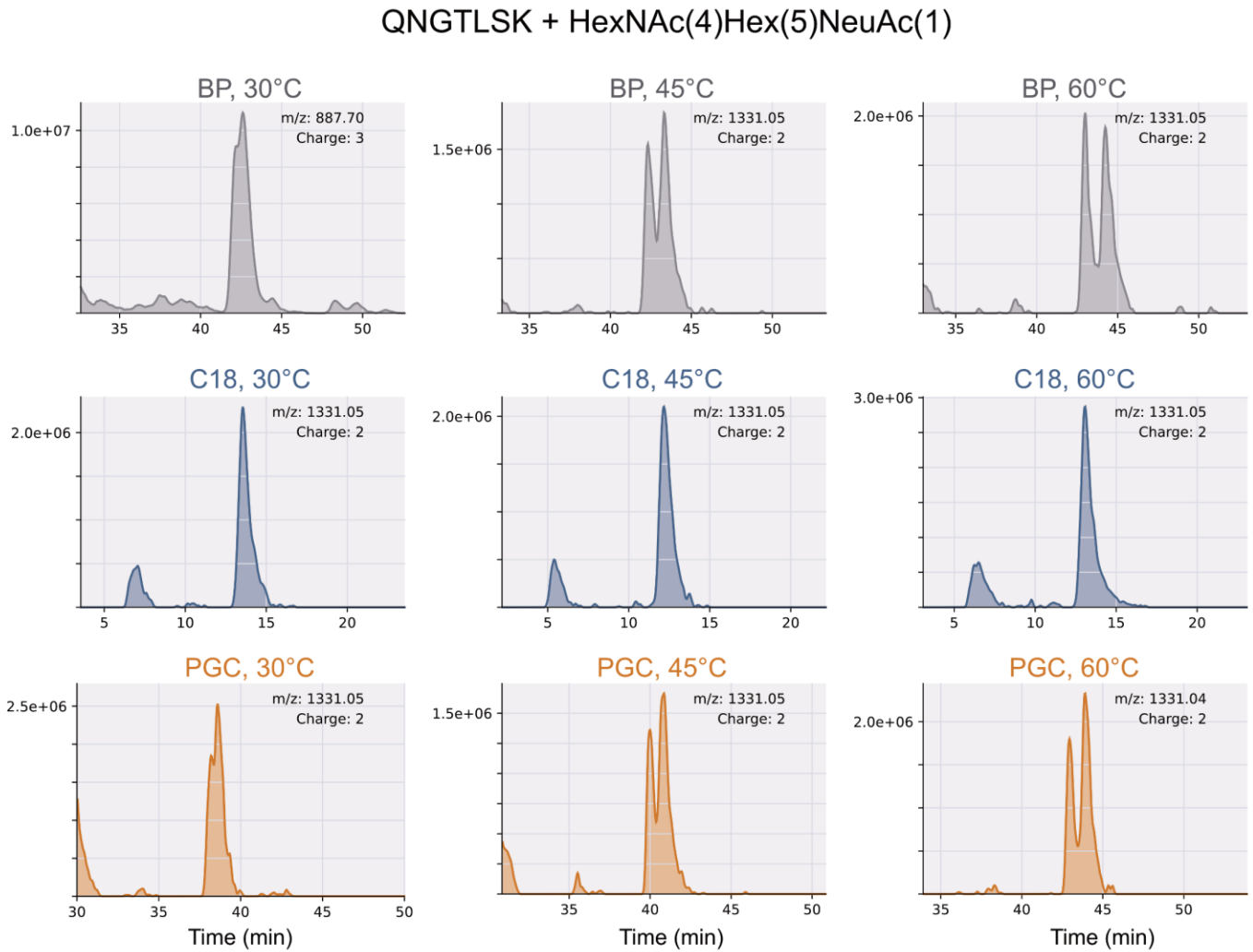


Outcomes

- PGC compliments RPLC separations, providing access to the hydrophilic portion of the glycoproteome
- Selective retention reduces charge state suppression
- PGC improved microheterogeneity recognition

Delafield, D. G., et al. (2022). *Anal Bioanal Chem*. 414(18):5461-5472.

Isomeric Separation

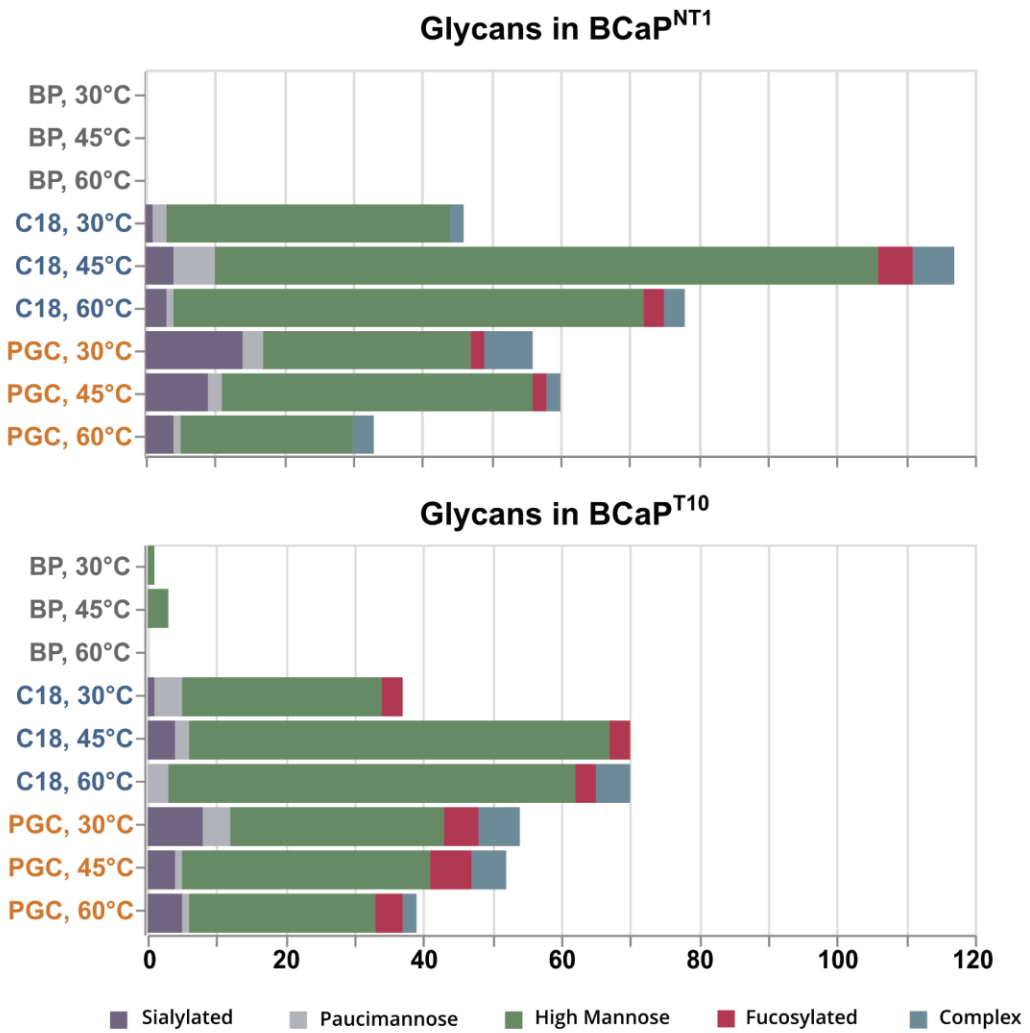


Outcomes

- PGC compliments RPLC separations, providing access to the hydrophilic portion of the glycoproteome
- Selective retention reduces charge state suppression
- PGC improved microheterogeneity recognition
- PGC demonstrates temperature-dependent liquid-phase resolution - can reveal isomeric species.

Delafield, D. G., et al. (2022). *Anal Bioanal Chem*. 414(18):5461-5472.

Temperature Affects Glycopeptide Identifications



Delafield, D. G., et al. (2022). *Anal Bioanal Chem*. 414(18):5461-5472.

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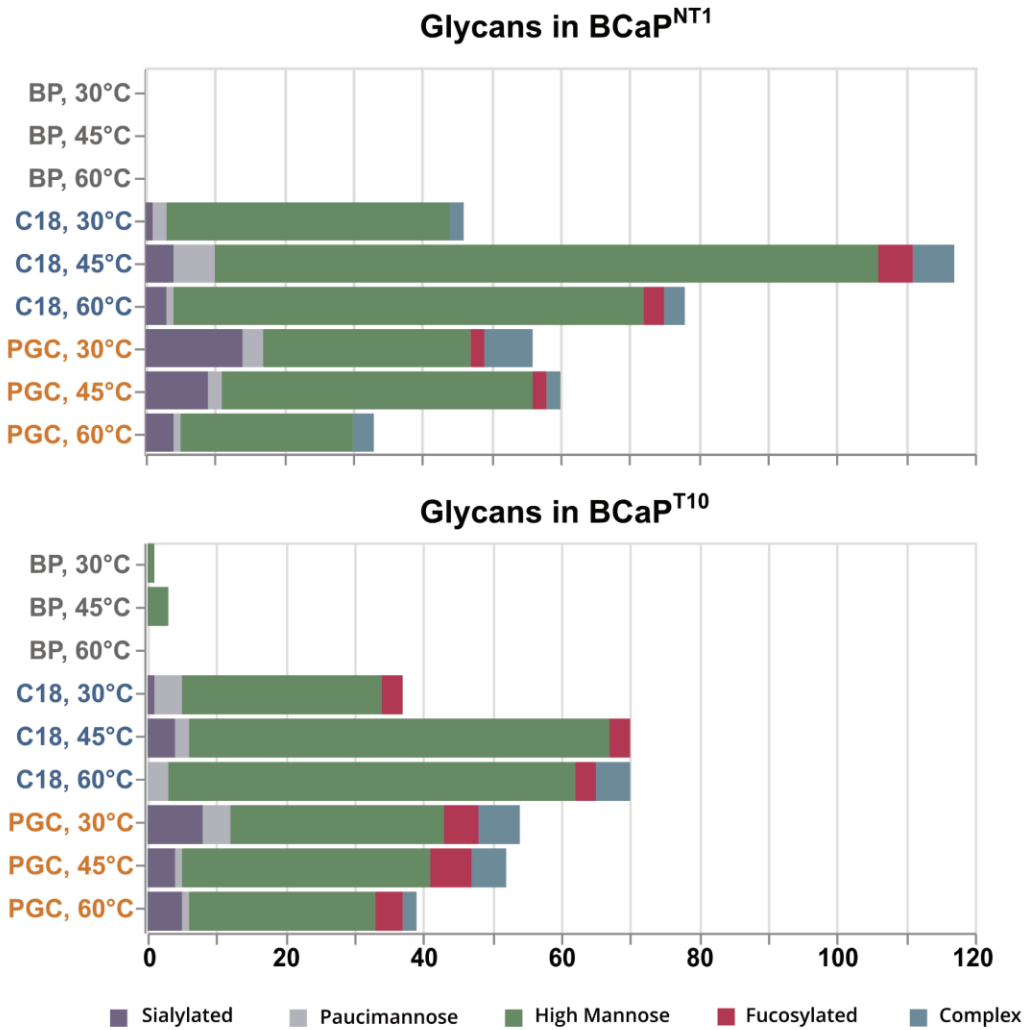

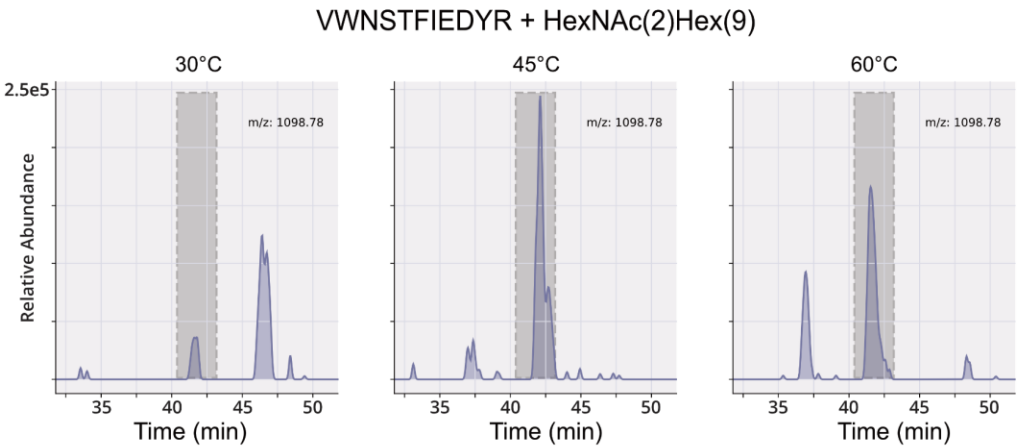

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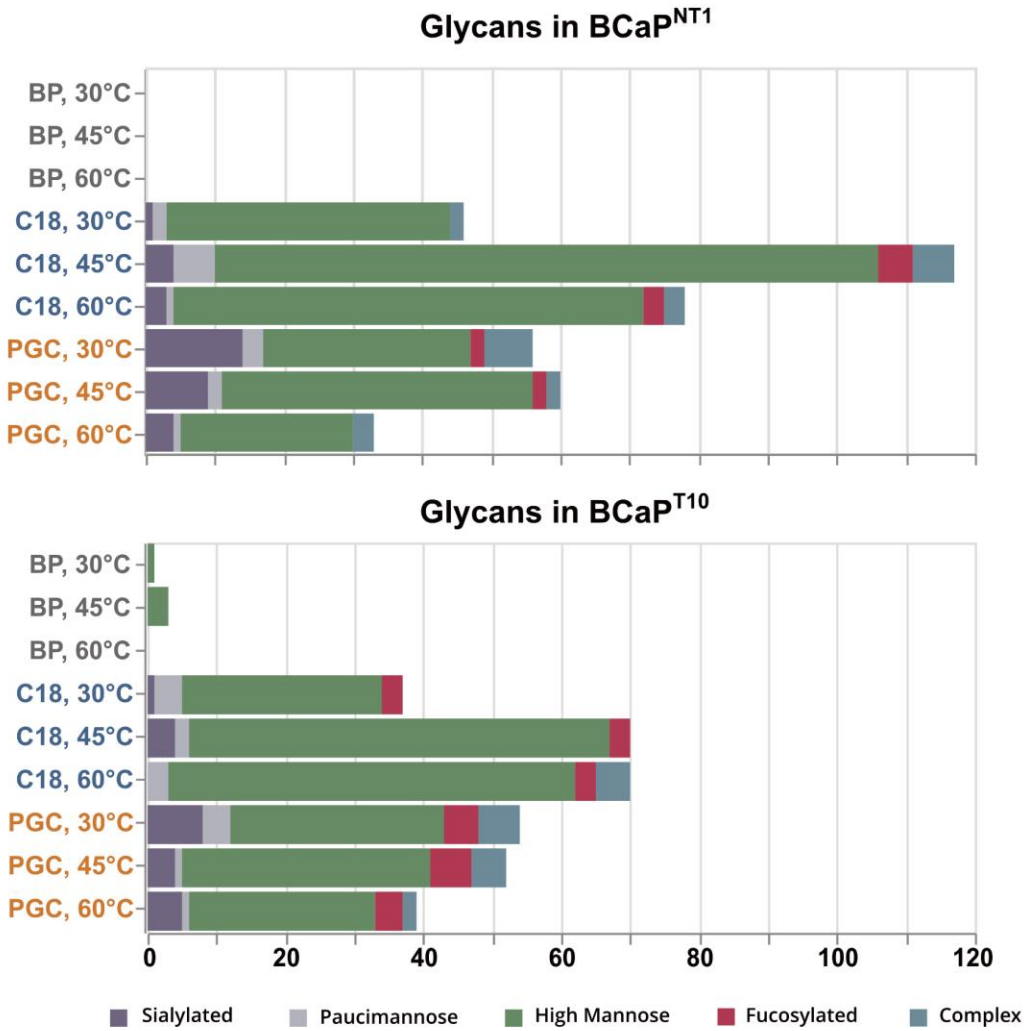
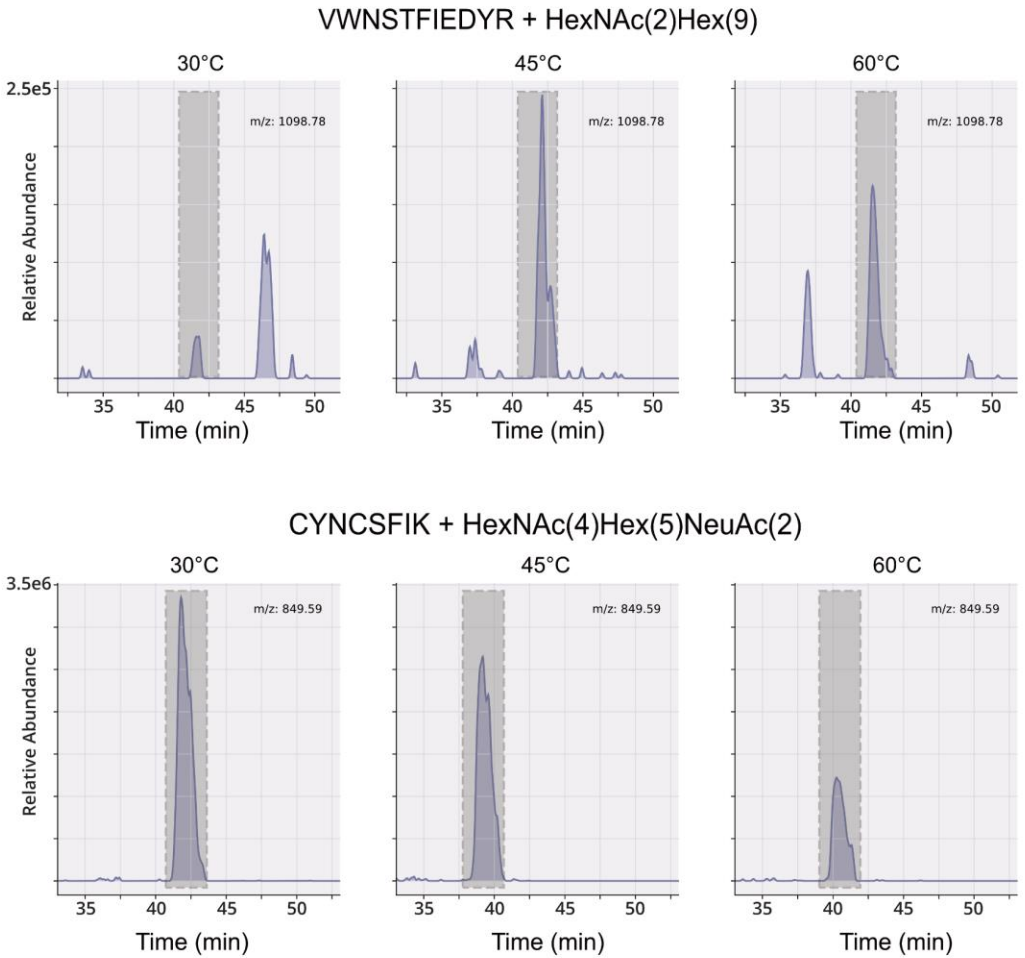
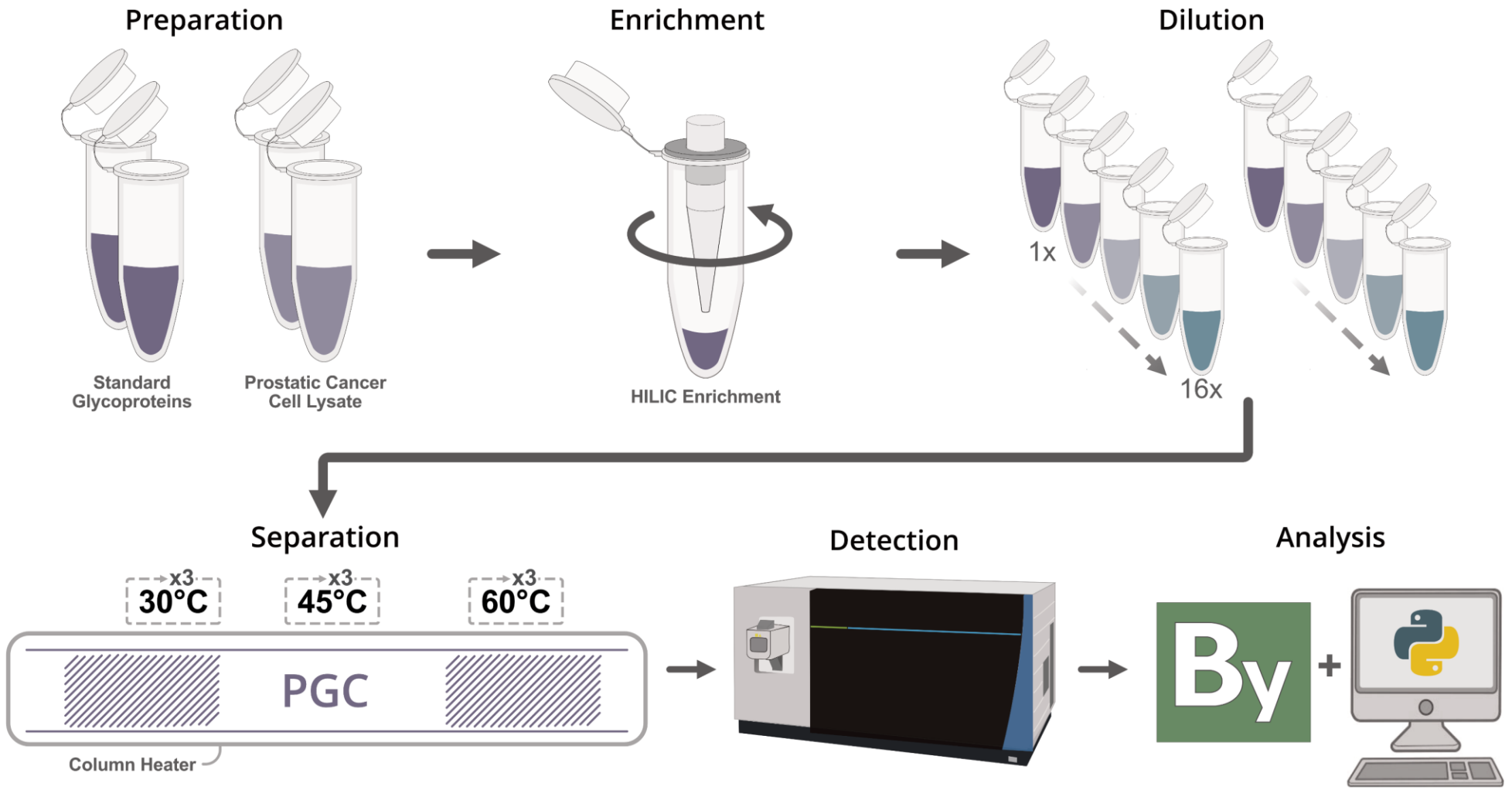




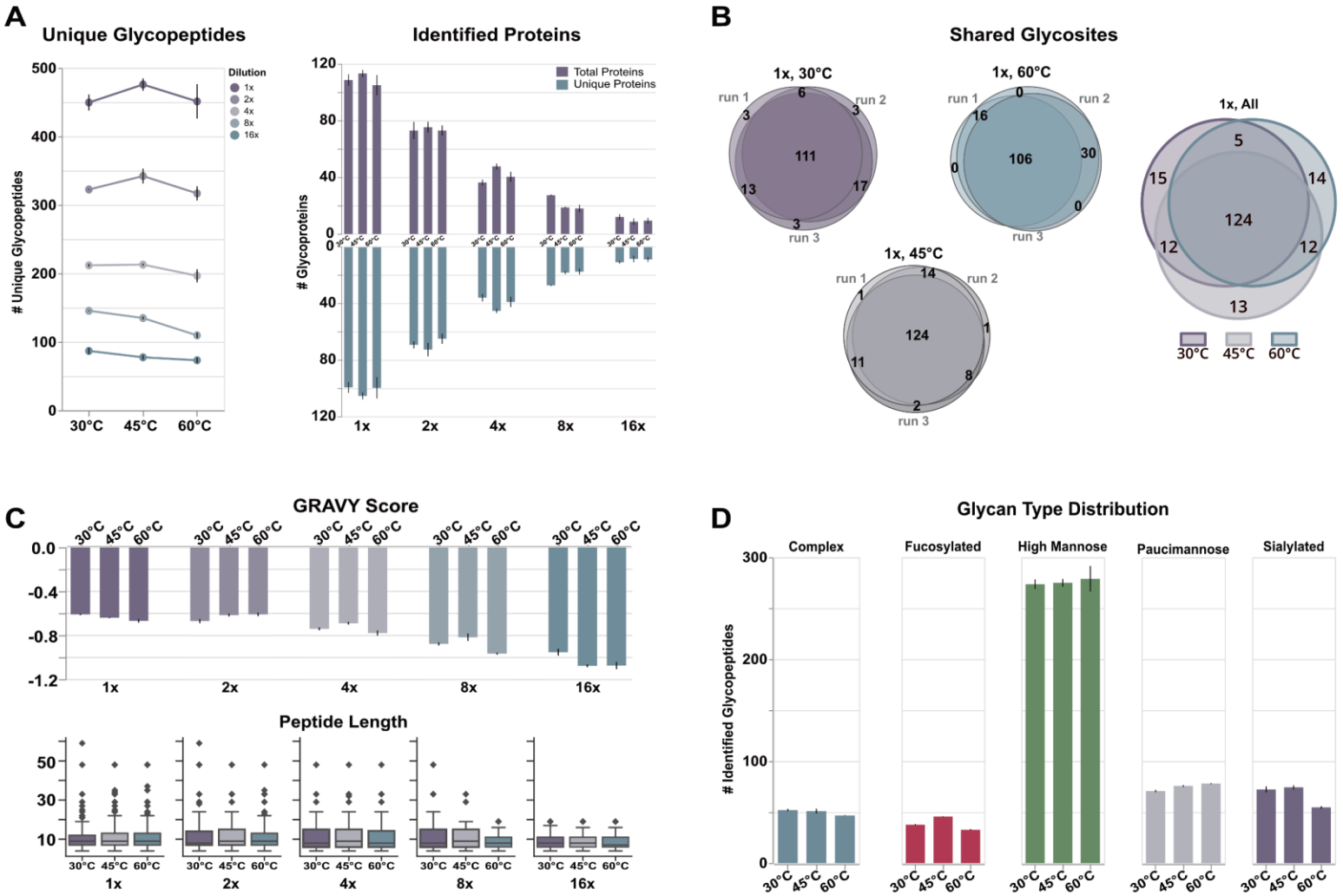
Delafield, D. G., et al. (2022). *Anal Bioanal Chem*. 414(18):5461-5472.

Investigating Temperature-based Aberrations



Delafield, D. G., et al. (2023) *JASMS*. 34(1):64-74.

Investigating Temperature-based Aberrations

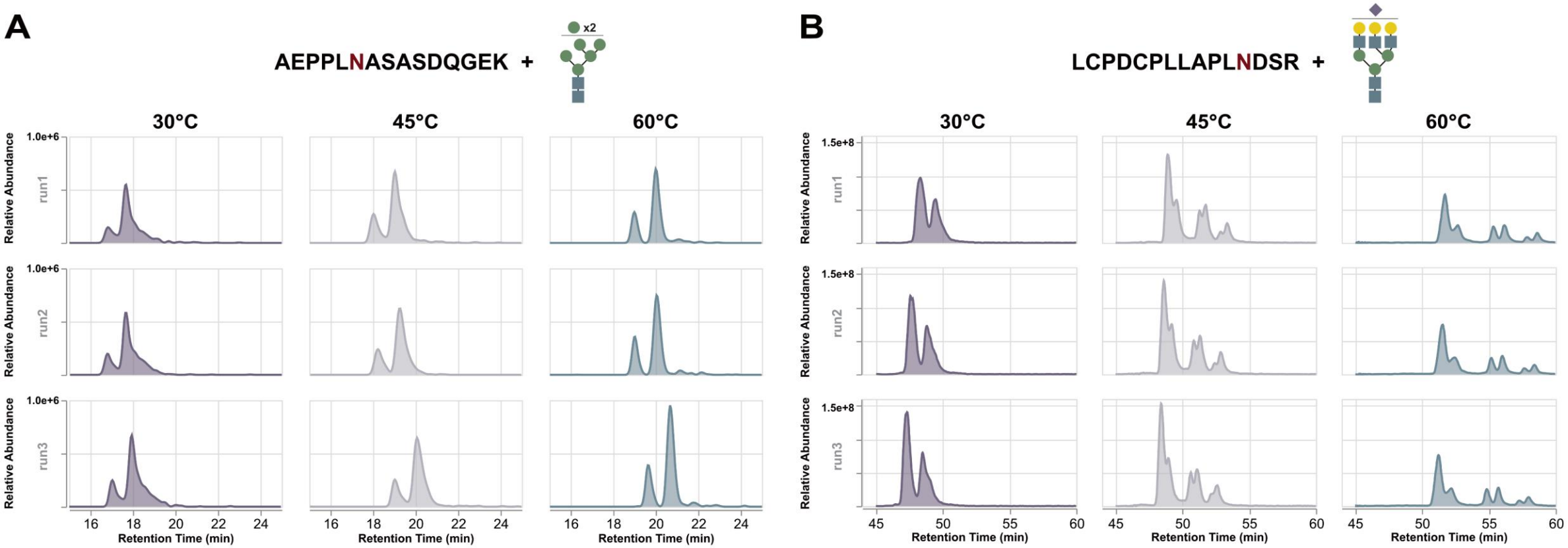


Complications

- Data rearticulate identification improvements at 45°C and drop off at 60°C
- Analytical variation and heterogeneity are not a factor
- Mannose type glycopeptides seem to 'survive' at high temperatures, others do not

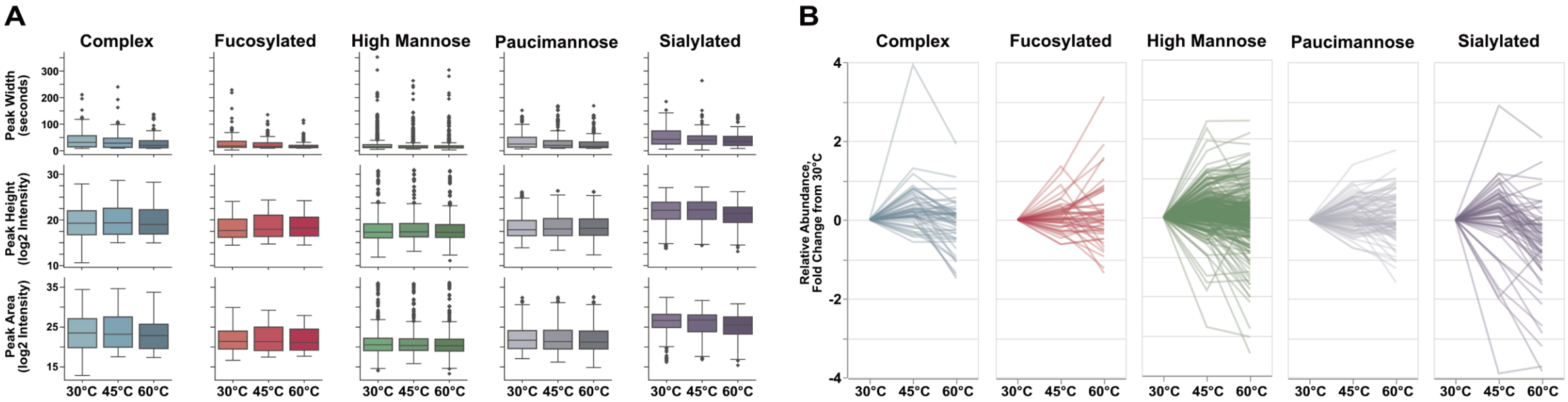
Delafield, D. G., et al. (2023) *JASMS*. 34(1):64-74.

Temperature Inspired Chromatographic Changes



Delafield, D. G., et al. (2023) *JASMS*. 34(1):64-74.

Resolution vs. Signal Intensity



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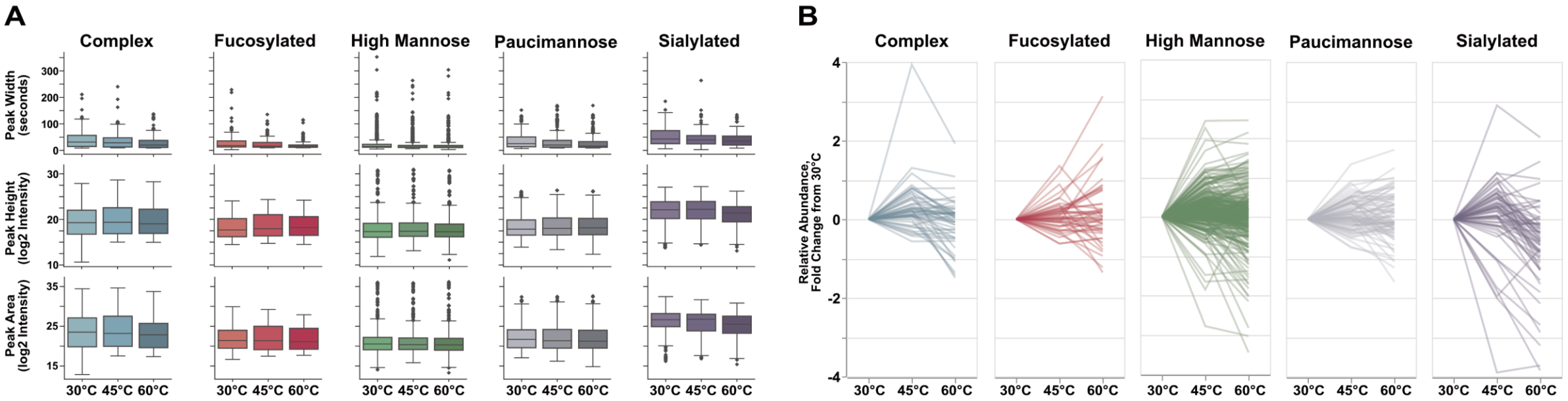
Resolution vs. Signal Intensity



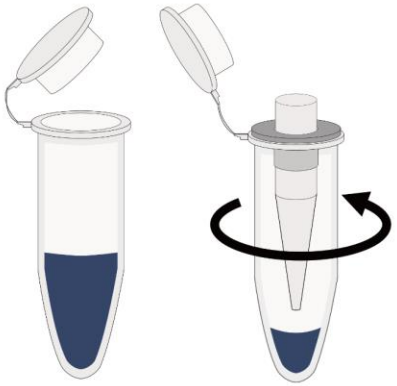
Conclusions

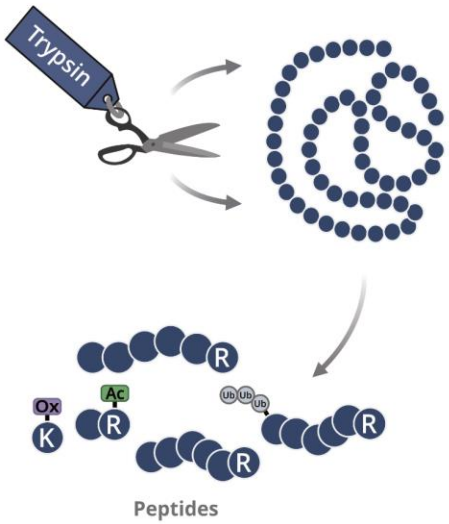
- PGC separations demonstrate great utility in glycopeptide analyses, but must be tailored to experimental goals
- Glycopeptide discovery is most efficient at 45°C where reporting signal is highest
- Isomeric separation is best at ≥60°C but adversely affects labile glycan moieties

Delafield, D. G., et al. (2023) *JASMS*. 34(1):64-74.

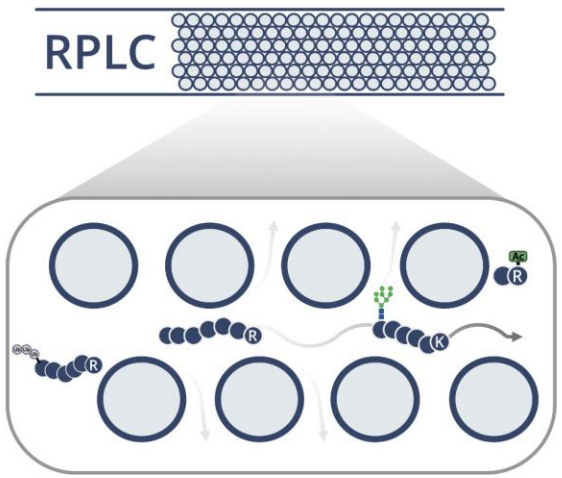
MS-Based Proteomics

Preparation

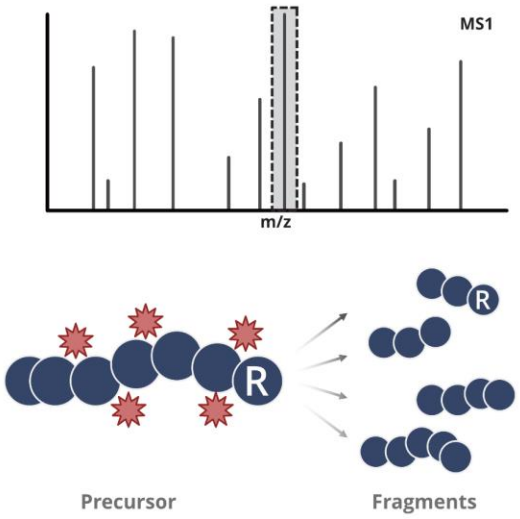




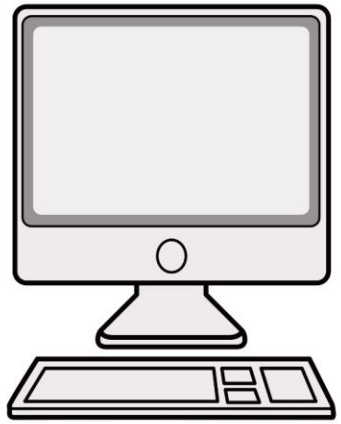
Separation

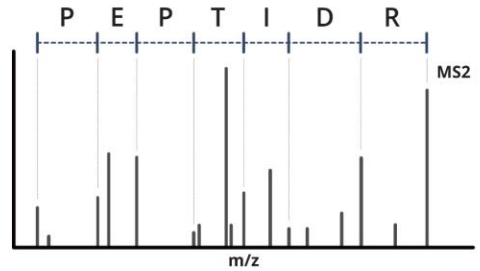


Detection



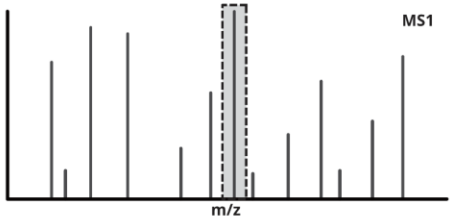
Analysis

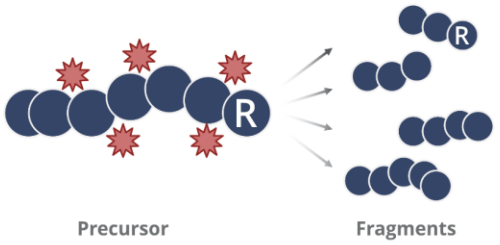




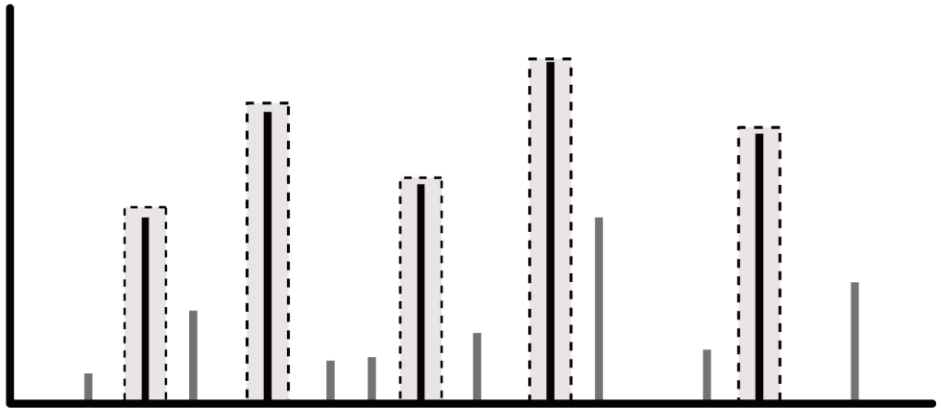
Dependent vs. Independent Acquisition

Detection



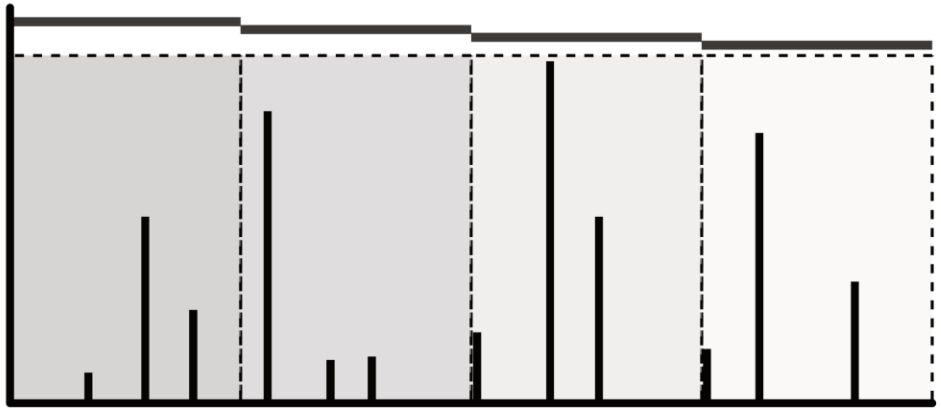


Data-Dependent Acquisition (DDA)



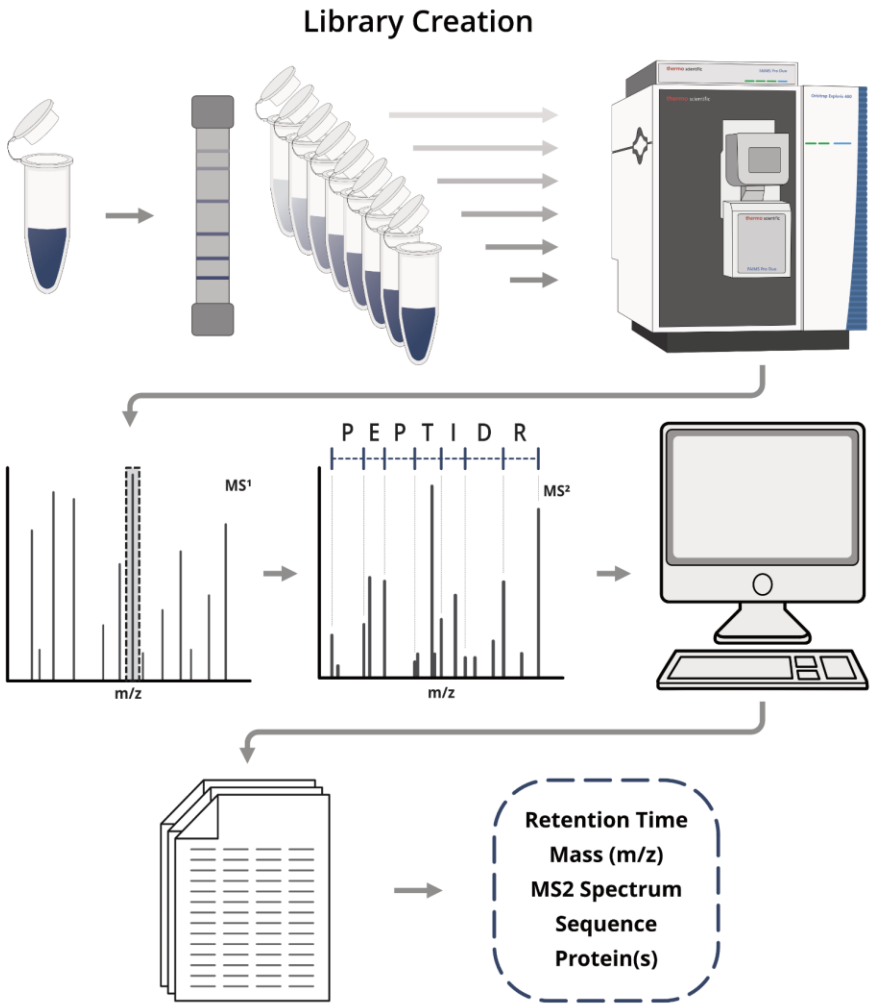
Confident and clean but biased towards highly-abundant analytes

Data-Independent Acquisition (DIA)

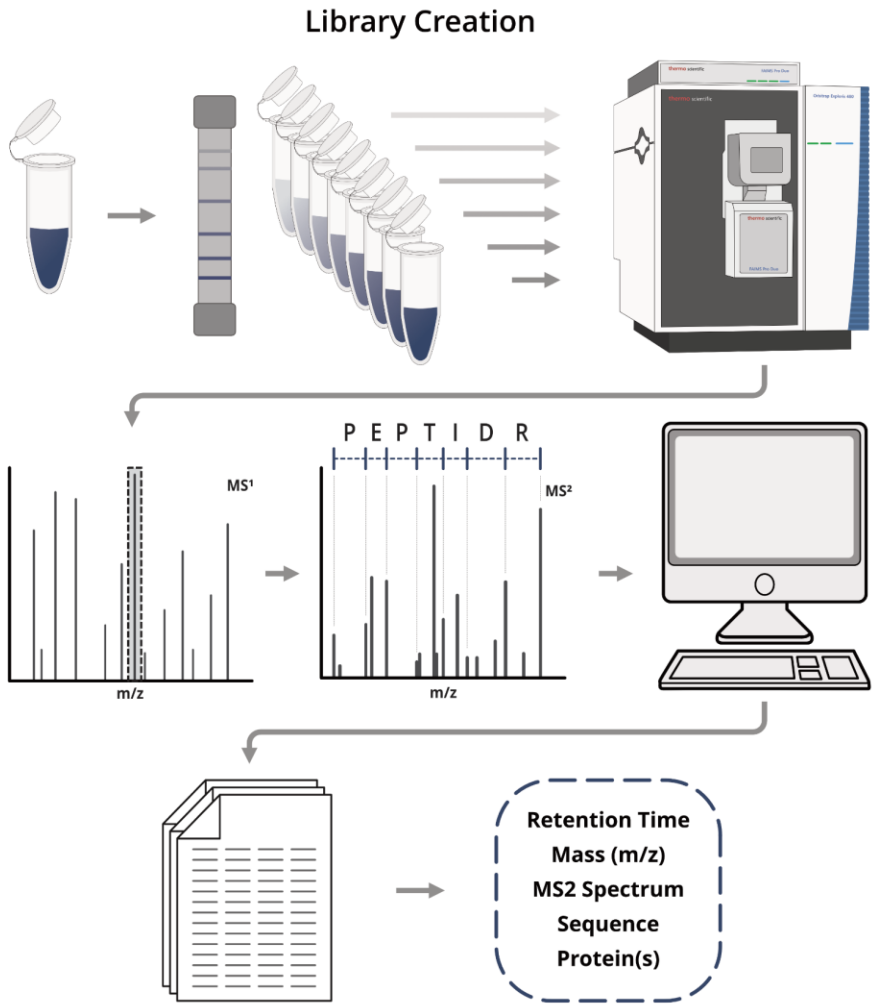


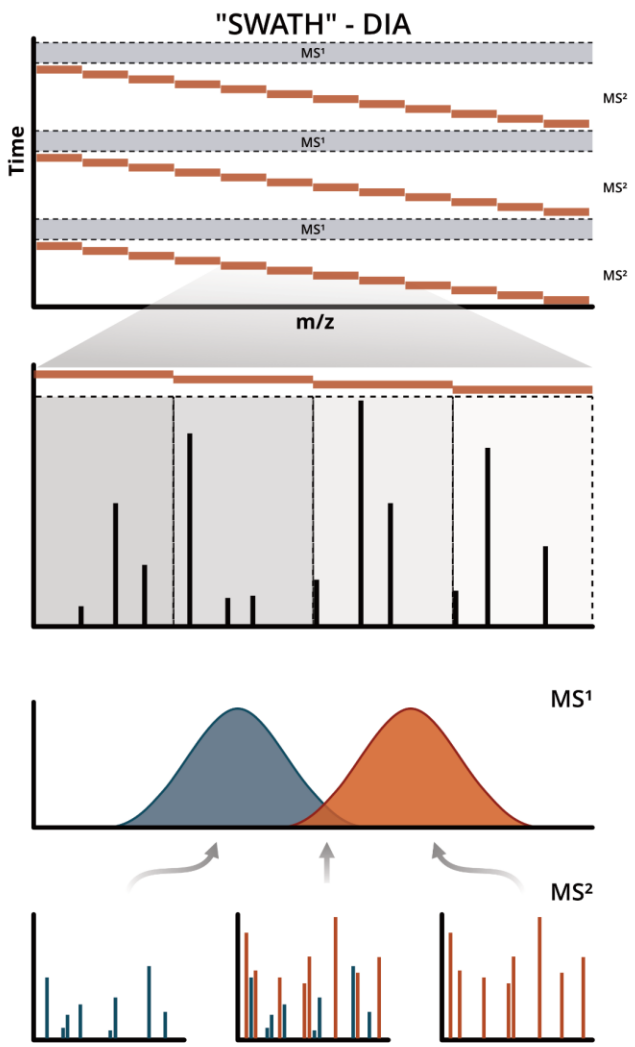
Unbiased and higher throughput but data processing is more complex

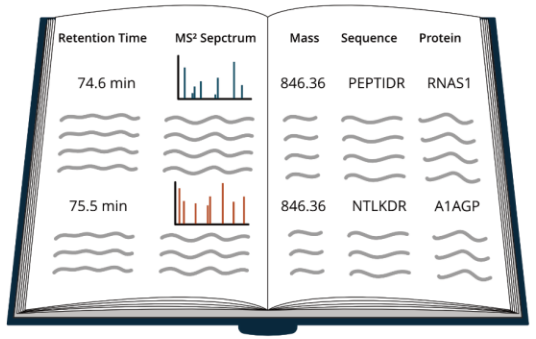
DIA and Spectral Libraries



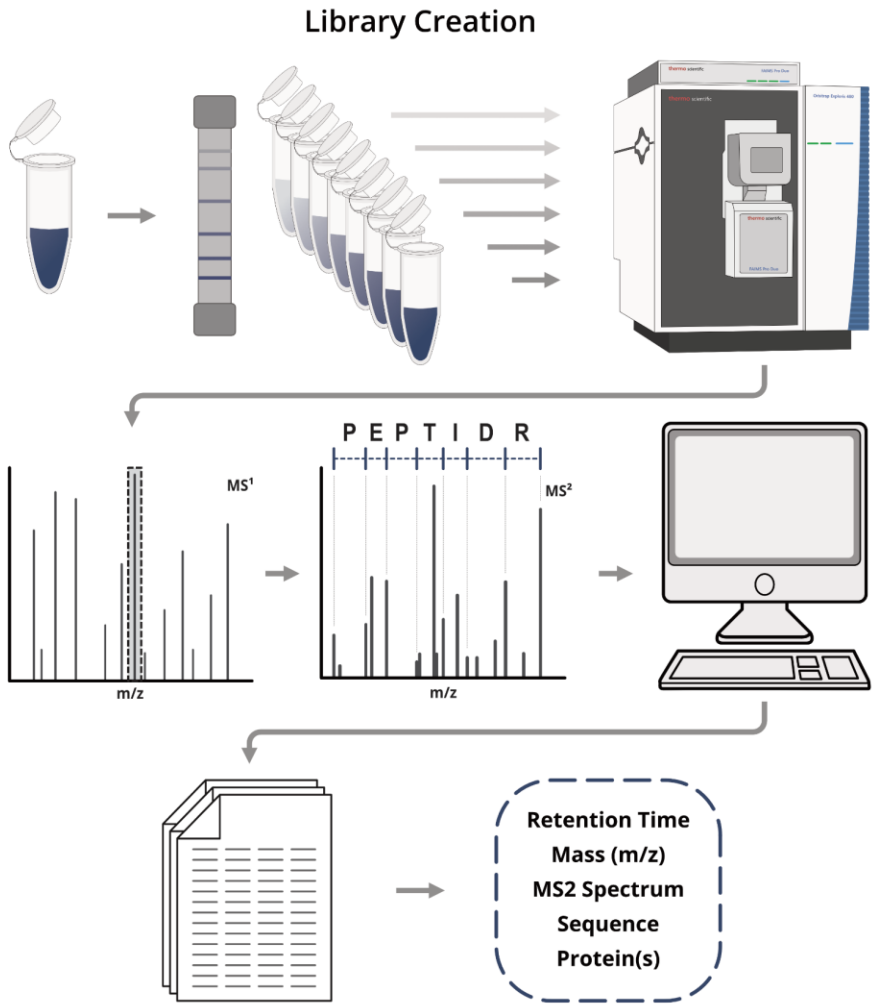
DIA and Spectral Libraries

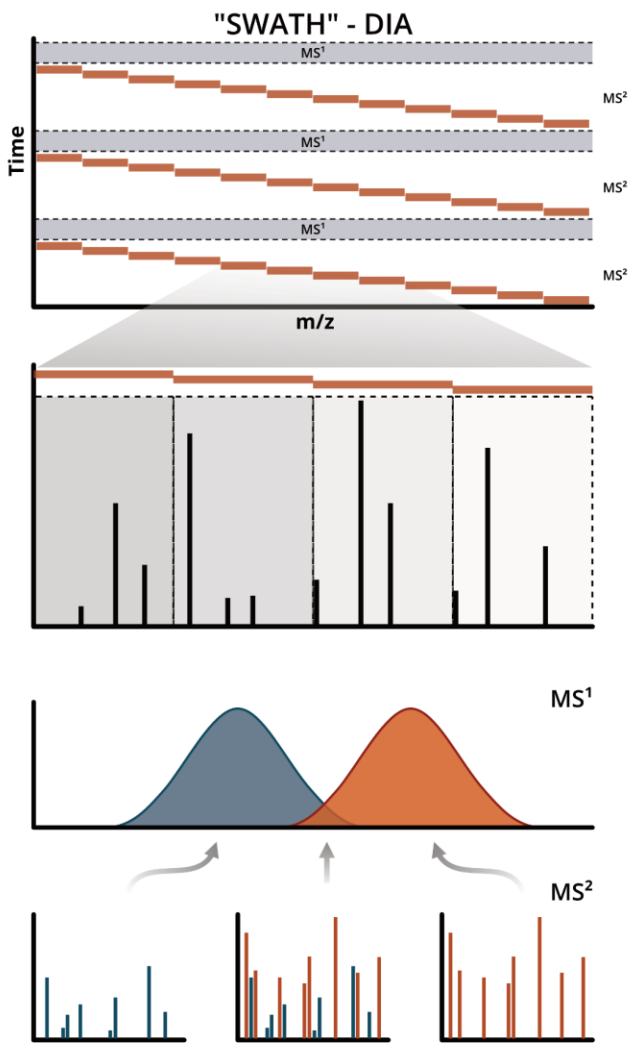


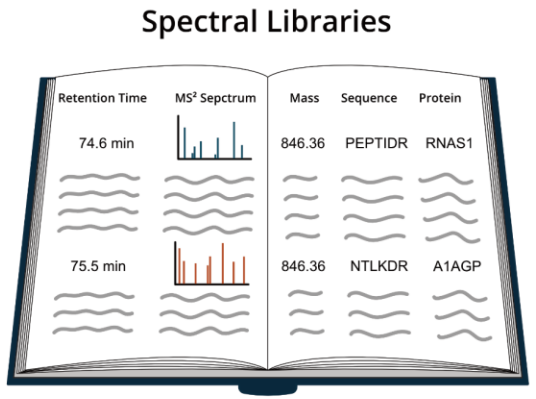




DIA and Spectral Libraries

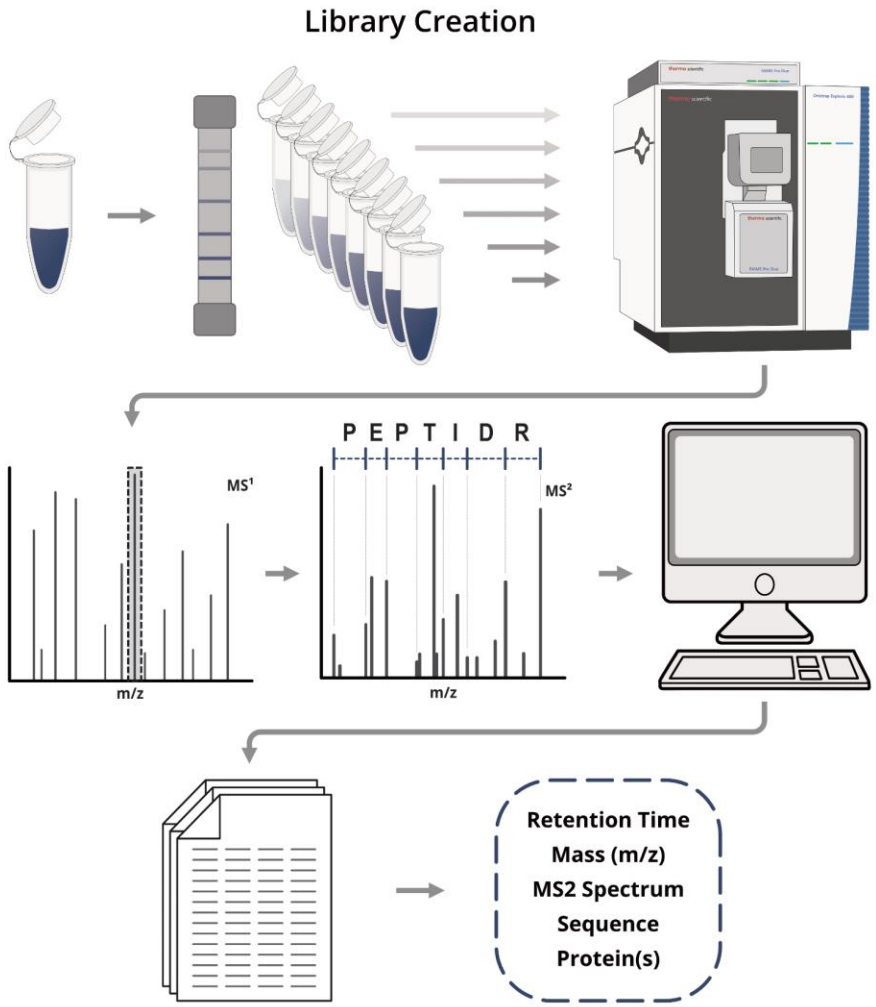


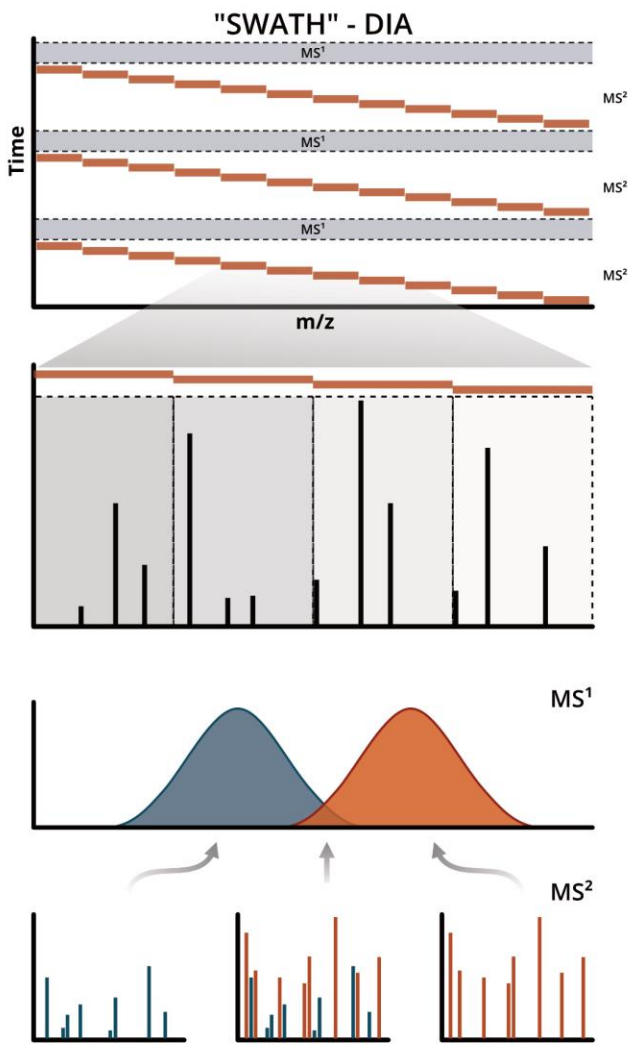


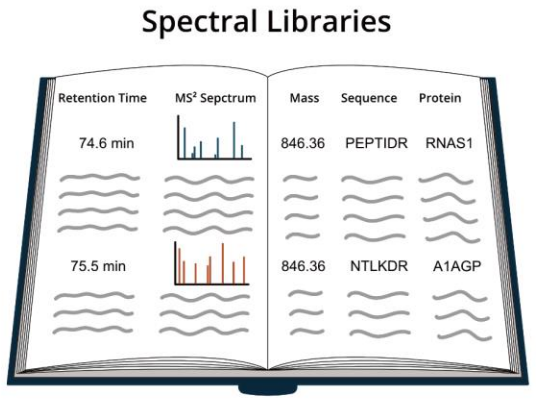


+ Efficient DIA deconvolution
+ Sample-specific, evidence-based
- Increases sample requirement
- Profiling depth limited by quality of library

DIA and Spectral Libraries







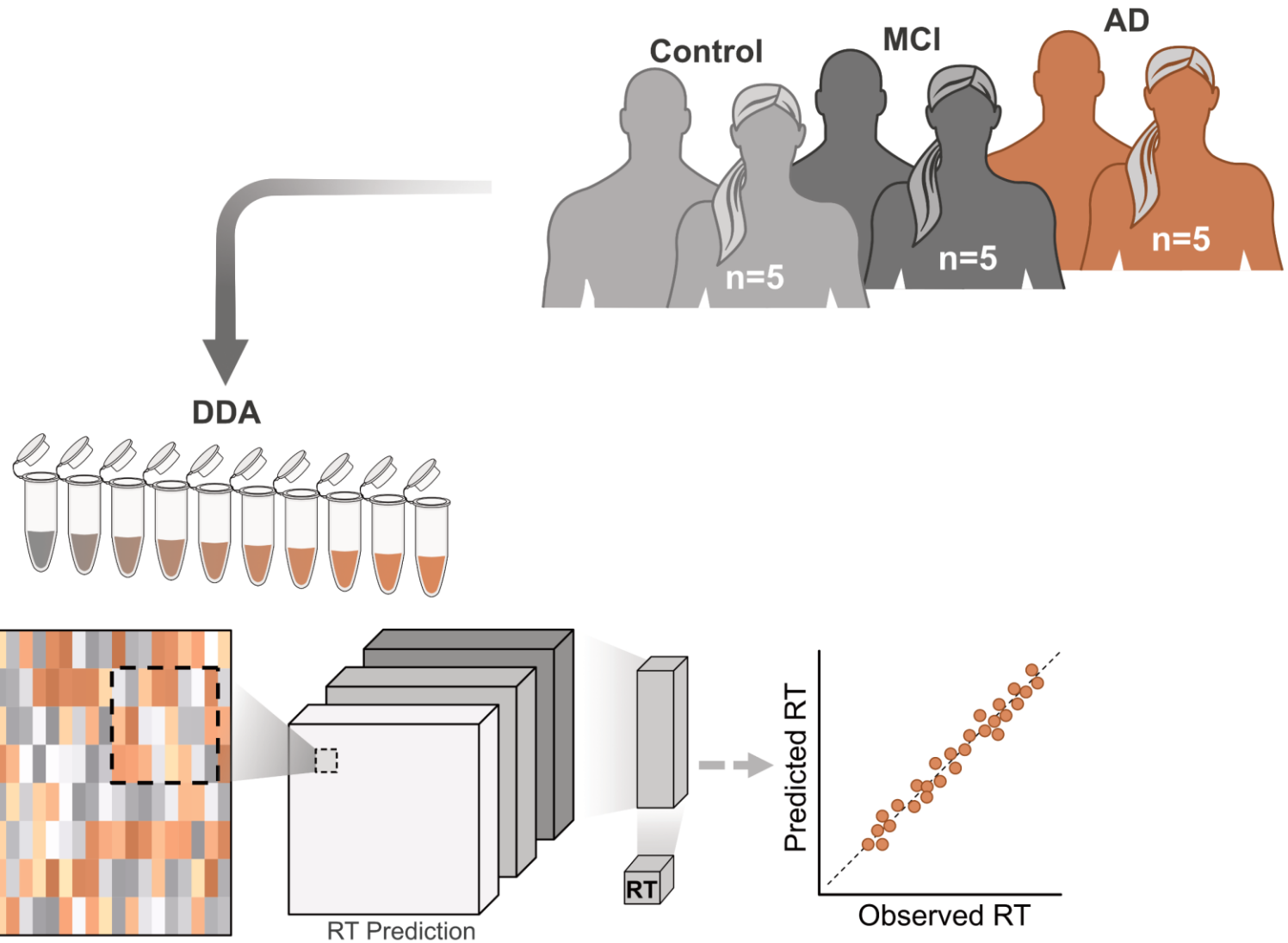
+ Efficient DIA deconvolution
+ Sample-specific, evidence-based
- Increases sample requirement
- Profiling depth limited by quality of library

Is it possible to adapt spectral libraries from existing analyses to a new experiment?

Questions and Experimental Goals

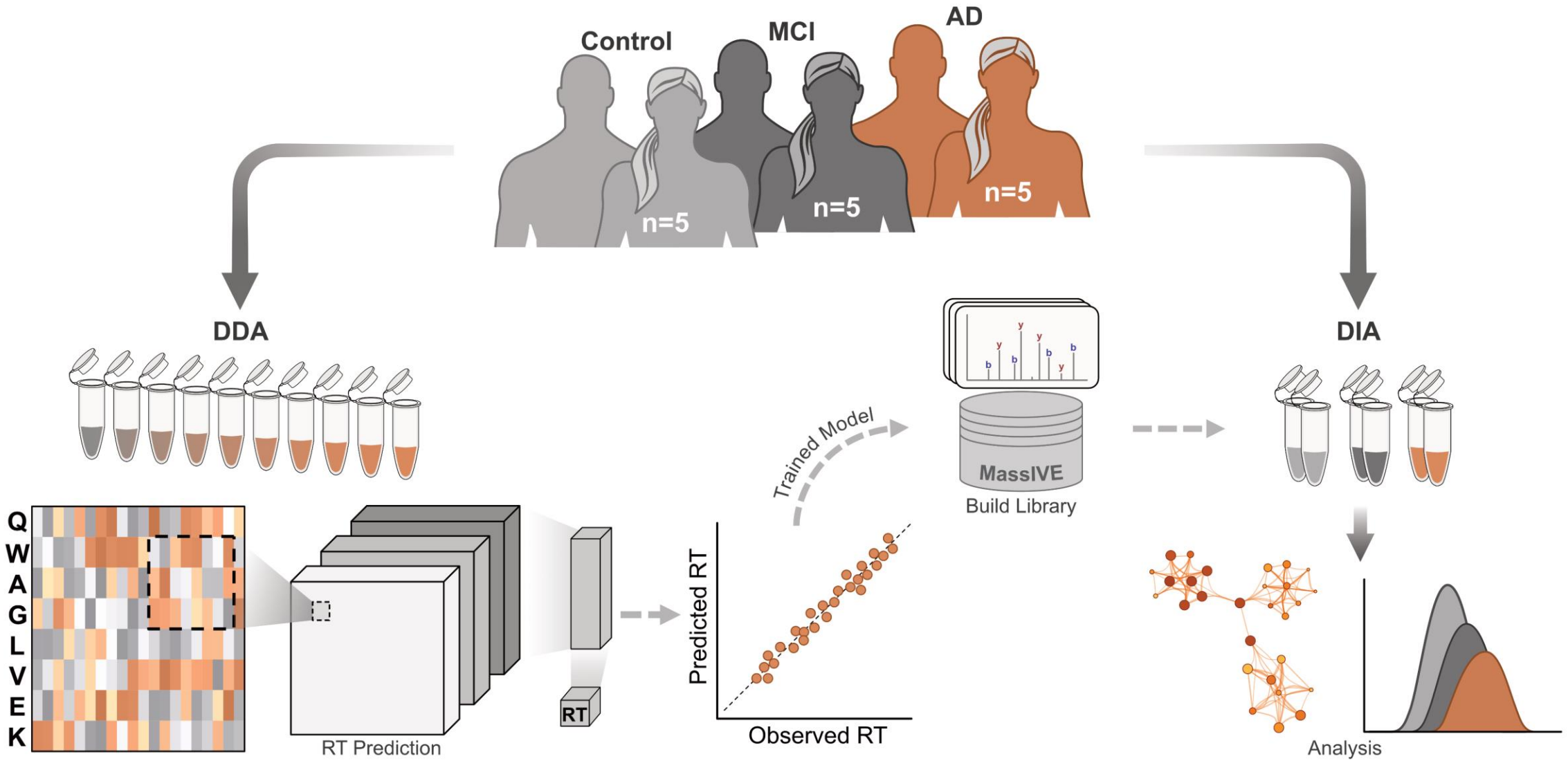
- Can we validate the ability to correlate prior proteomic measurements to new experimental conditions?
- Can we re-purpose prior measurements for biomolecular discovery? How does it compare to traditional approaches?
- Is quantitation accurate and reliable?
- Can we utilize this information to discover proteomic dysregulation in clinically-relevant applications?

Parallel Workflow



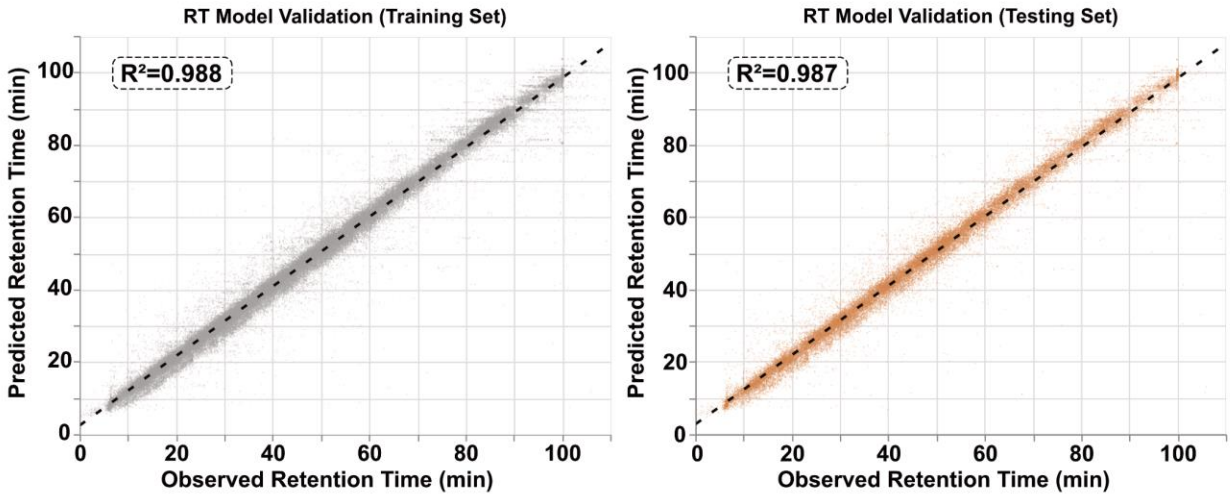
Chunwei Ma, Yan Ren, Jiarui Yang, Zhe Ren, Huanming Yang, and Siqi Liu Anal. Chem. 2018
D.G. Delafield*, X. Zhong*, et al. In submission.

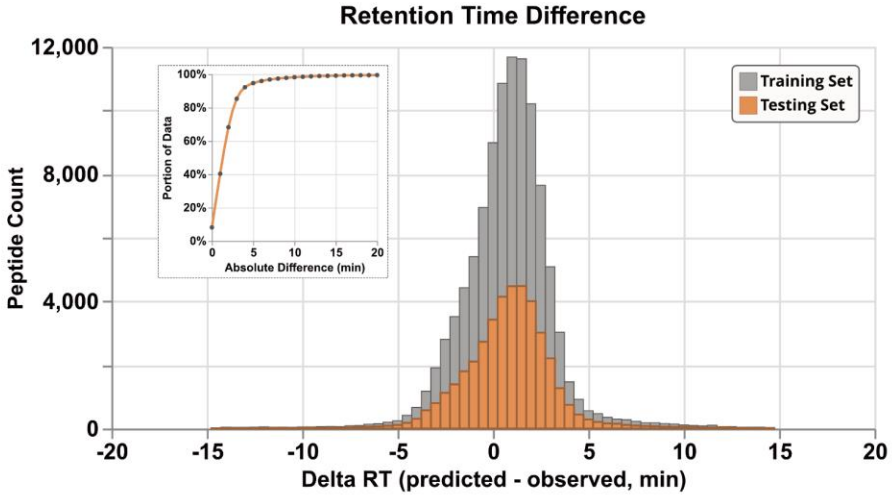
Parallel Workflow

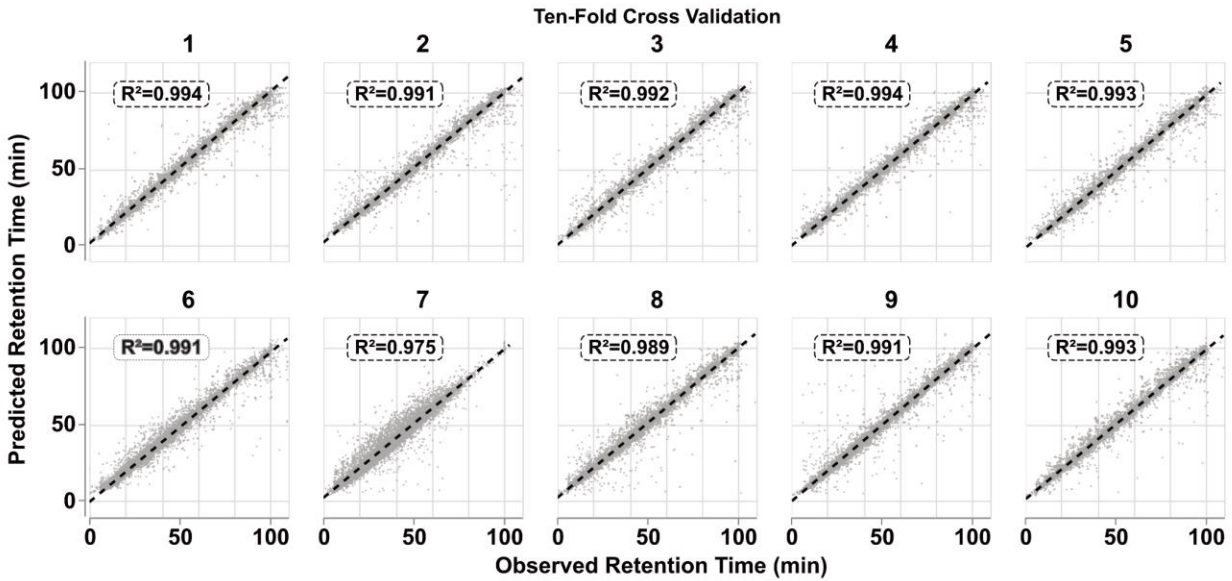


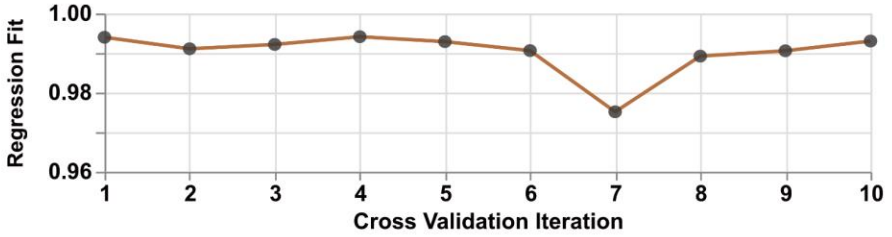
Chunwei Ma, Yan Ren, Jiarui Yang, Zhe Ren, Huanming Yang, and Siqi Liu Anal. Chem. 2018
D.G. Delafield*, X. Zhong*, et al. In submission.

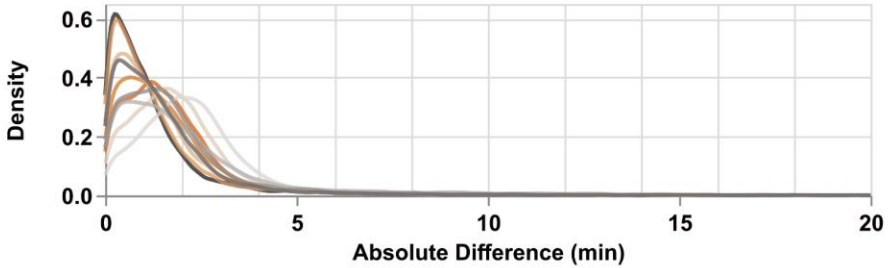
Model Validation





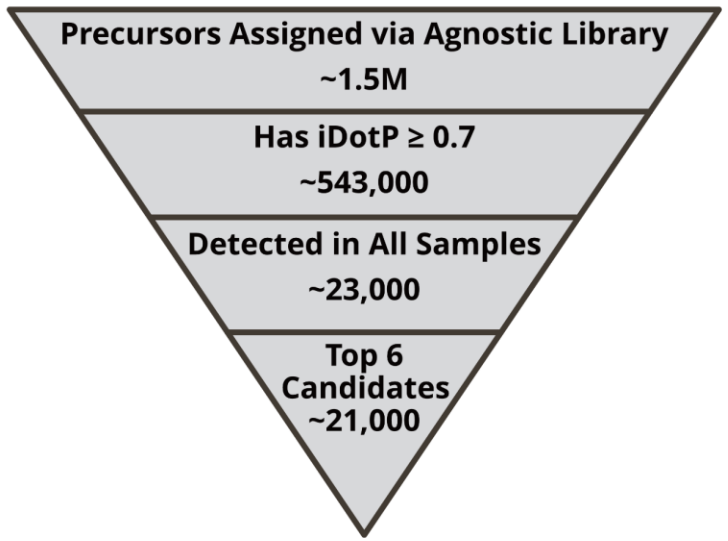


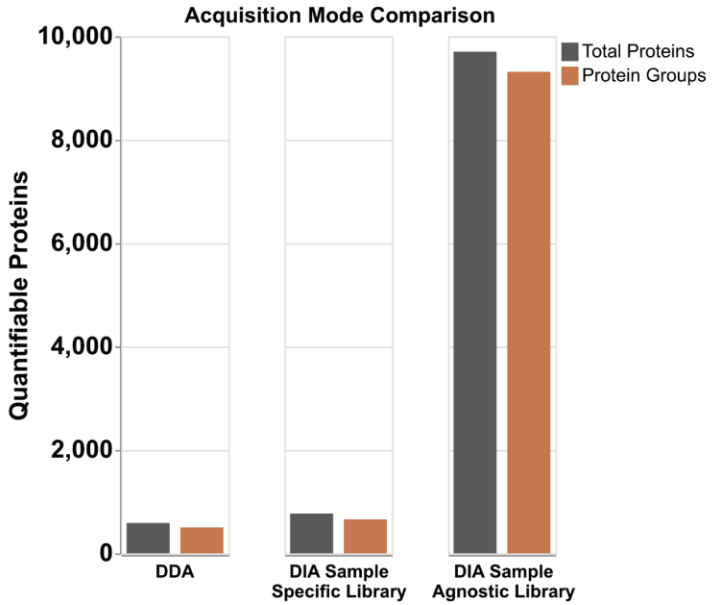


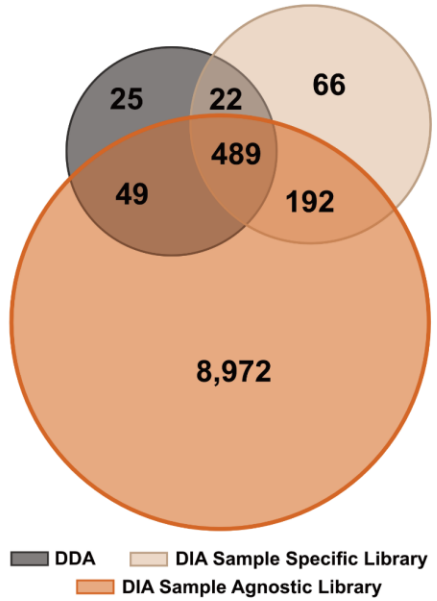


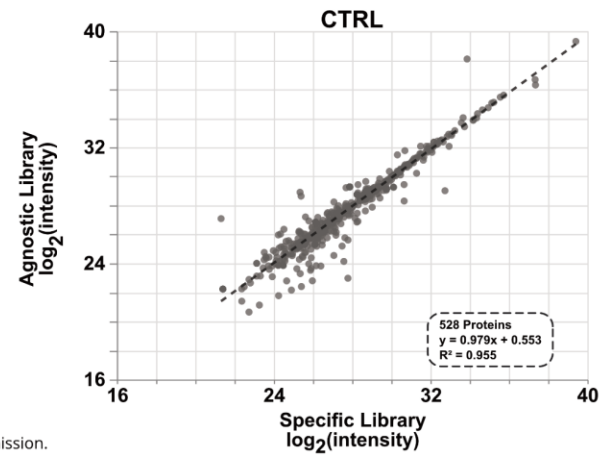
D.G. Delafield*, X. Zhong*, et al. In submission.

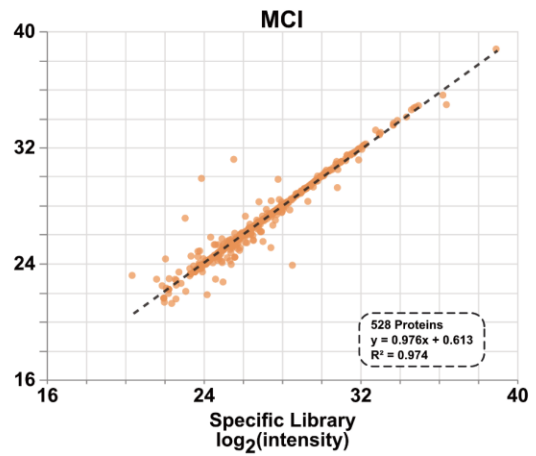
Quantitative Comparison

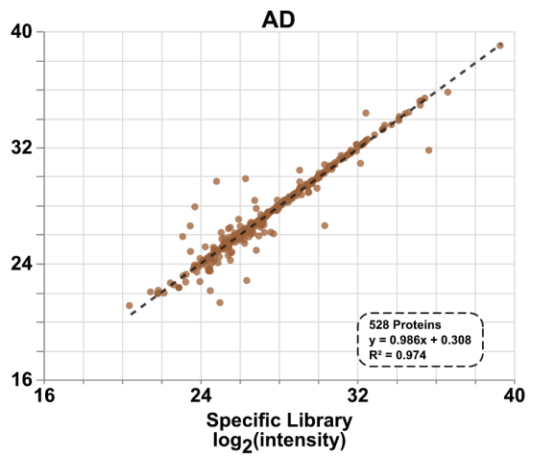






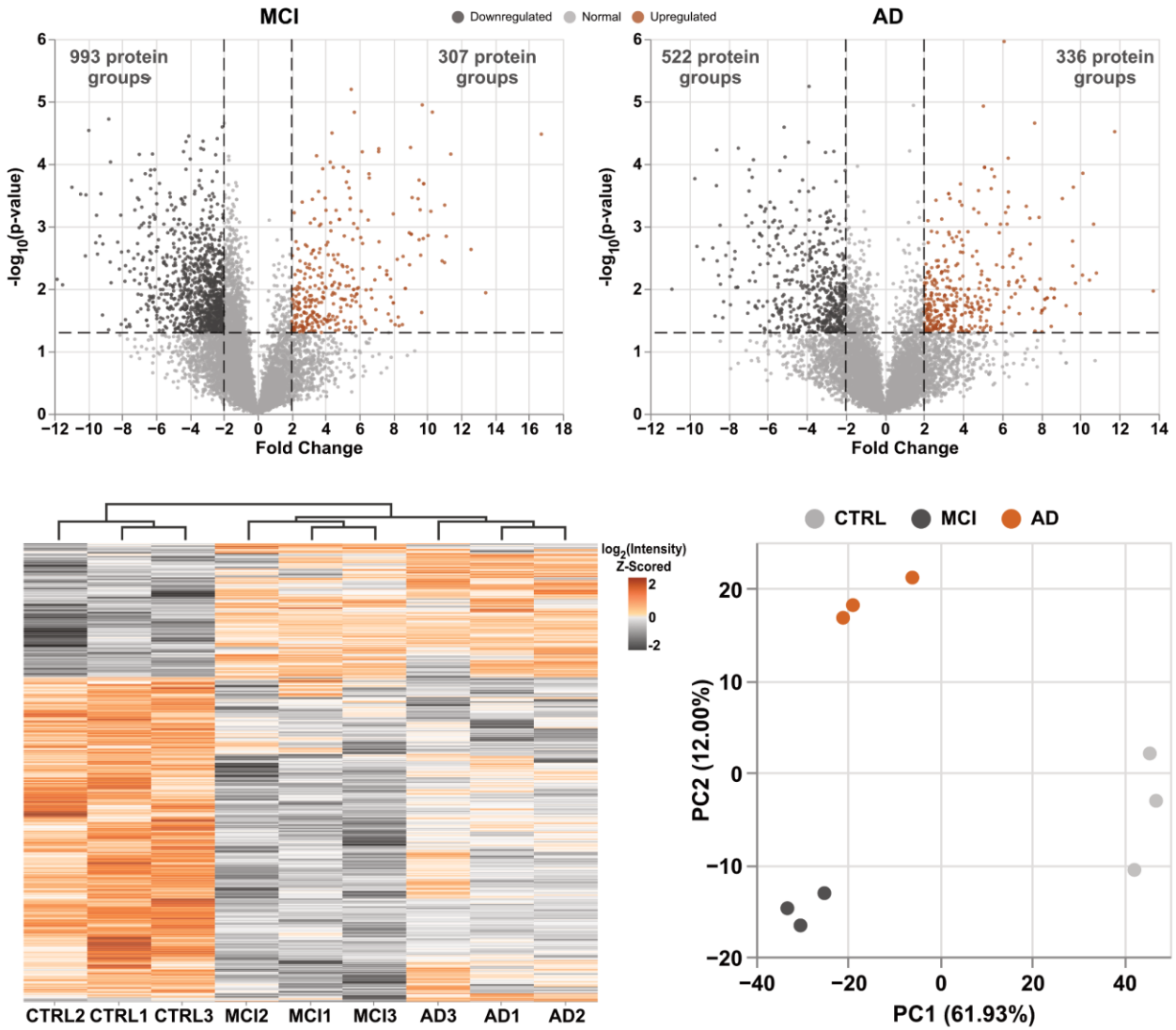






D.G. Delafield*, X. Zhong*, et al. In submission.

Disease State Discrimination

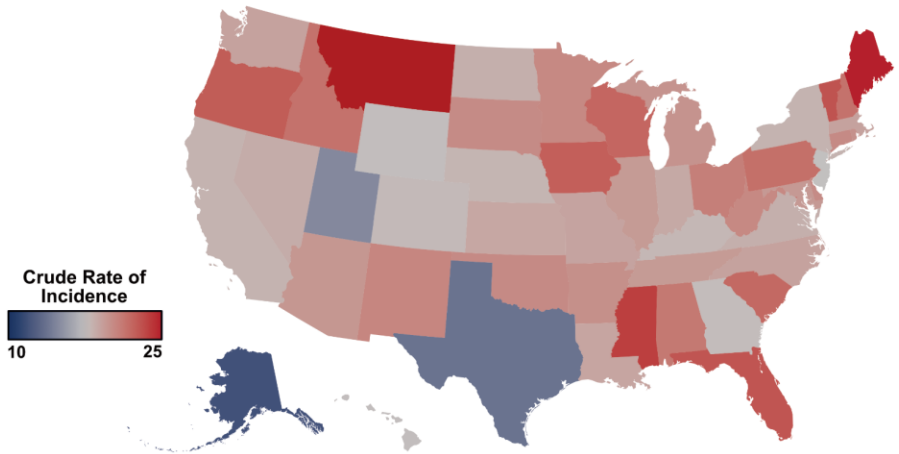


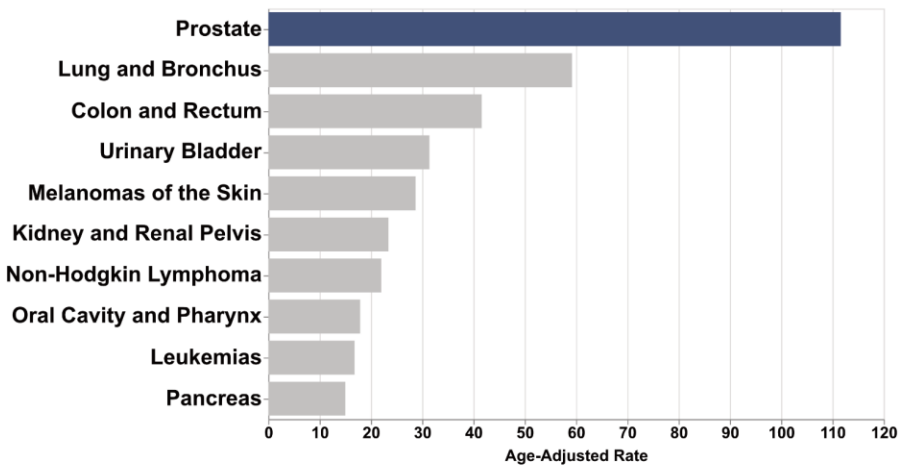
Outcomes

- Using machine learning, spectral libraries can be effectively calibrated to a new experiment.
- Agnostic libraries provide significantly improved profiling depth.
- We revealed 1,642 significantly dysregulated proteins, highlighting remodeling of extracellular matrix and tubulin dysruption.
- We advocate for a stronger community-driven approach to translate proteomic analyses to clinical application.
- Presents a path forward when utilizing novel chromatographic separations where peptide retention times are significantly different.

D.G. Delafield*, X. Zhong*, et al. In submission.

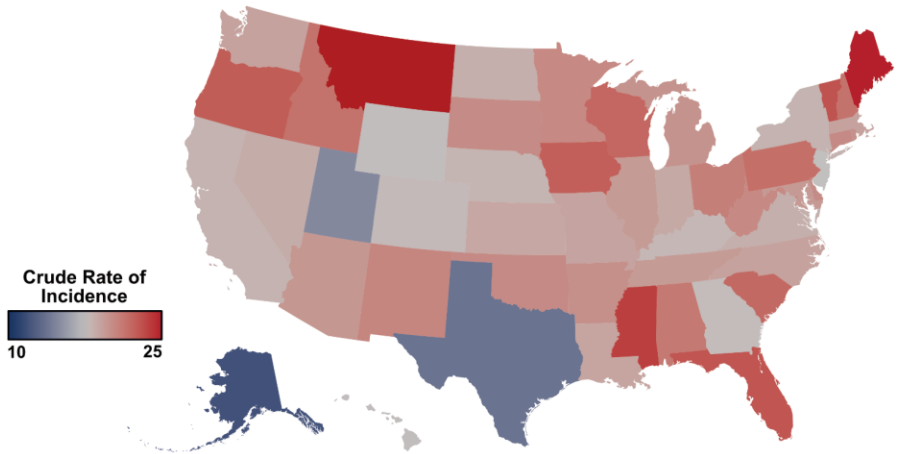
Prostate Cancer Incidence and Outlook

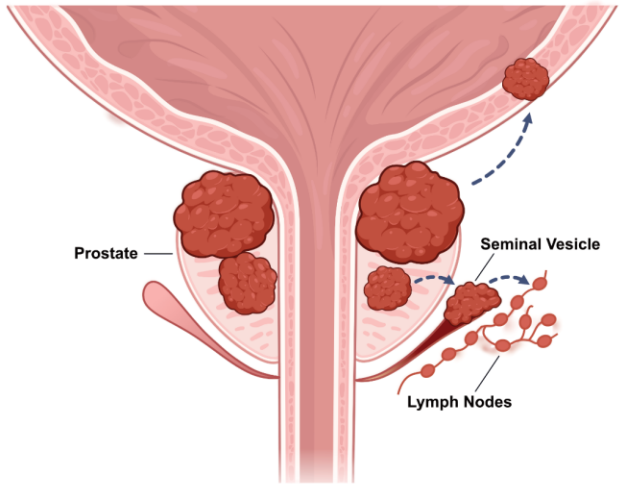


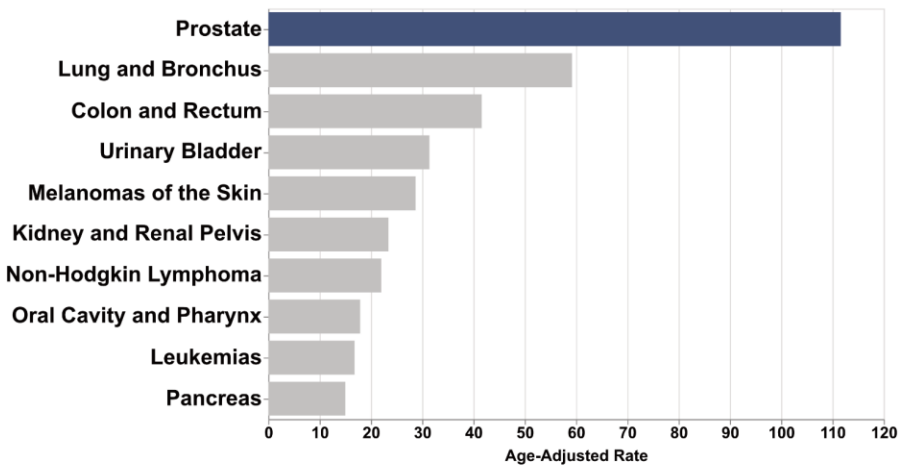


U.S. Cancer Statistics Working Group. U.S. Cancer Statistics. Centers for Disease Control and Prevention and National Cancer Institute. June 2022.

Prostate Cancer Incidence and Outlook







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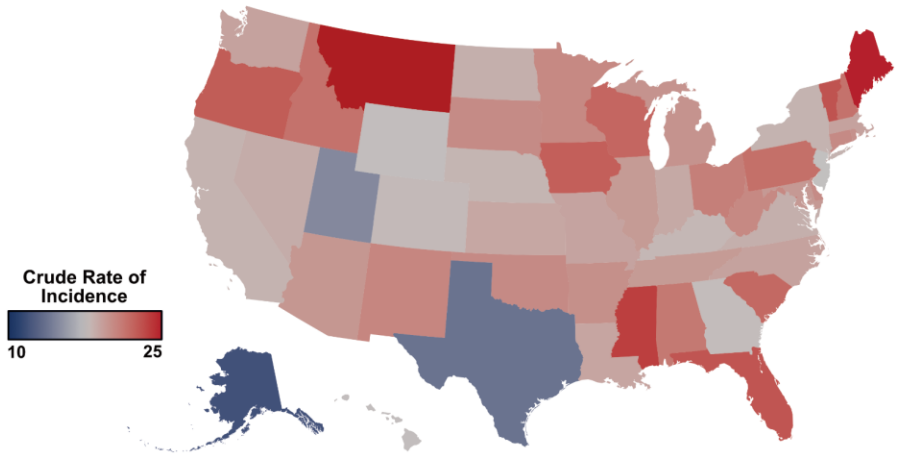
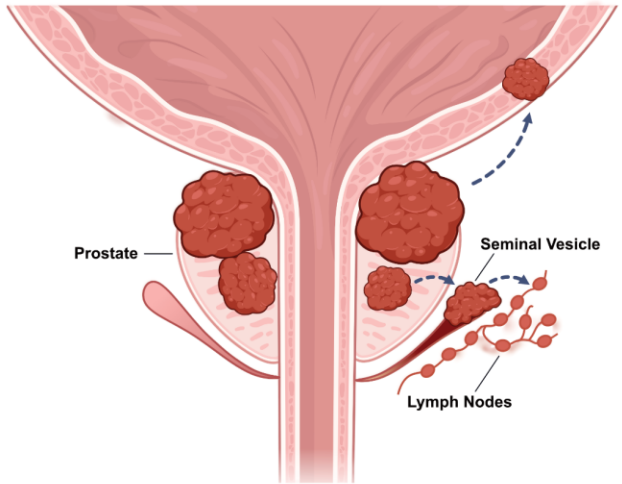


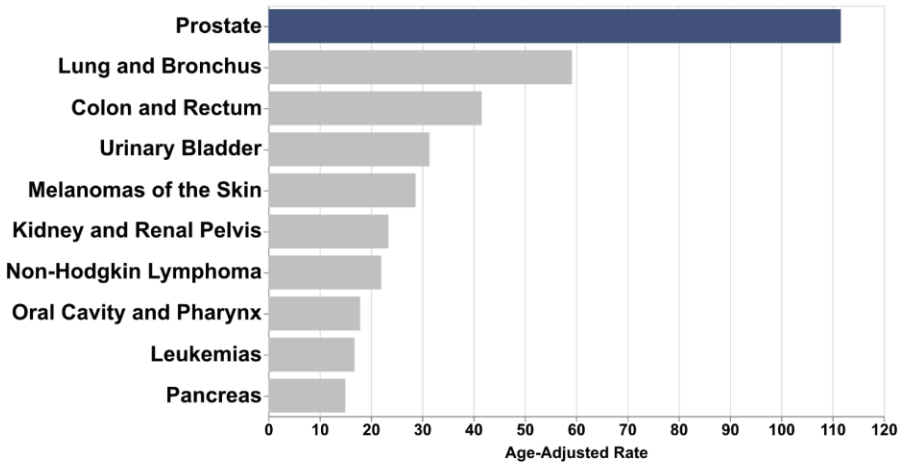


Urgent, infrequent, hesitant urination

↓

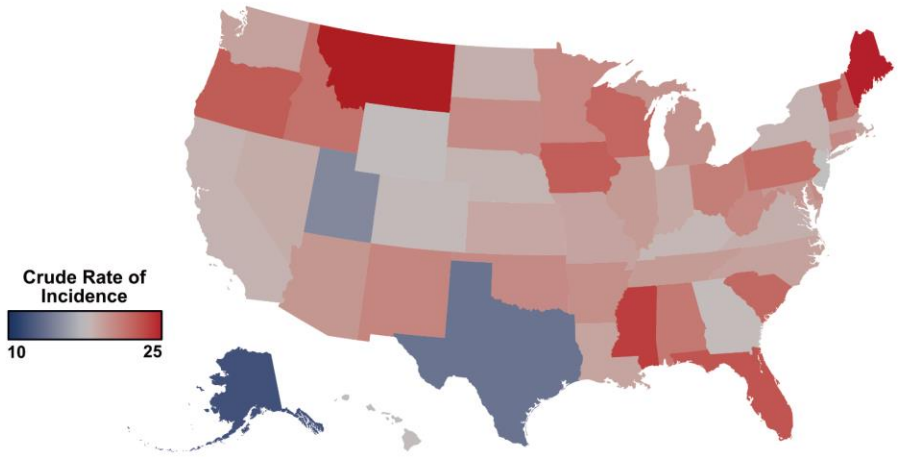
Enlarged prostate? (DRE)

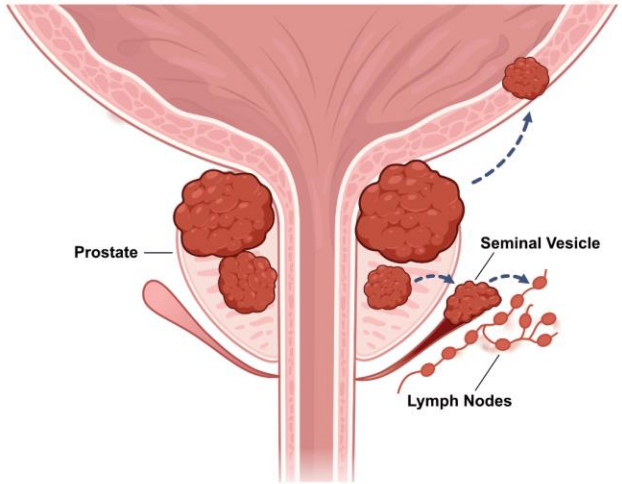
- Benign Prostate Hypertrophy (BPH)
- Prostate Cancer (PCa)
  - ↓
  - Gleason Scoring (Stage I - IV)

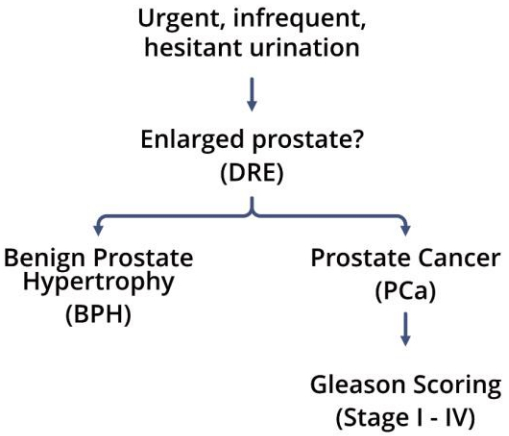


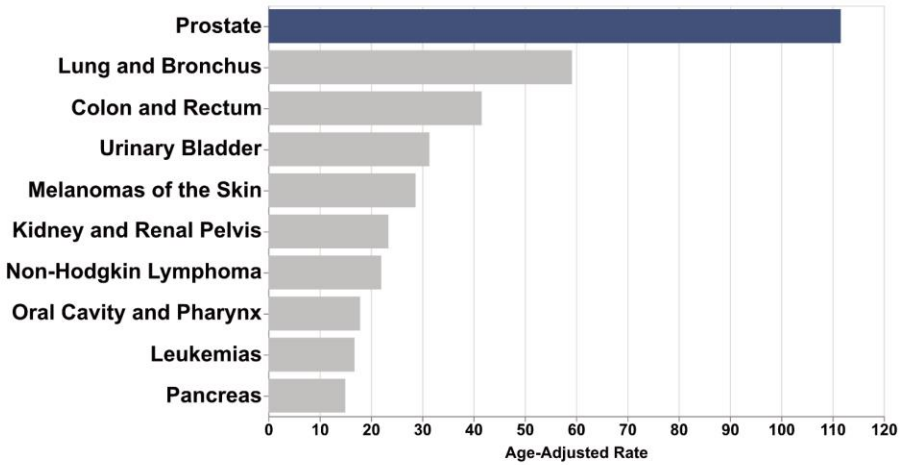
U.S. Cancer Statistics Working Group. U.S. Cancer Statistics. Centers for Disease Control and Prevention and National Cancer Institute. June 2022.

Prostate Cancer Incidence and Outlook





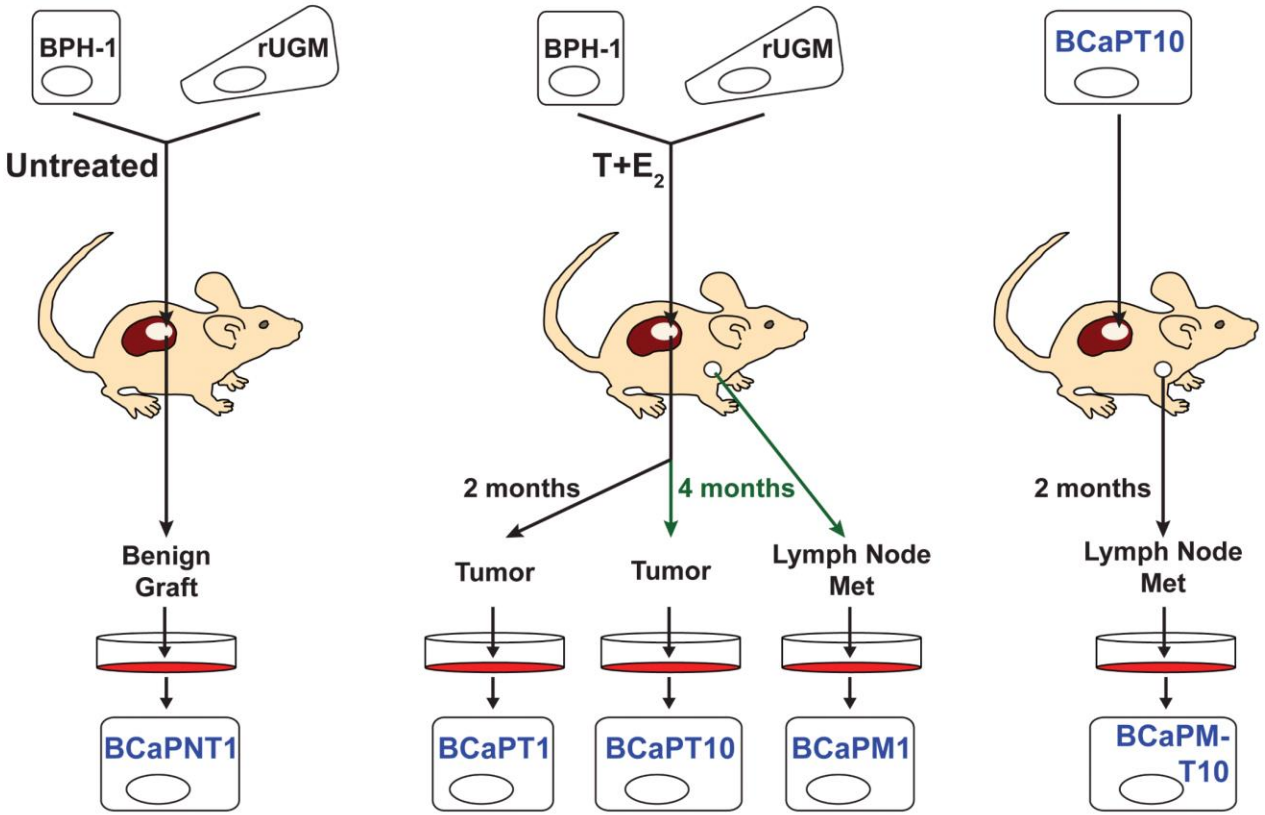




- Leading rates of incidence and mortality necessitate preventative screening
- Urological screening (e.g., DRE) remain the most direct means of PCa detection but still require biopsy and serological confirmation
- Therapeutic monitoring and PCa progression dependent on a single biomarker
- There is substantial need to uncover protein panels that provide unambiguous diagnosis, stratification, and monitoring

U.S. Cancer Statistics Working Group. U.S. Cancer Statistics. Centers for Disease Control and Prevention and National Cancer Institute. June 2022.

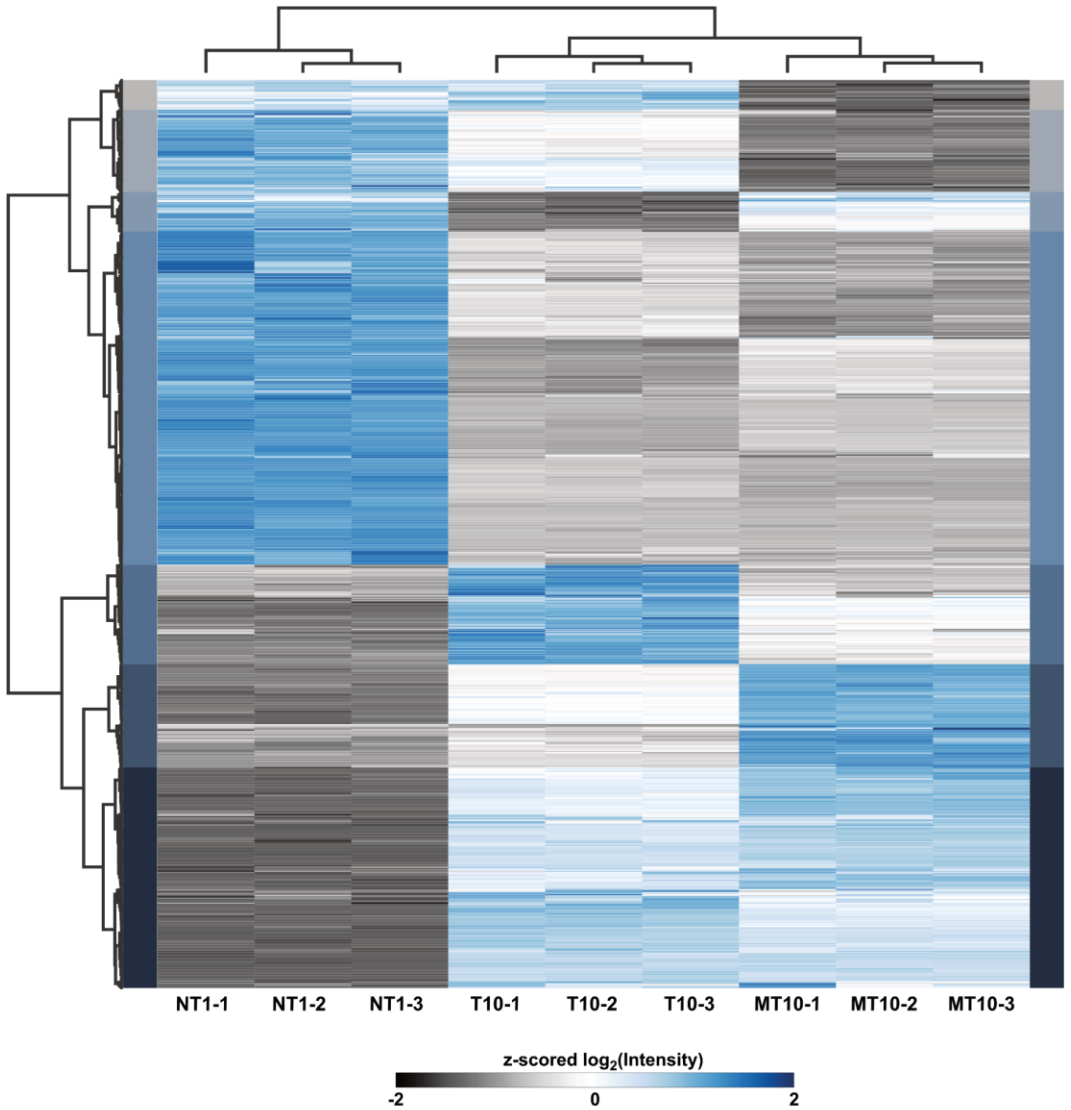
BPH-1-Derived Cancer Progression Cell Model



- Early investigations demonstrated Benign Prostatic Hypertrophy (BPH) can progress to cancer in presence of hormones
- Ricke and colleagues developed cell line model of cancer progression that mimics the genetic, phenotypic, and molecular characteristics of PCa
- Proteomic analyses of discrete phenotypes will help illuminate the proteomic organization specific to PCa progression

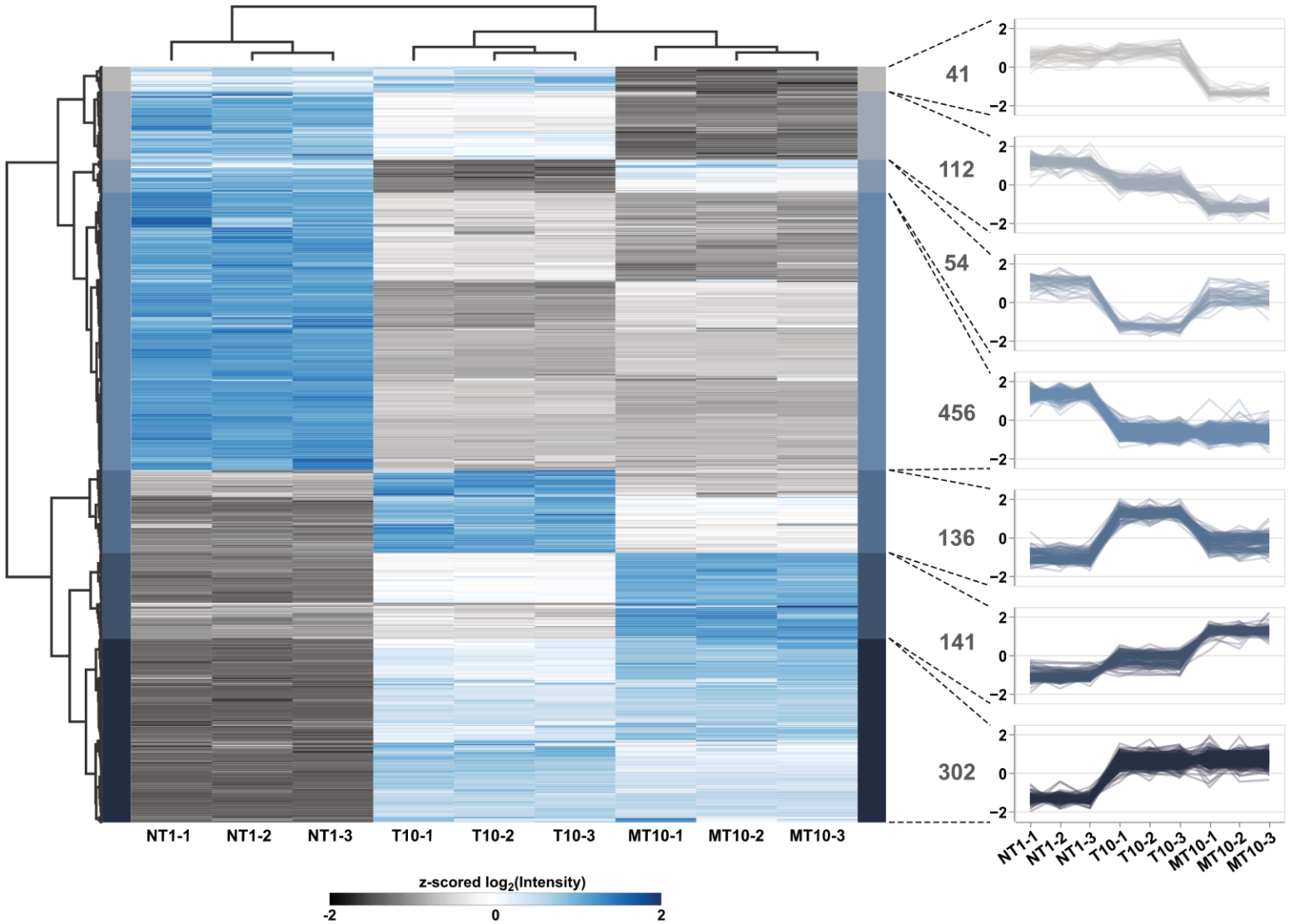
Liu, T. T., et al. (2019). "Modeling human prostate cancer progression in vitro." Carcinogenesis **40**(7): 893-902.

PCa Proteomic Fingerprinting



D.G. Delafield, *et al*. **In Preparation**.

PCa Proteomic Fingerprinting



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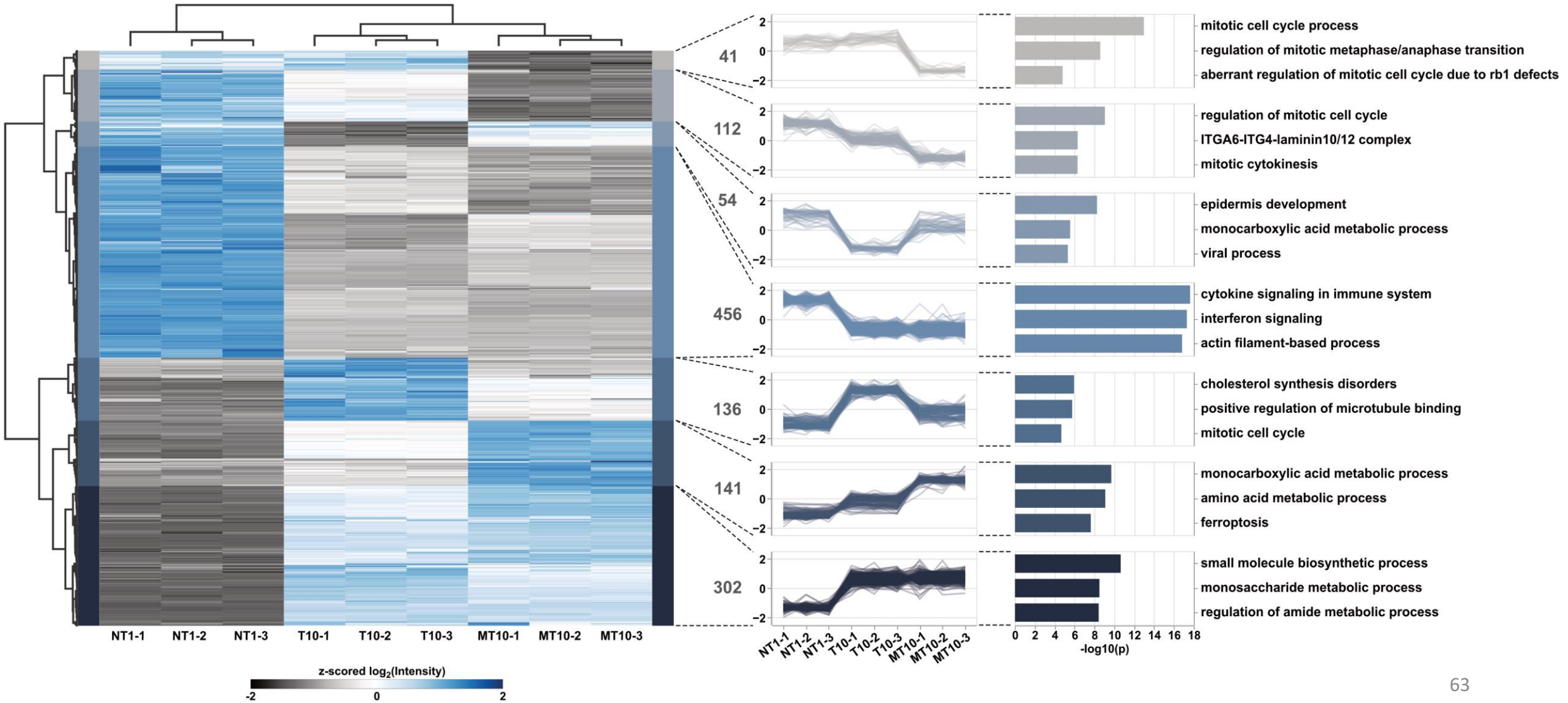
PCa Proteomic Fingerprinting



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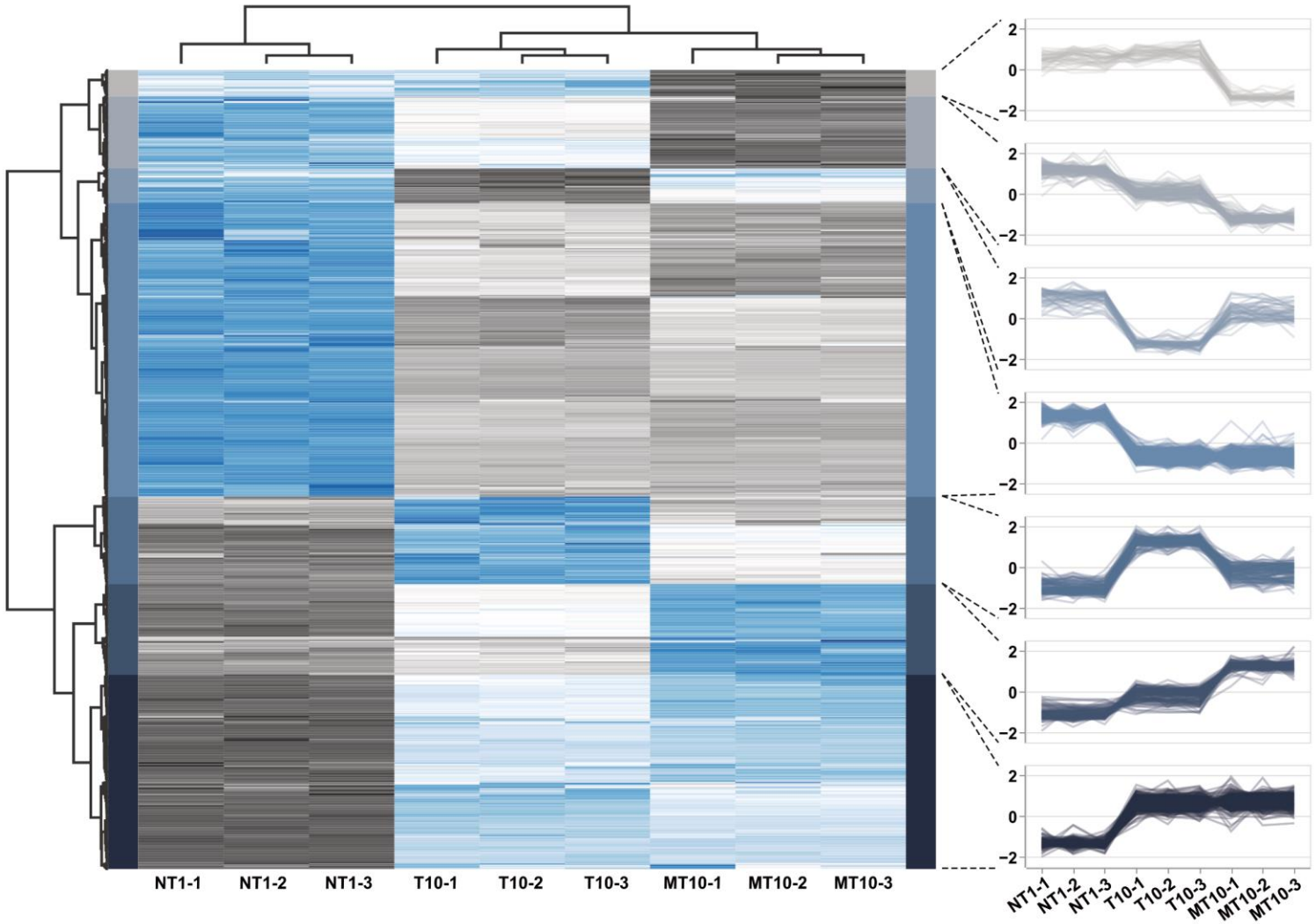
Future Directions

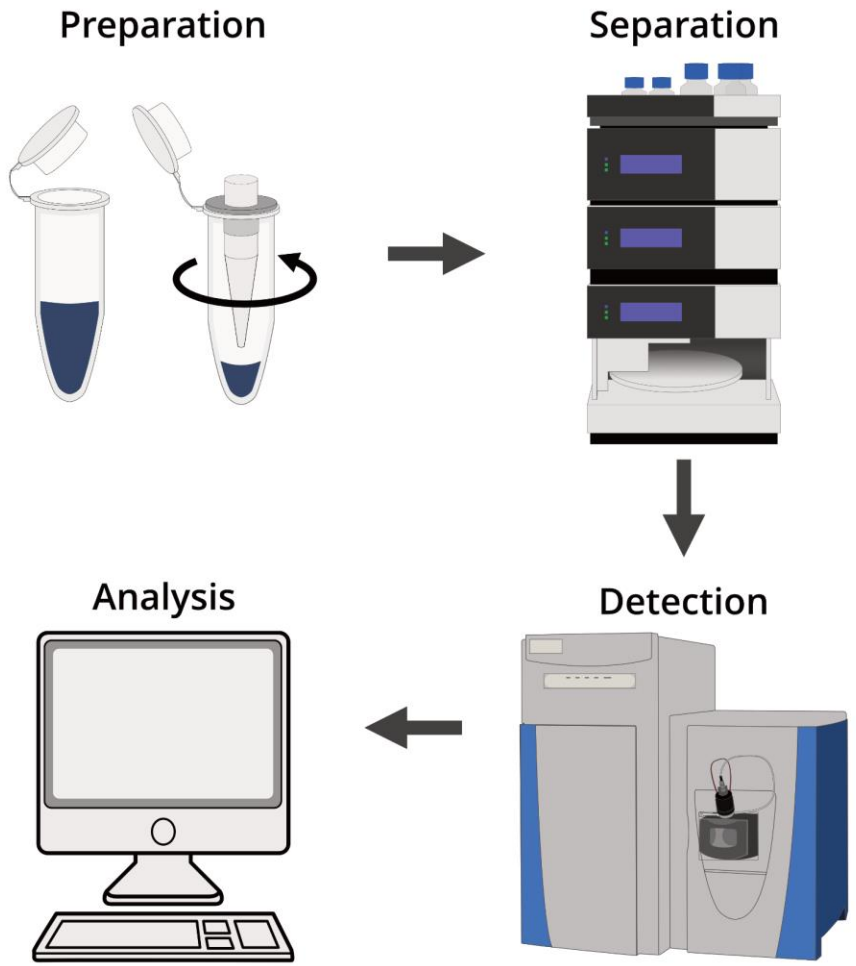


Takeaways

- DIA analyses present unparalleled access to the human proteome and enable reproducible protein quantitation.
- We provide early understanding of the biomolecular fingerprints of BCaP phenotypes.
- Conserved dysregulation of protein communities provide suitable targets for biomarker validation, markers of malignancy and therapeutic monitoring.

D.G. Delafield, *et al*. **In Preparation**.

Conclusions



- Mass-spectrometry based proteomics serves as a premier method for biomolecular investigations.
- Proteomics will forever be searching for a 'more complete' picture.
- Our interpretation of results and underlying biology are biased by the strategies employed.
- Alternative separations and acquisition strategies compliment our current understanding and traditional strategies.

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Zexin Zhu
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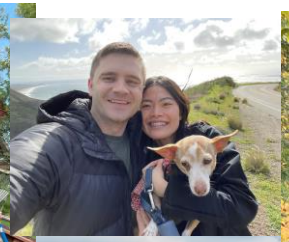
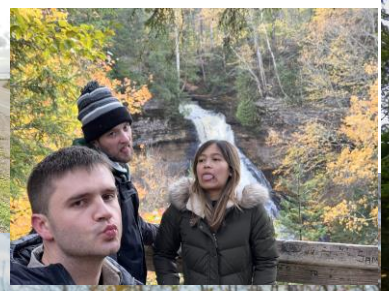
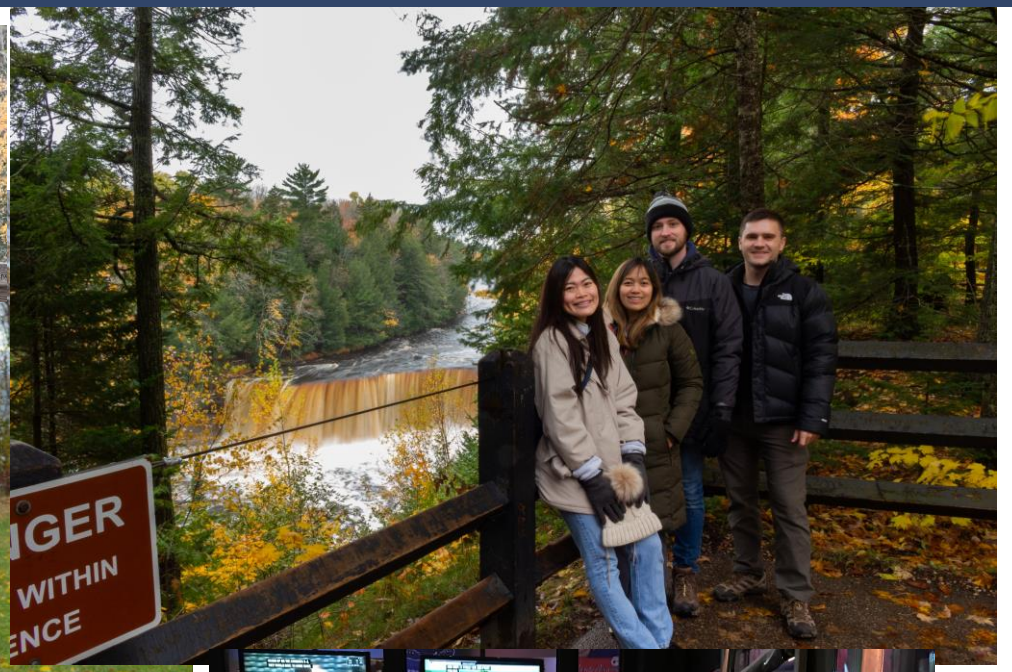
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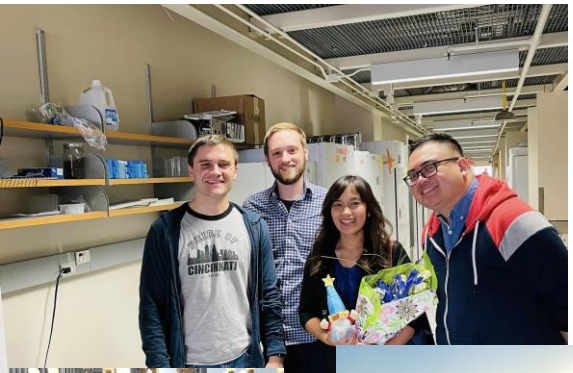
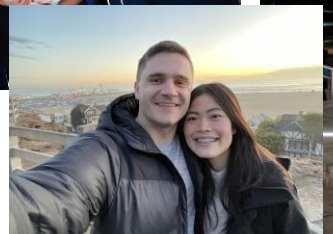
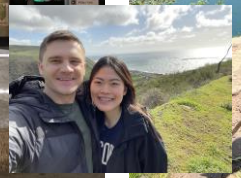
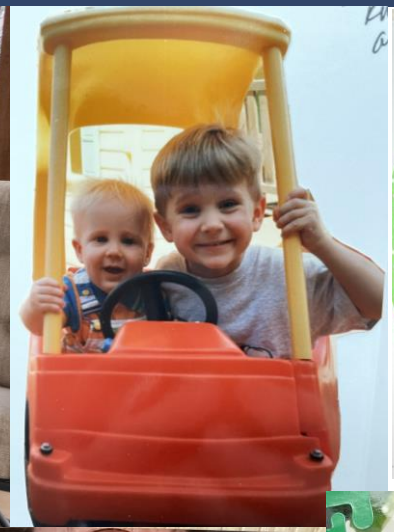
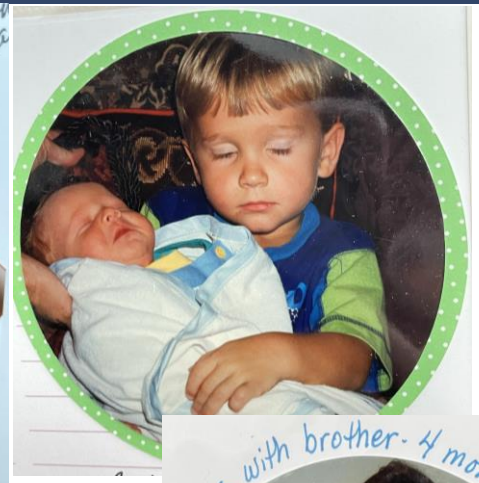


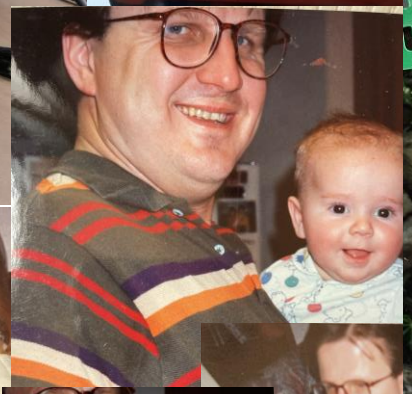


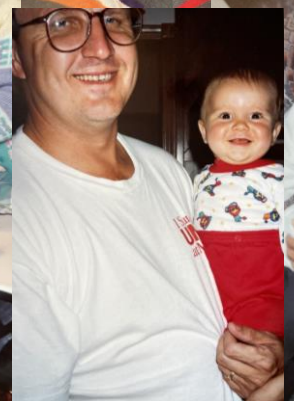






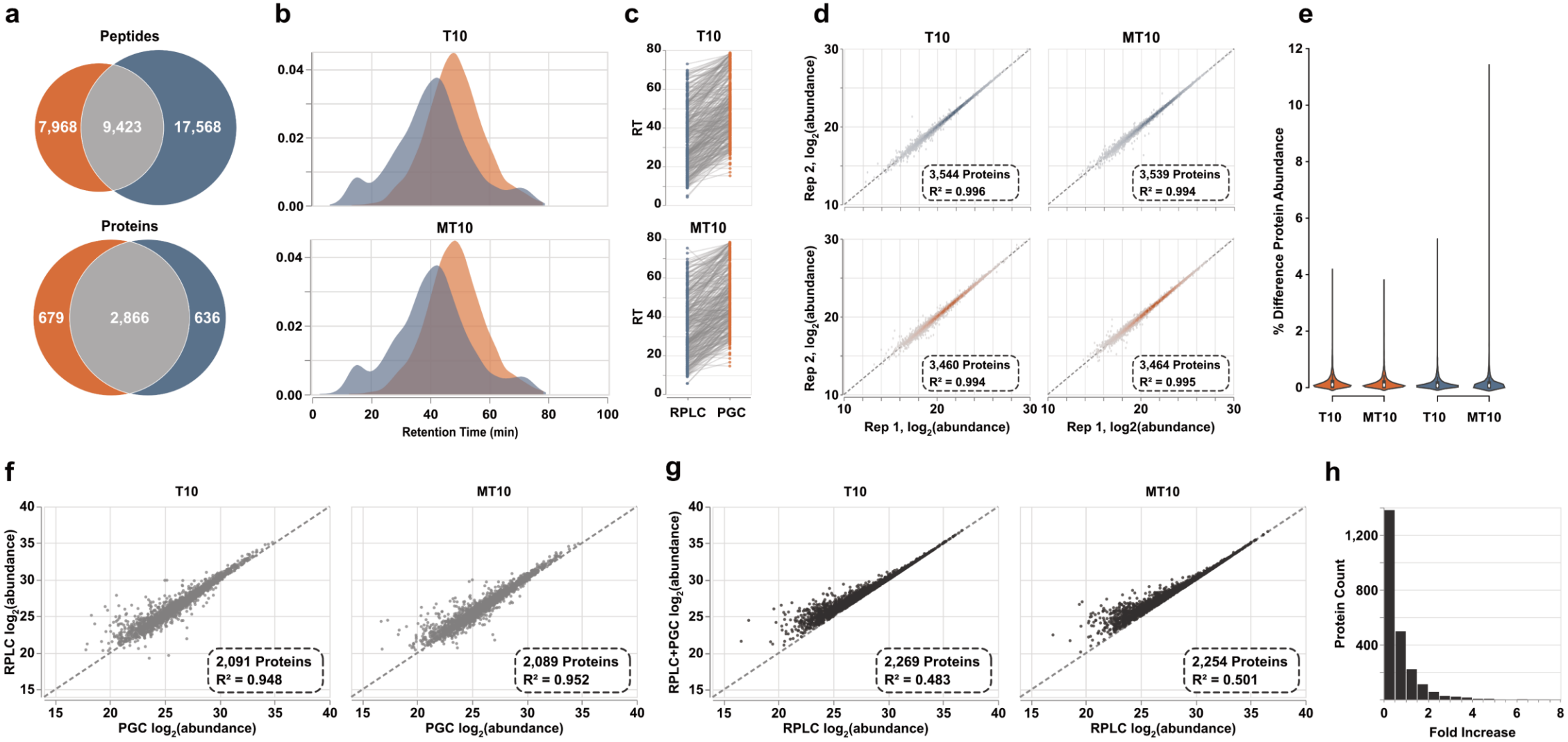

with brother- 4 months





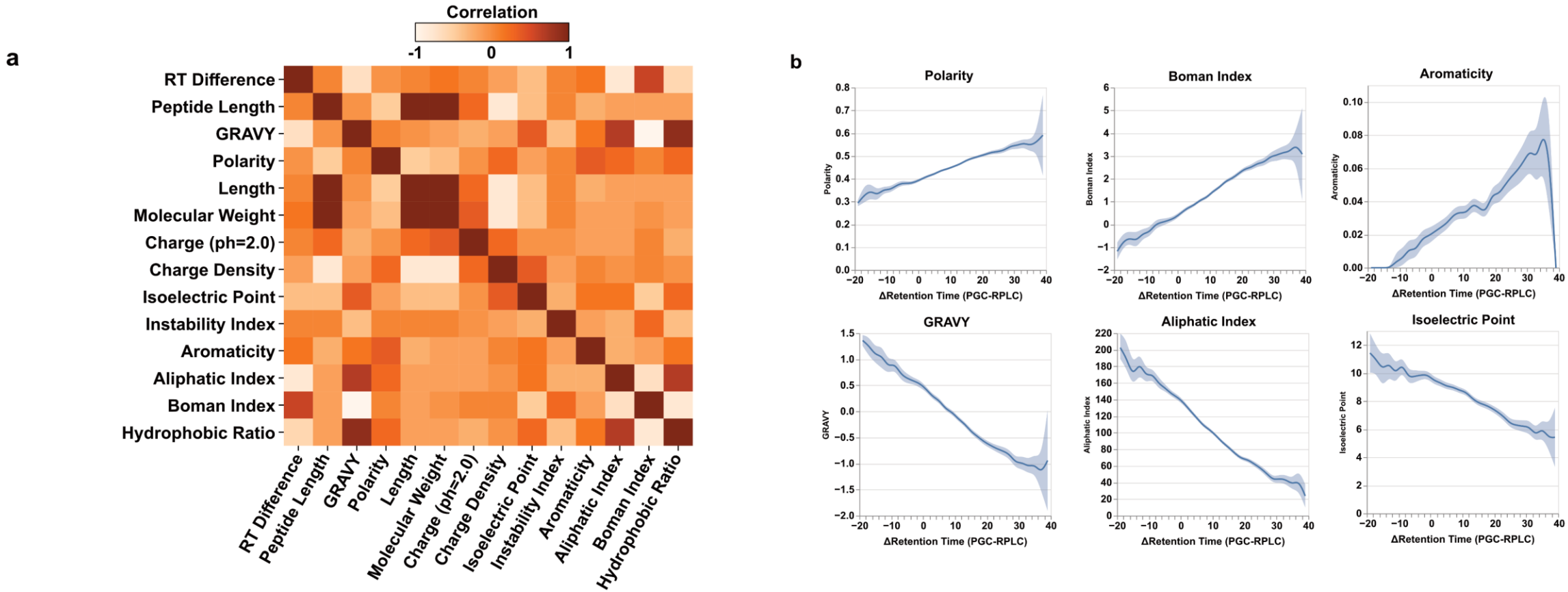
Supplemental

How PGC Separations Affect Proteomic Measurement



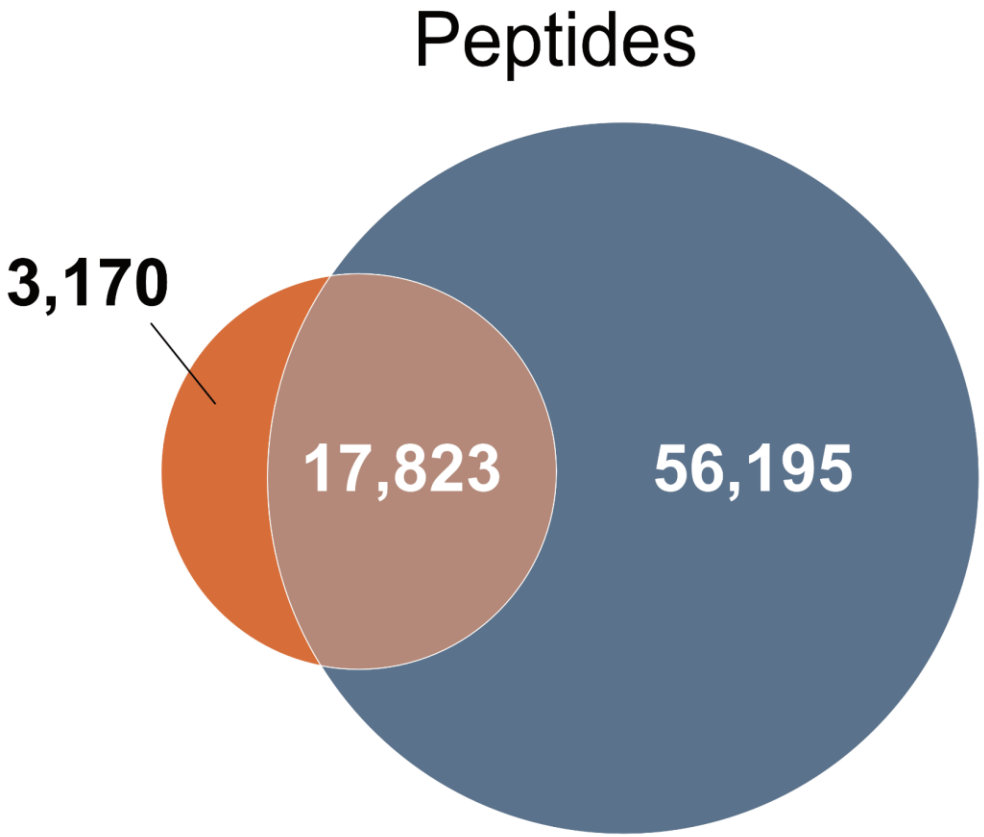
Delafield, D. G., et al. (2023). In Submission.

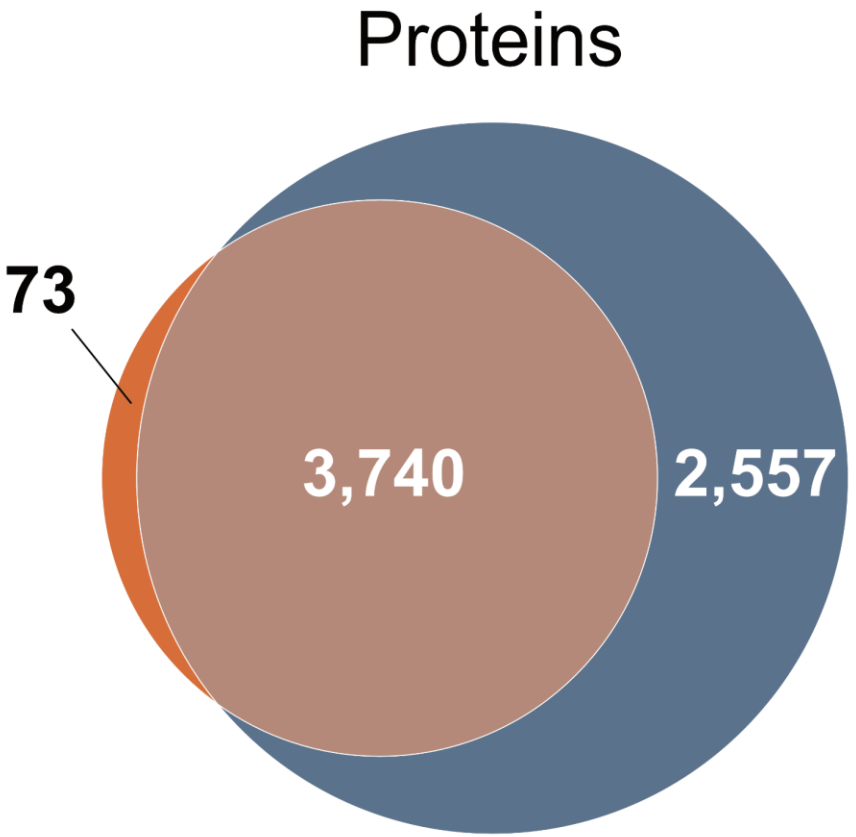
Peptide Character Affecting PGC Retention



Delafield, D. G., et al. (2023). In Submission.

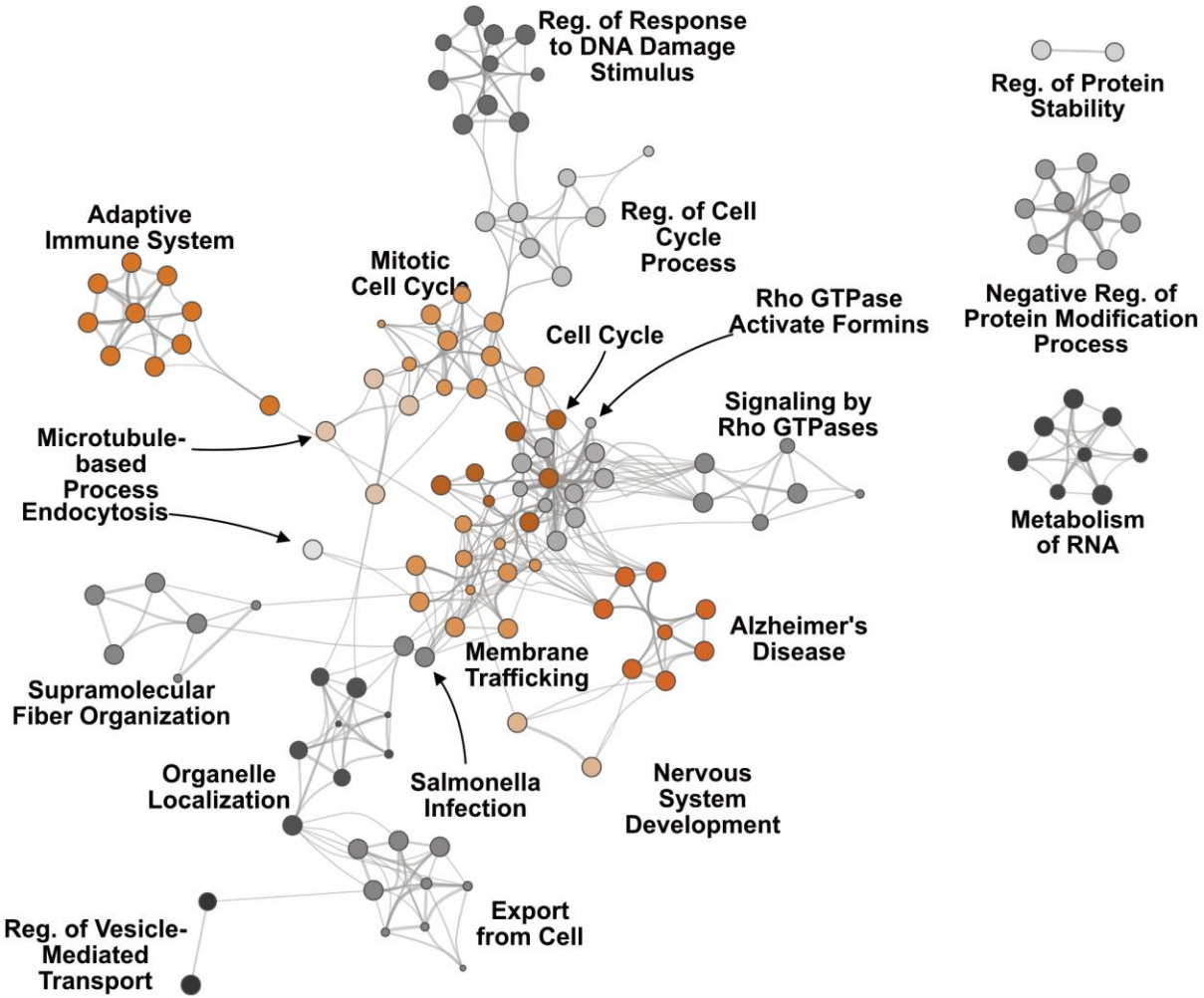
Library-Free Comparison, RPLC vs. PGC

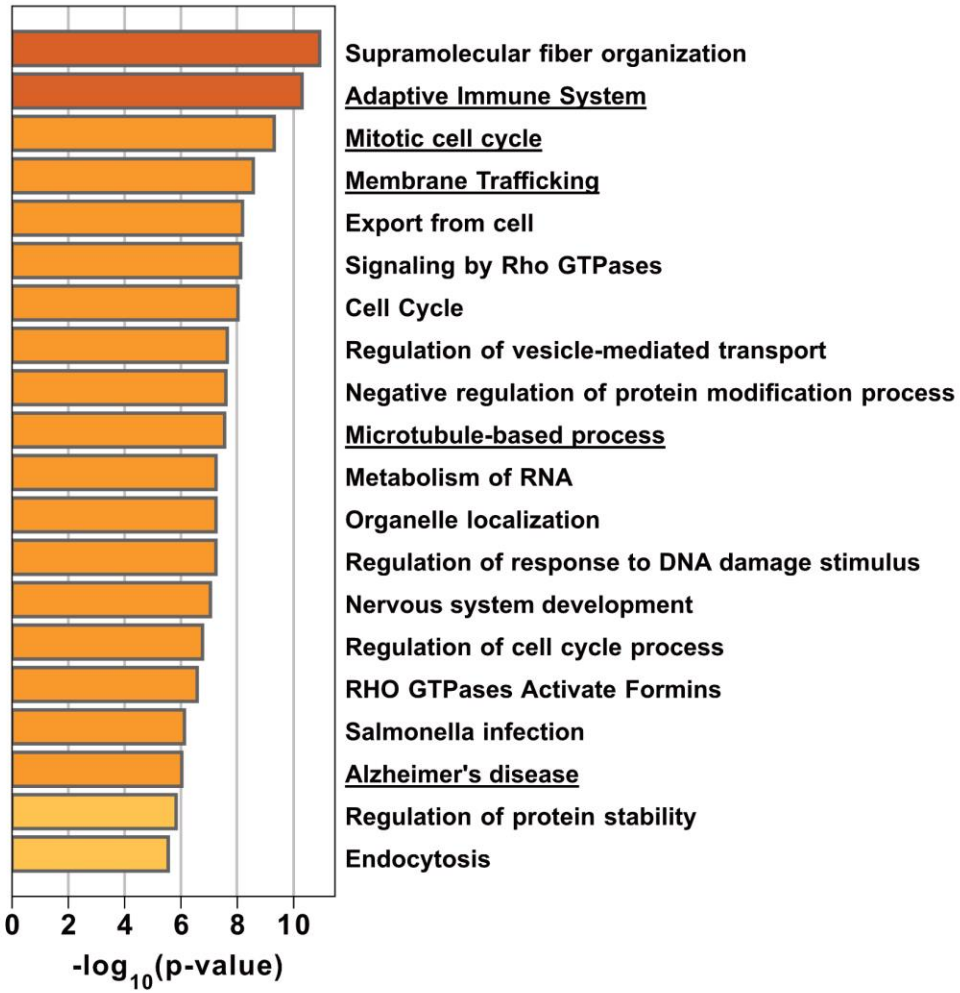




Delafield, D. G., et al. (2023). In Submission.

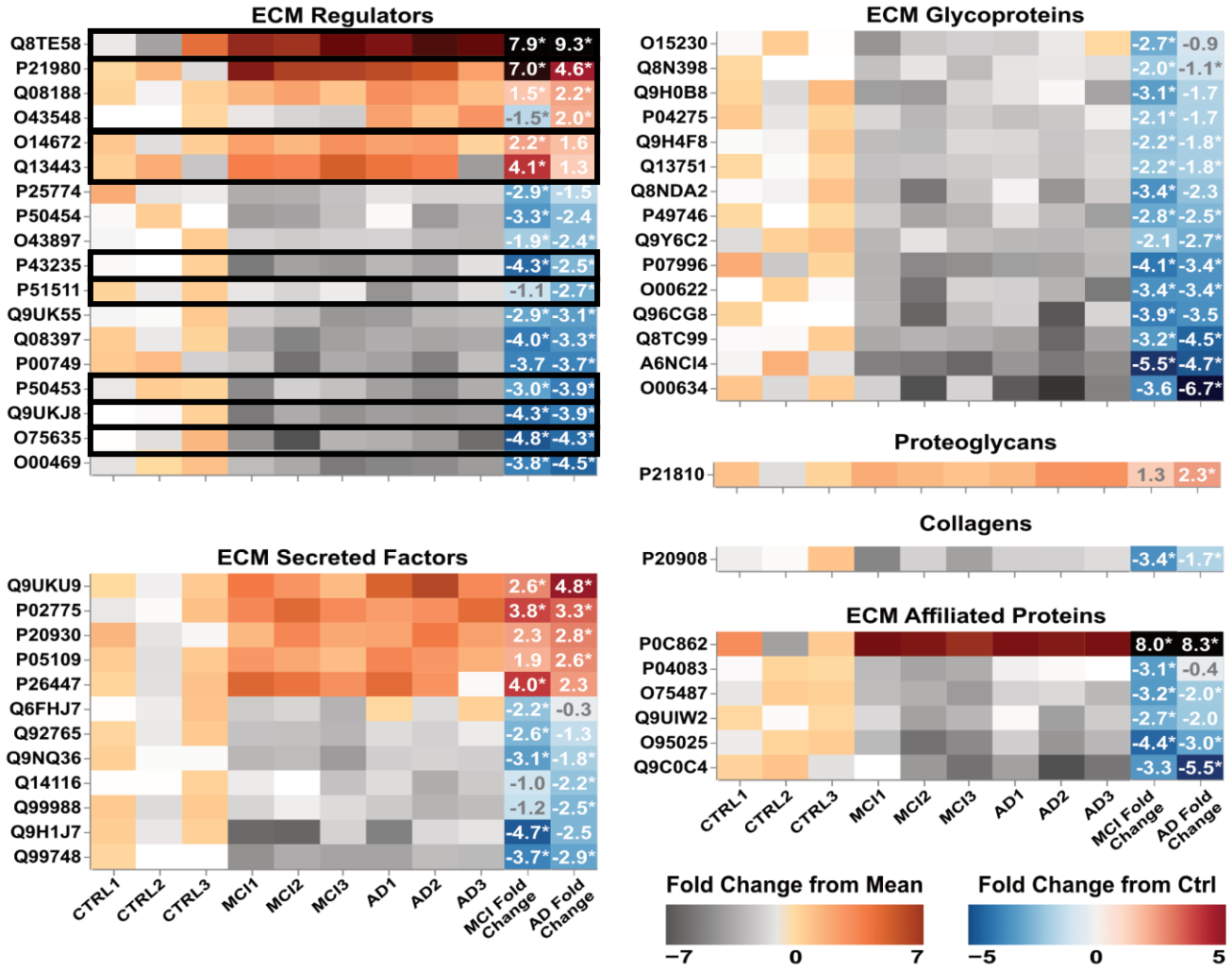
Dysregulated Pathways





Zhou Y, Zhou B, et al. Nat Commun. 2019 Apr 3;10(1):1523.
D.G. Delafield*, X. Zhong*, et al. In preparation.

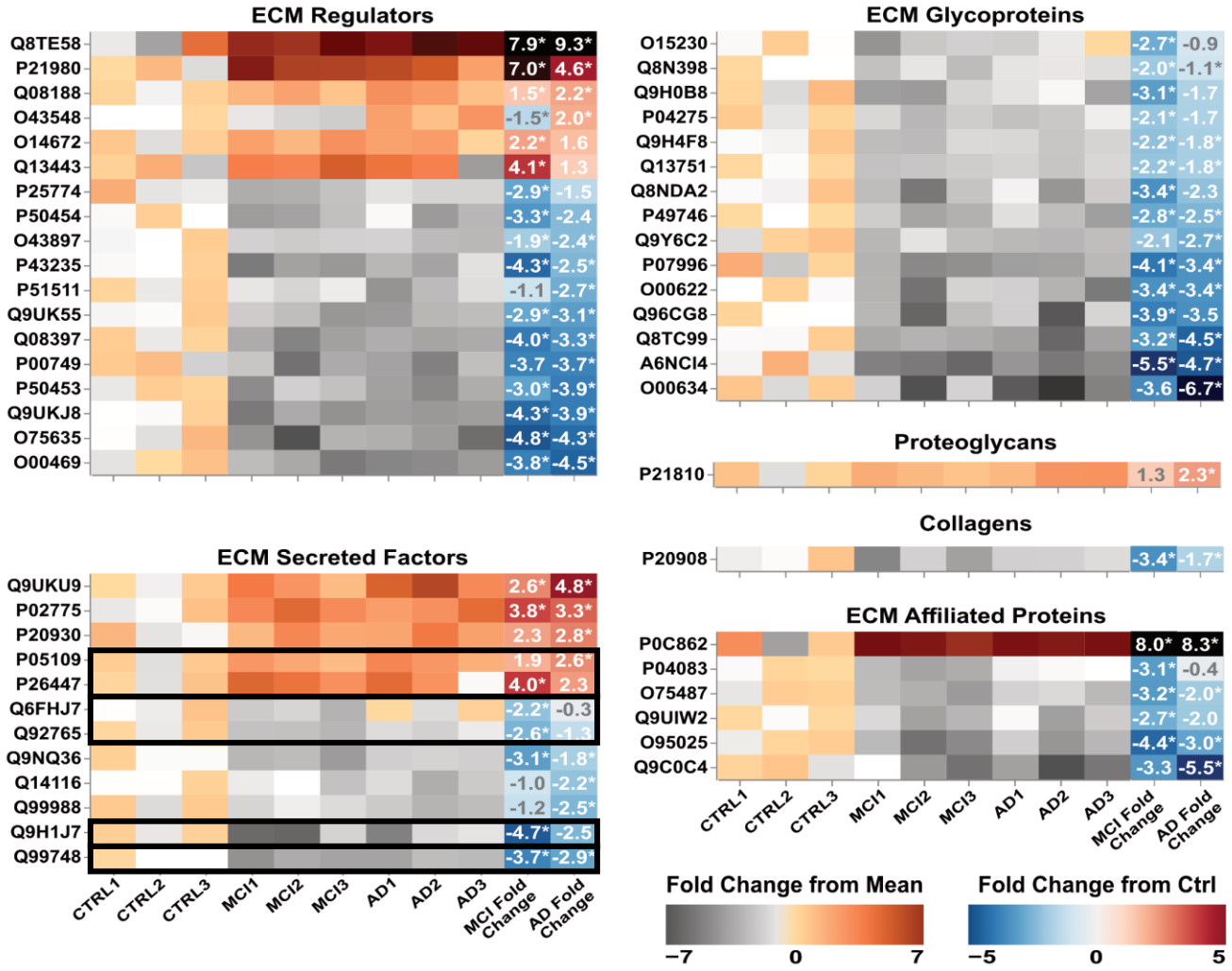
ECM Reorganization



Dysregulated Proteins

- Disentegrin and metalloproteinases
- Gamma glutamyltransferases
- Serpins

ECM Reorganization



Dysregulated Proteins

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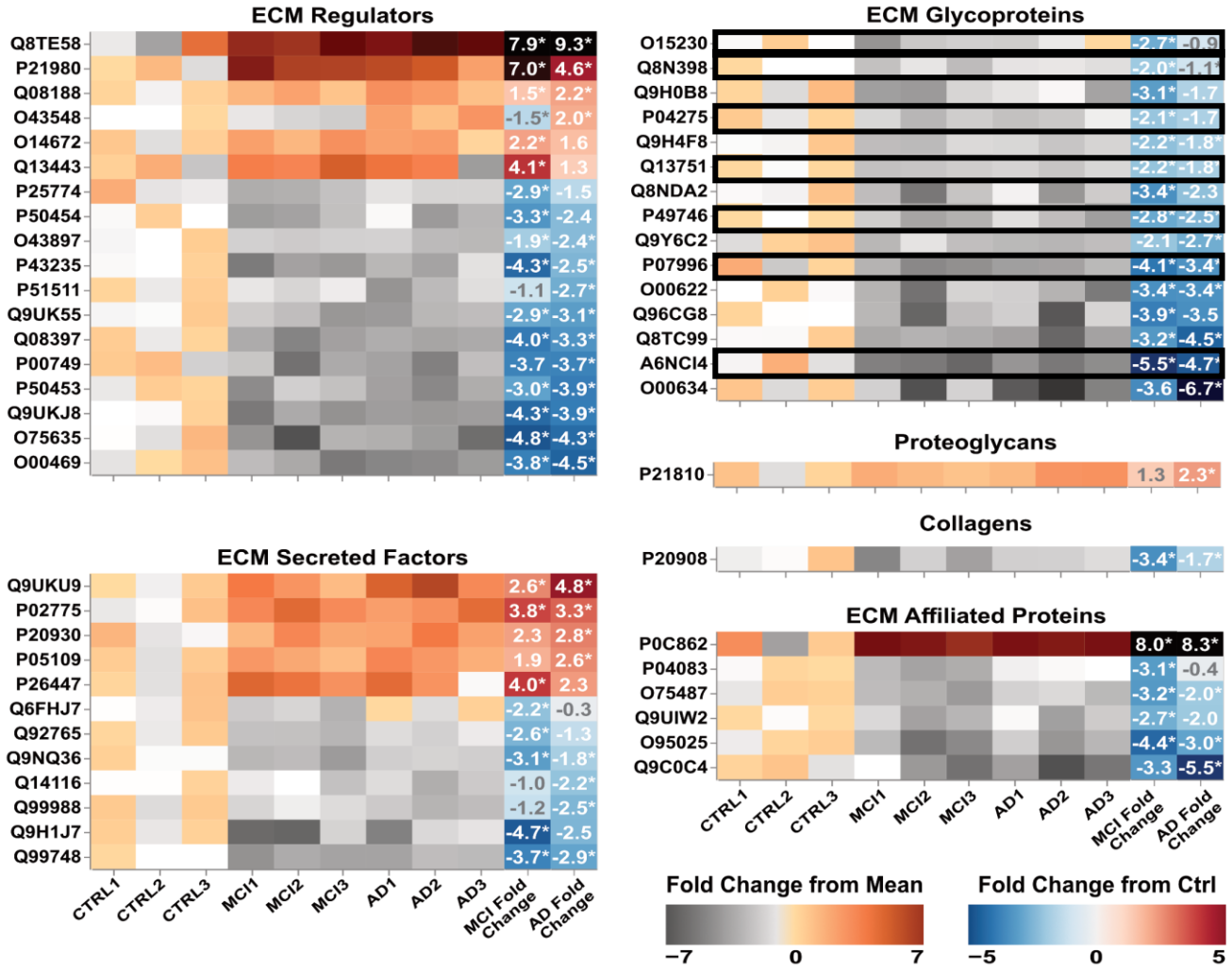
- Neurturin

ECM Reorganization



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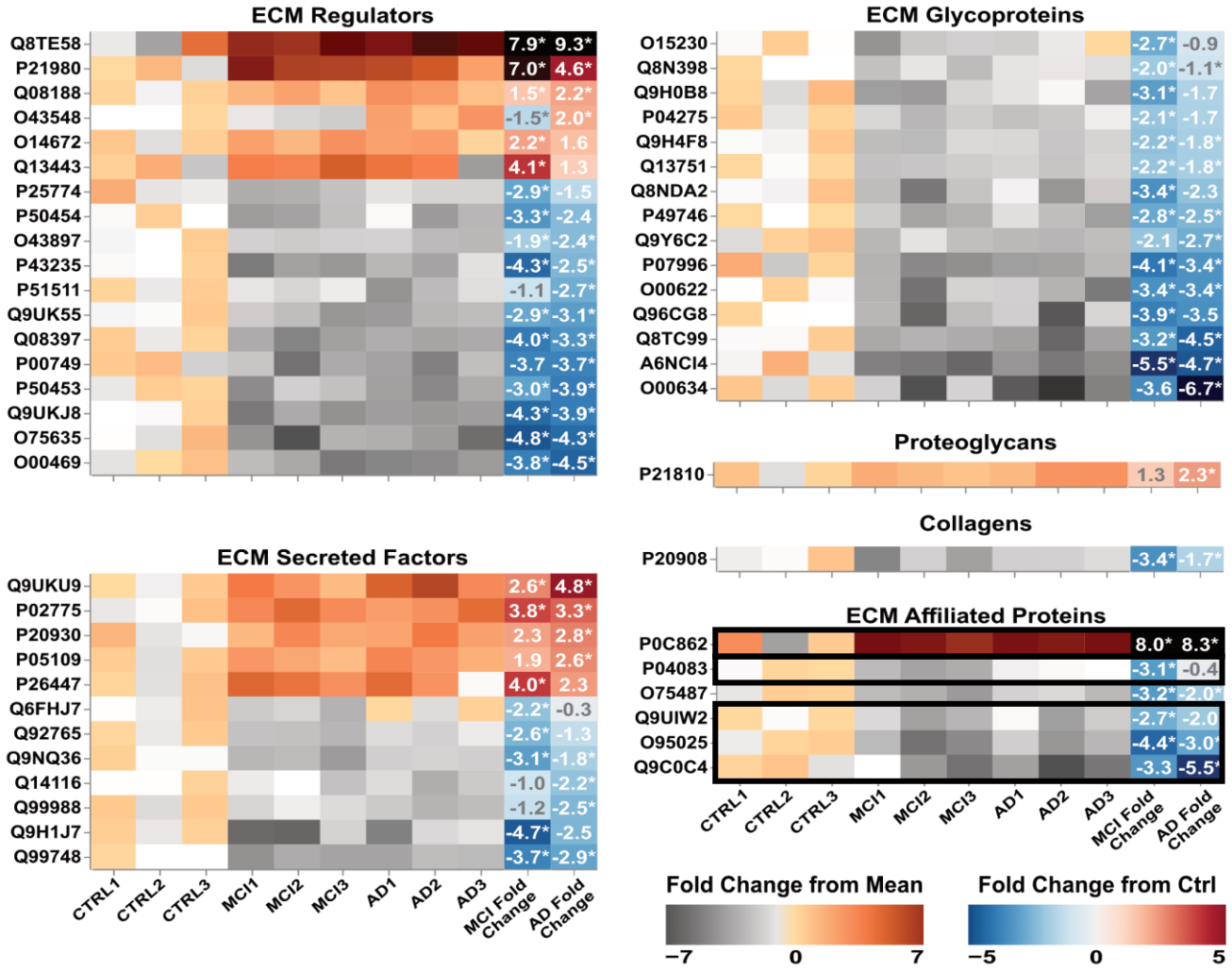
- Lamanin Subunits
- Thrombospondins

ECM Reorganization



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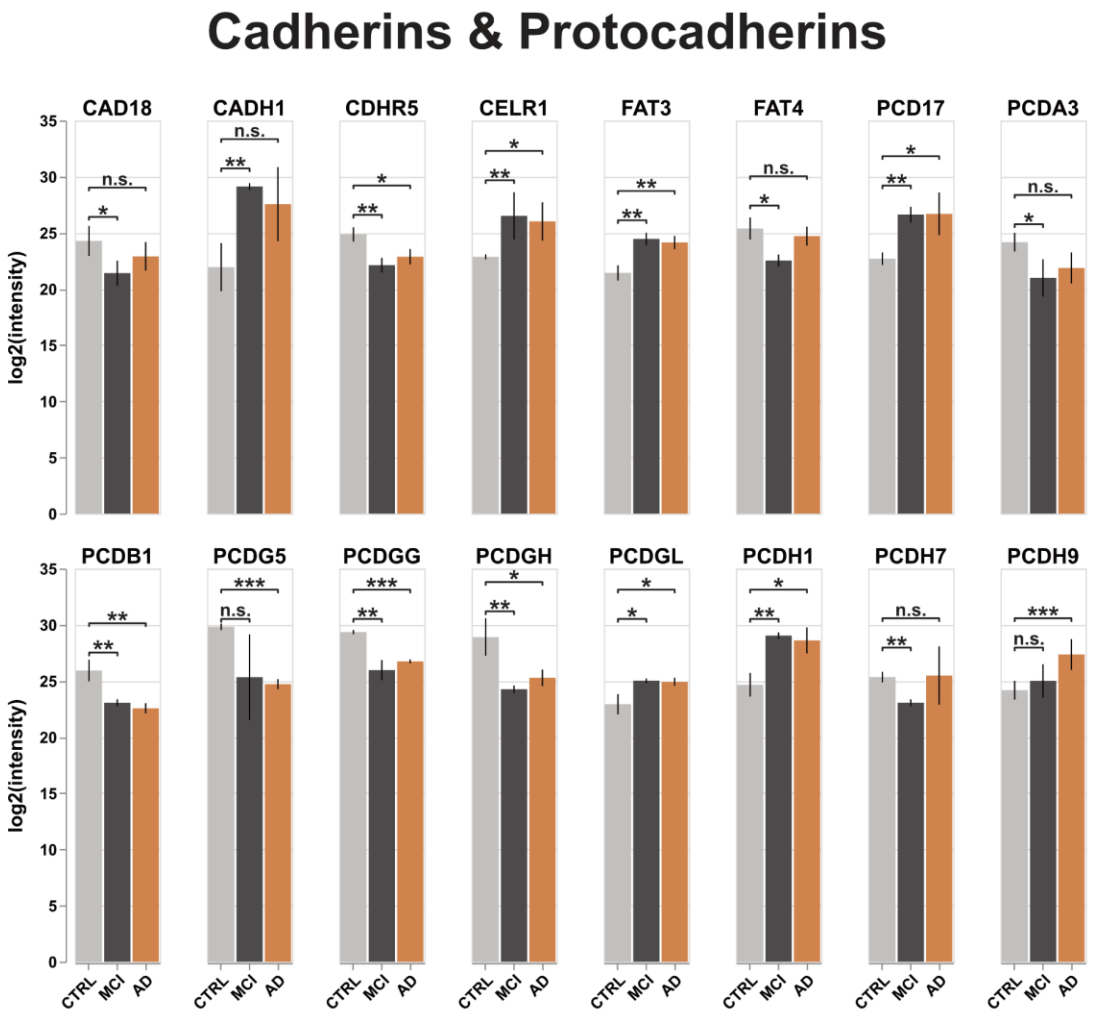
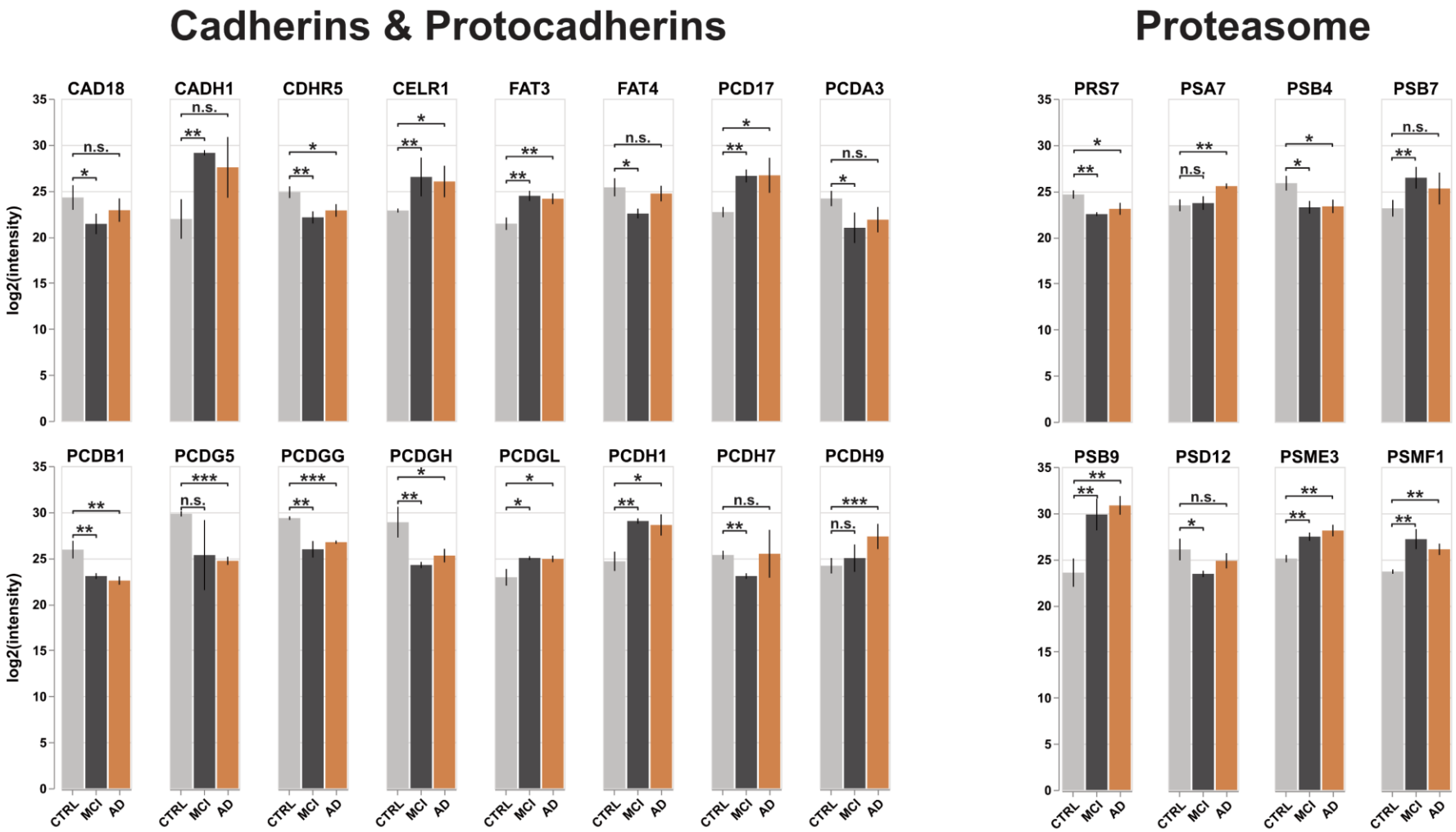
- Lamanin Subunits
- Thrombospondins
- Plexin and Semaphorins
- Annexin
- Complement C1q

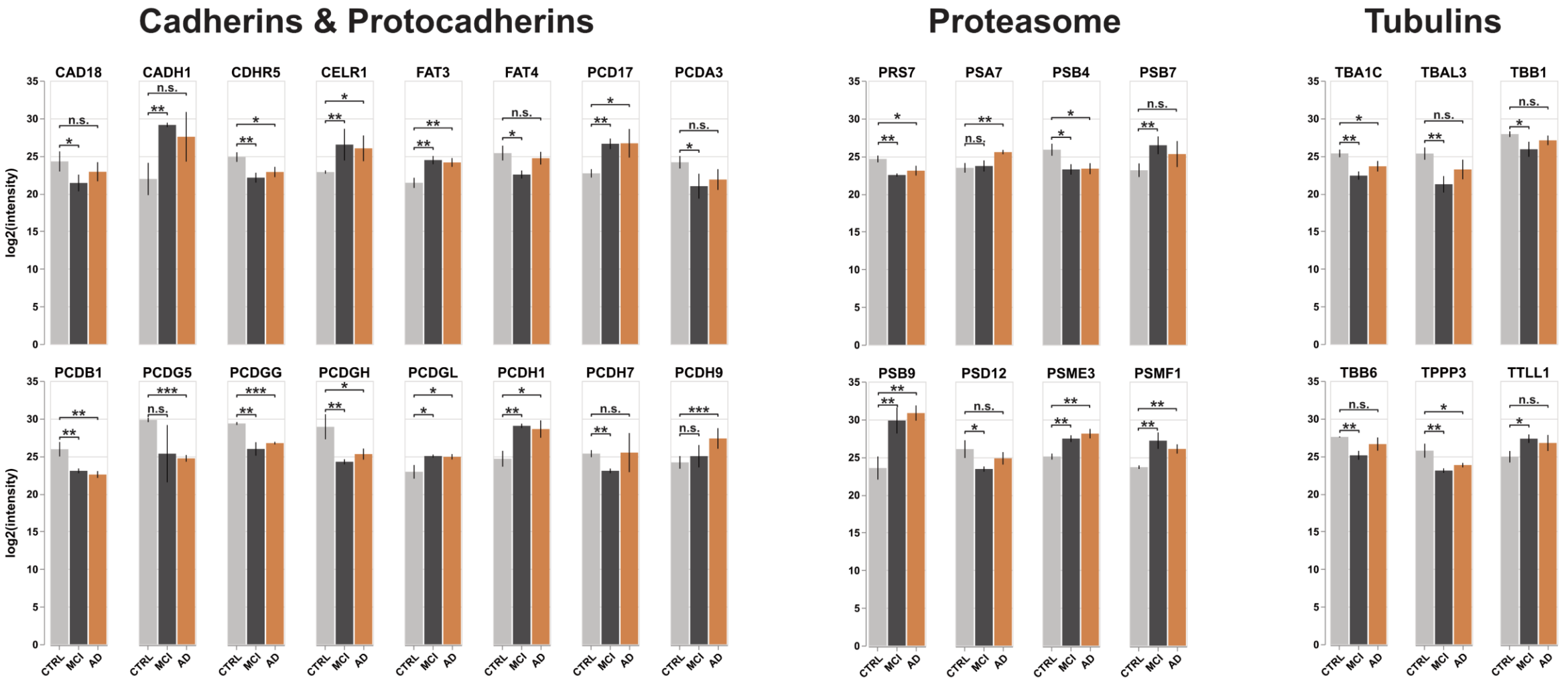
Protein Class Dysregulation



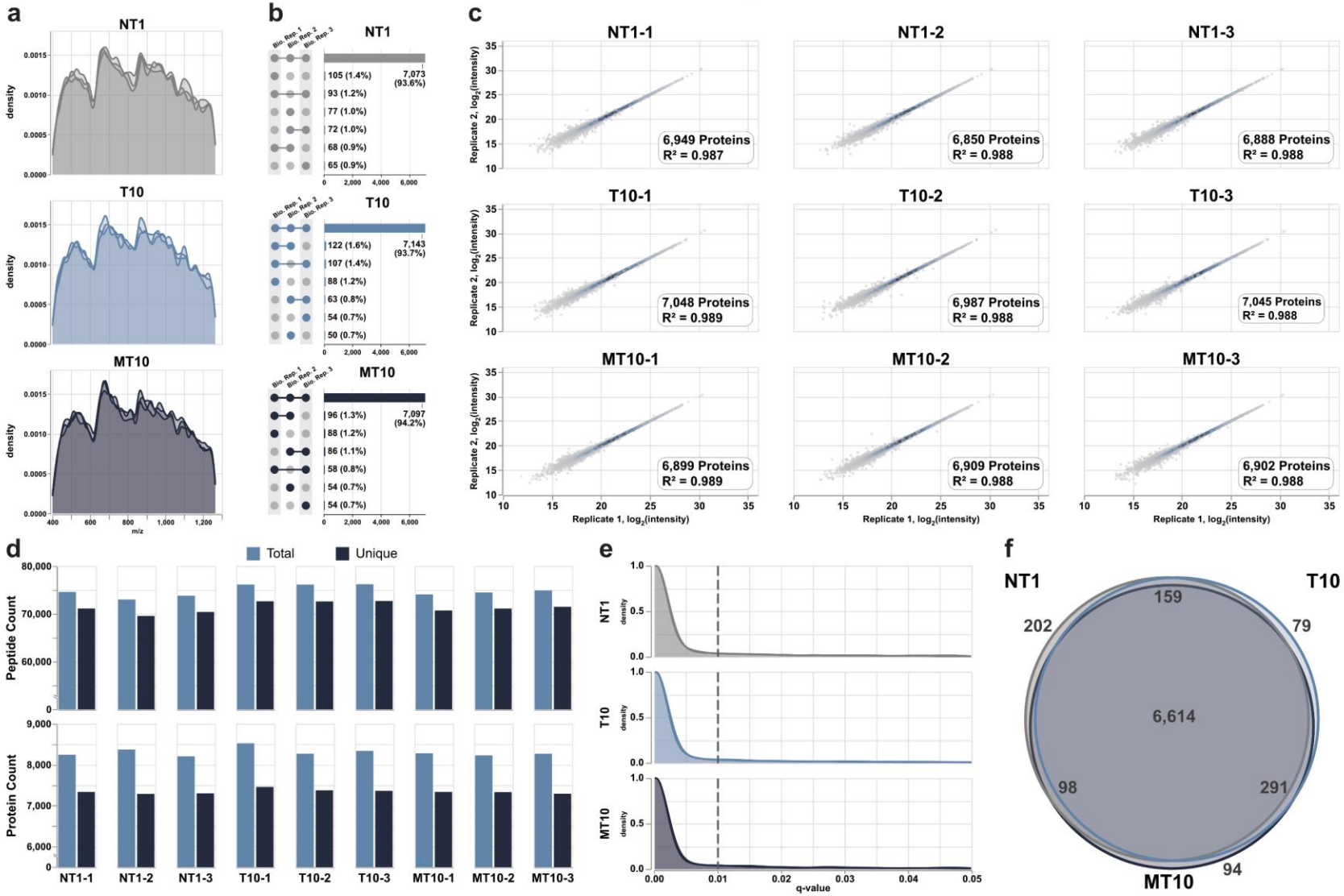
Protein Class Dysregulation



Protein Class Dysregulation

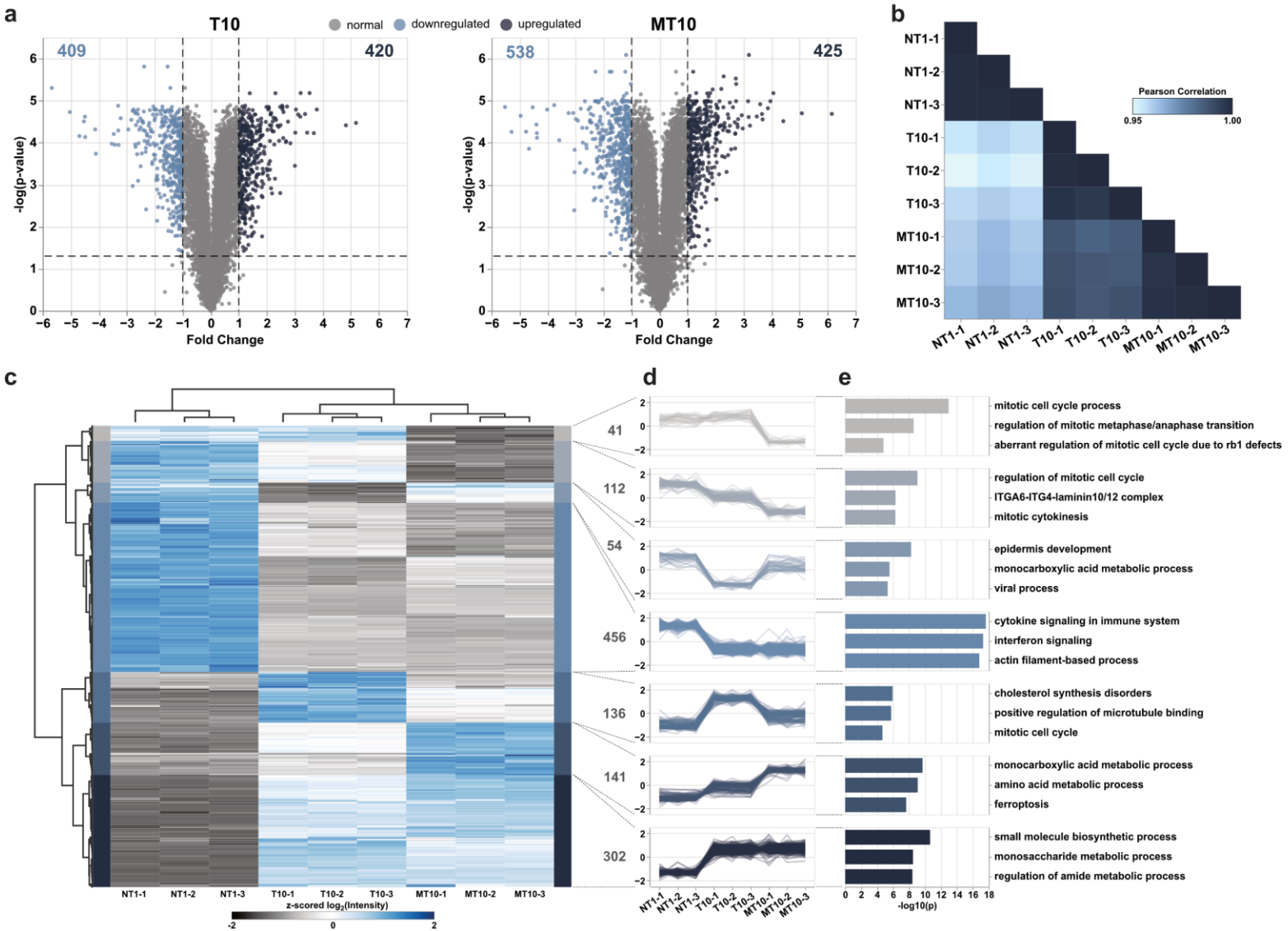


DIA Provides Extensive Profiling Access



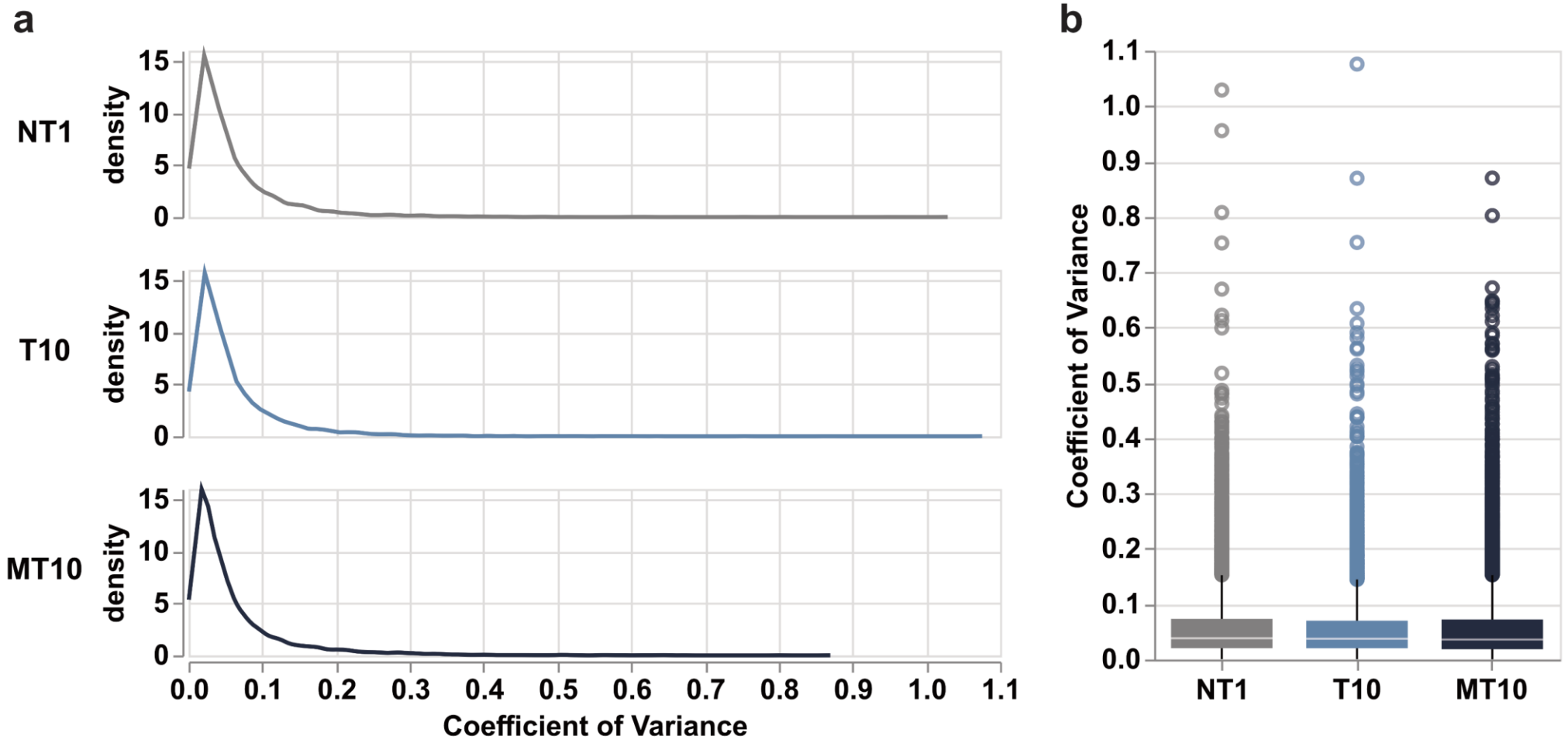
D.G. Delafield, *et al*. **In Preparation**.

DIA Provides Extensive Profiling Access



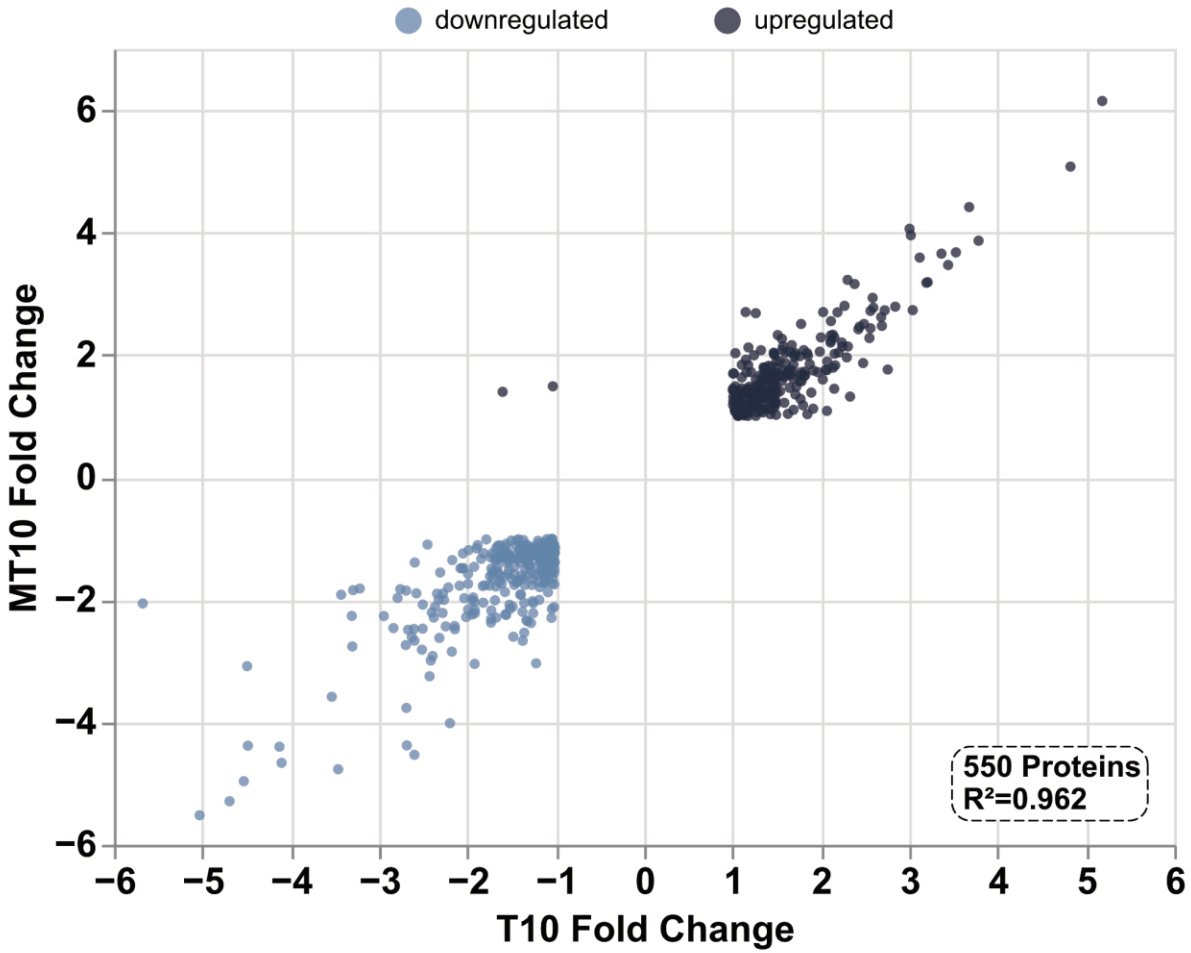
D.G. Delafield, *et al*. **In Preparation**.

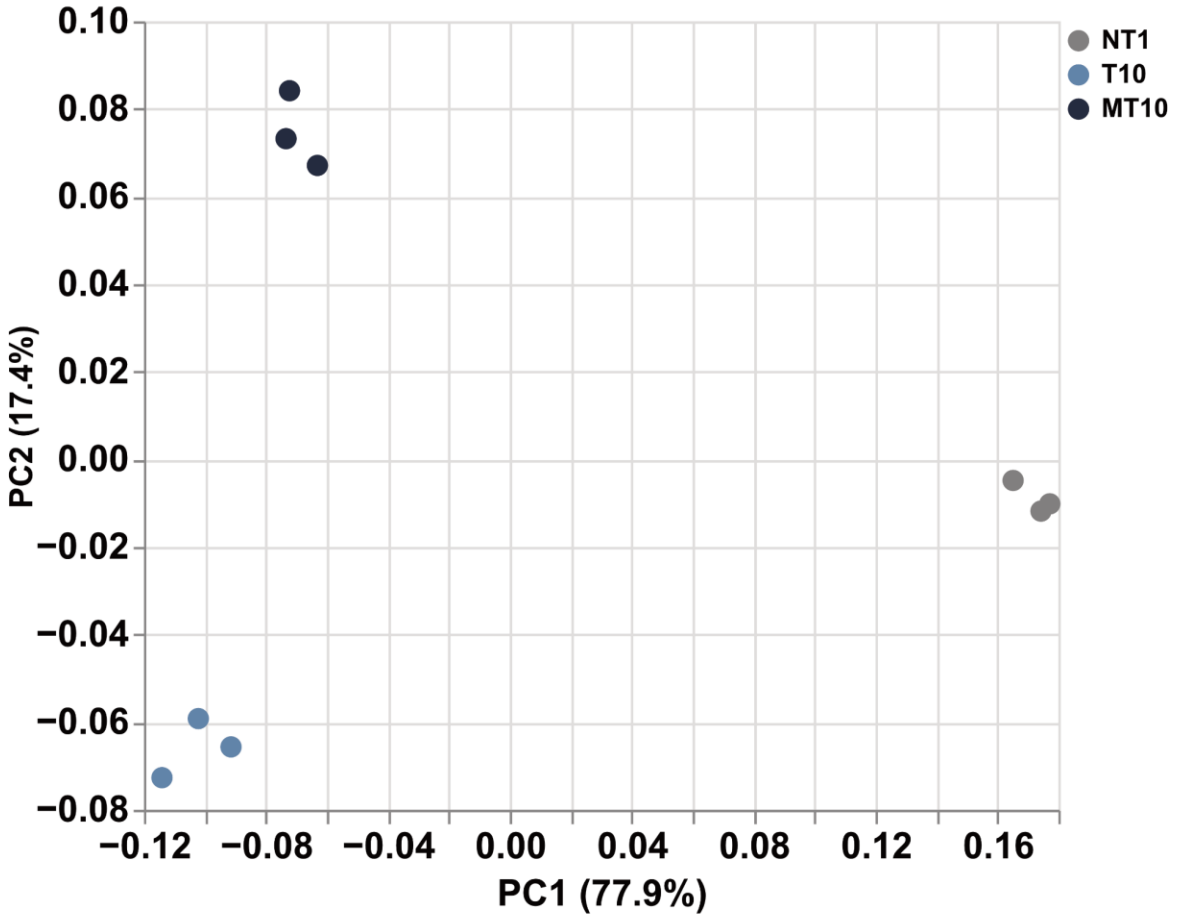
Variance in BCaP DIA Measurements



D.G. Delafield, *et al*. **In Preparation**.

Correlation and Dimensional Reduction





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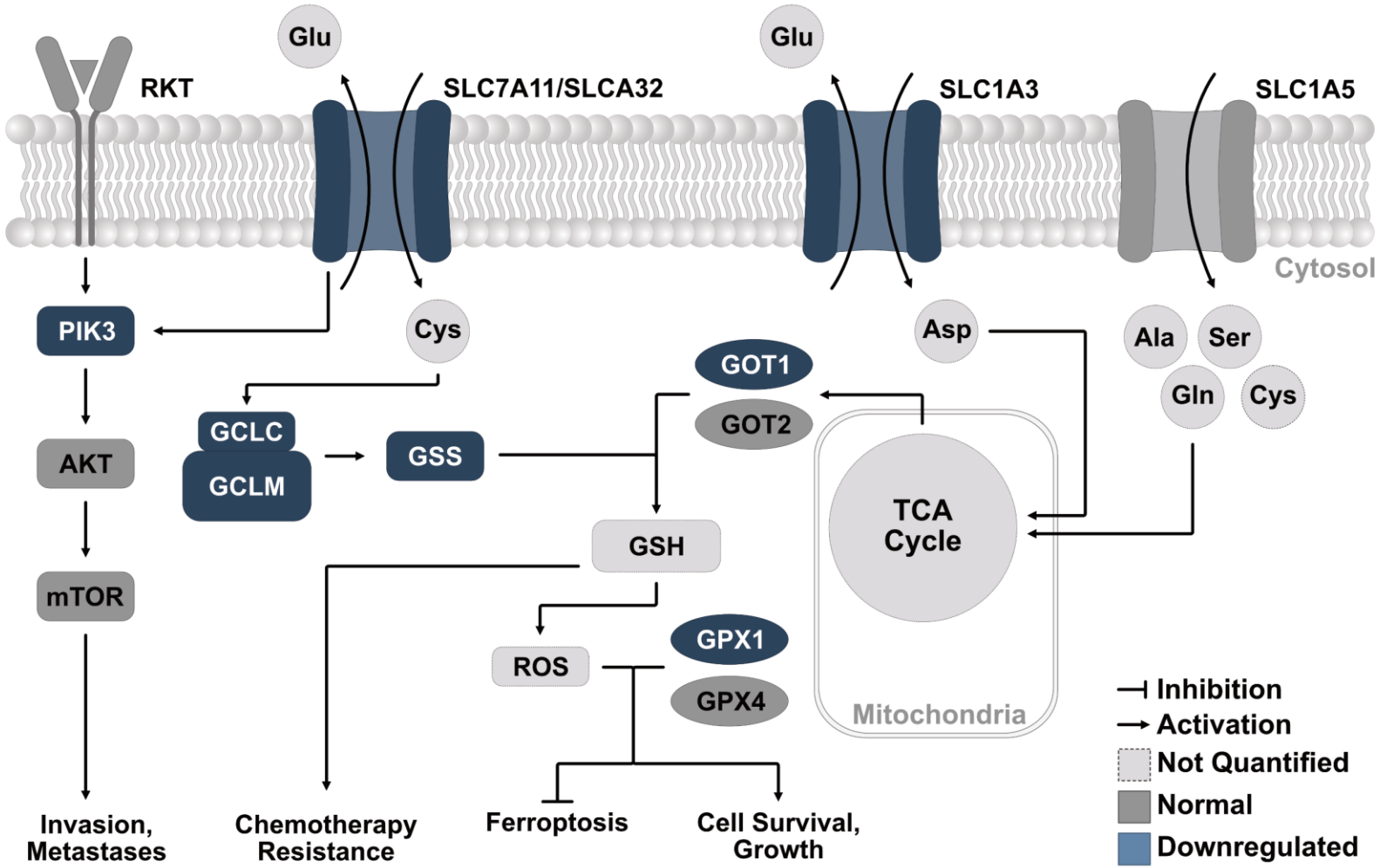
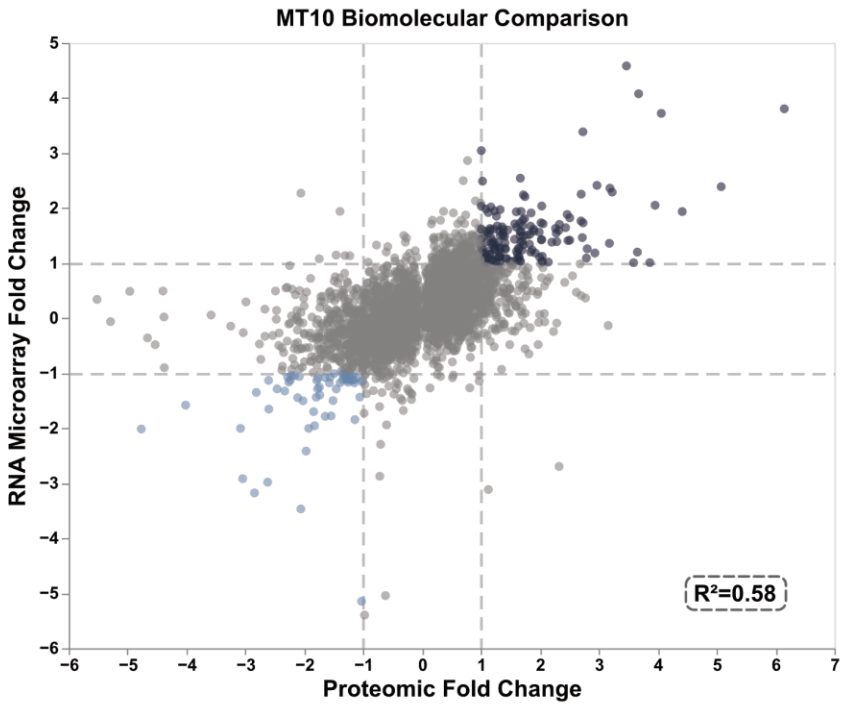
GSH Overproduction in PCa



- Proteomic analyses reveal upregulation of proteins involved in GSH production
- Increased GSH is known to confer chemotherapy resistance
- Normal and upregulated expression of glutathione peroxidase suggests inhibition of ferroptosis and increased survival
- Future investigations should seek to directly quantify GSH *in vivo*

D.G. Delafield, *et al*. **In Preparation**.

Illuminating Putative Biomarkers



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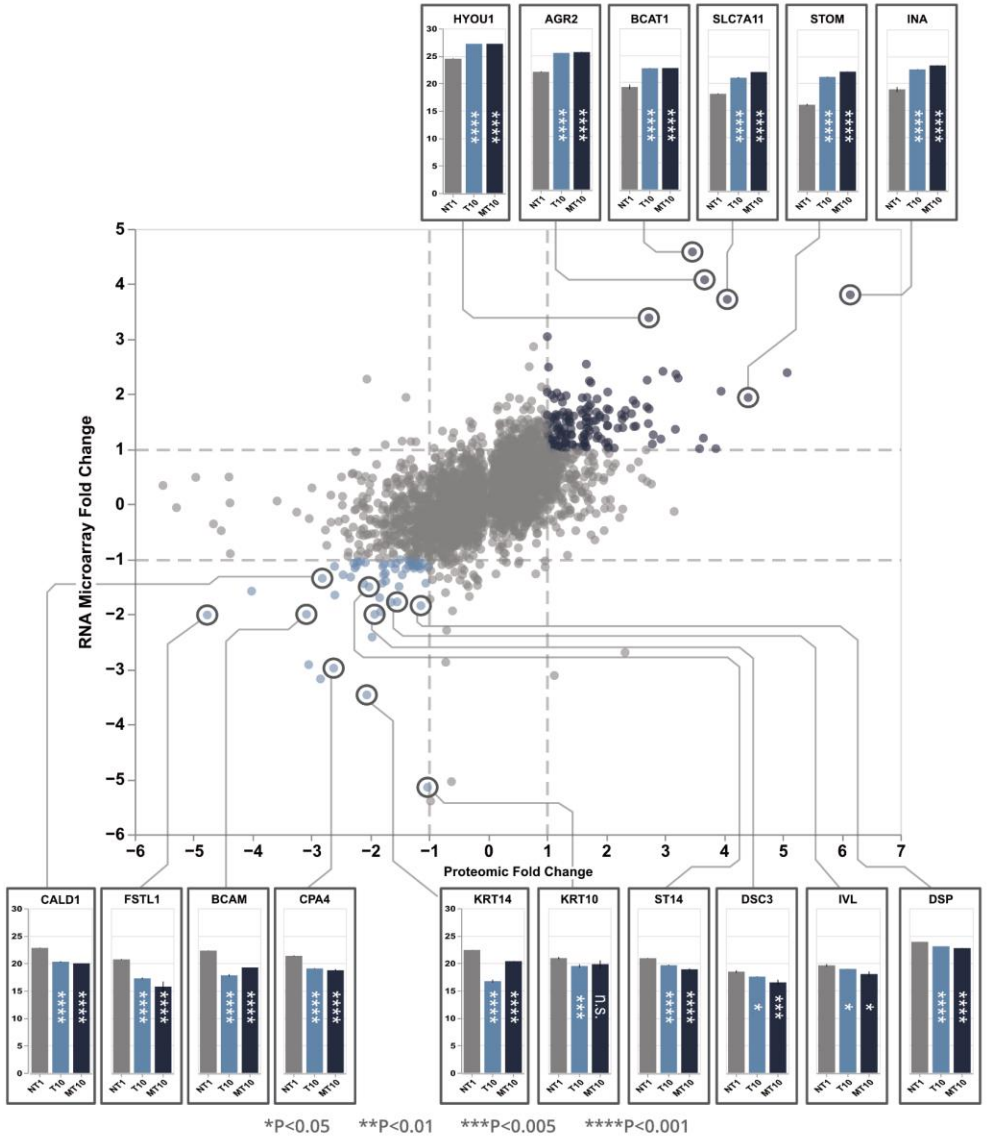
Illuminating Putative Biomarkers



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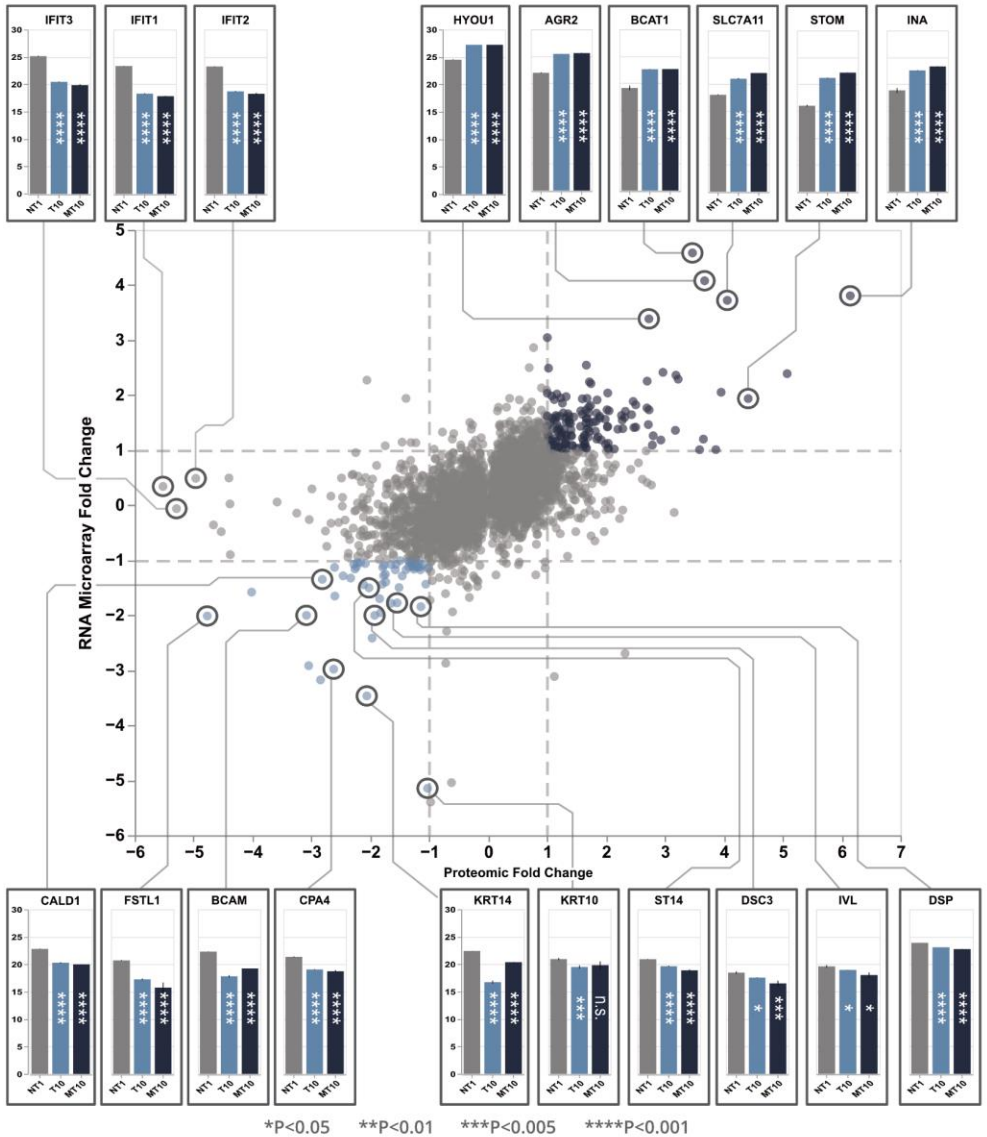
Illuminating Putative Biomarkers



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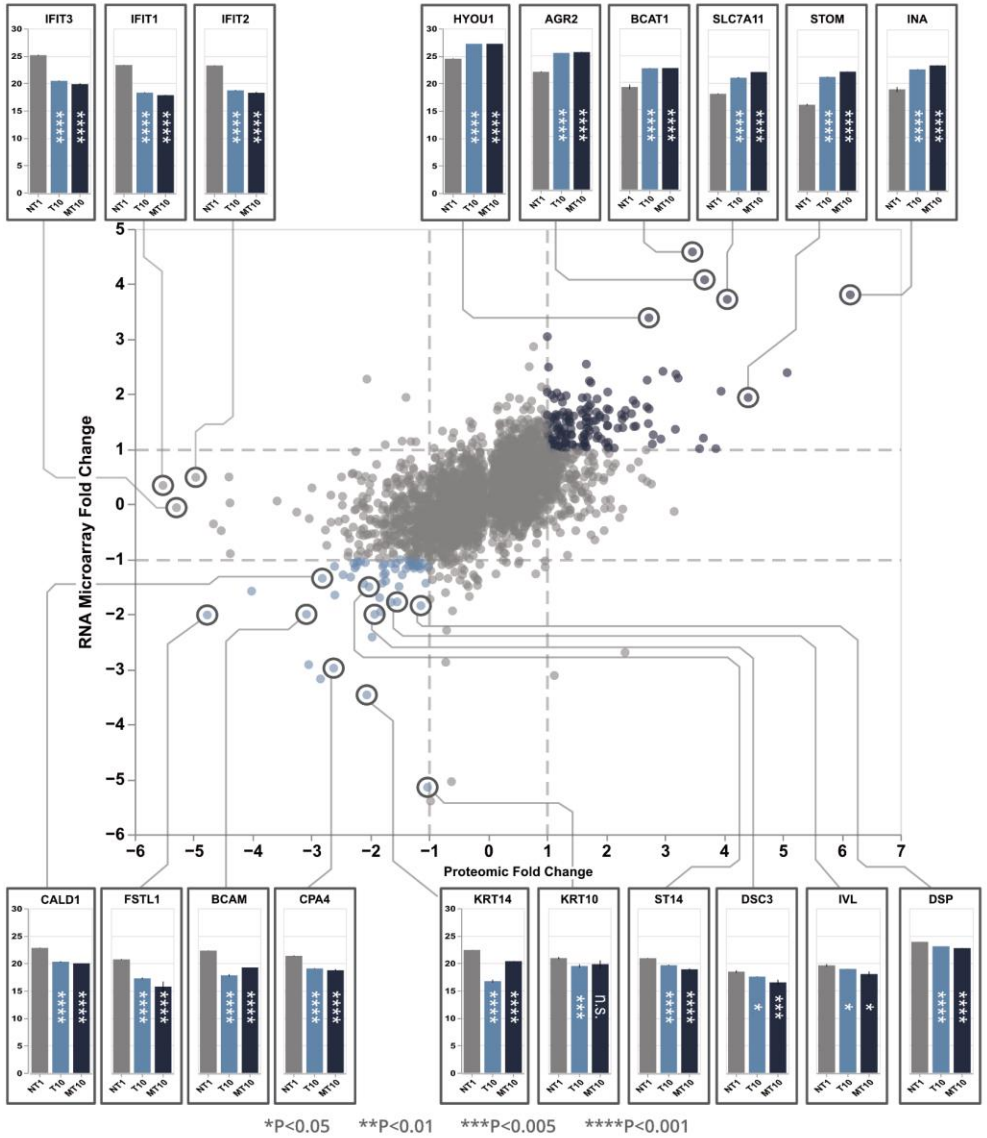
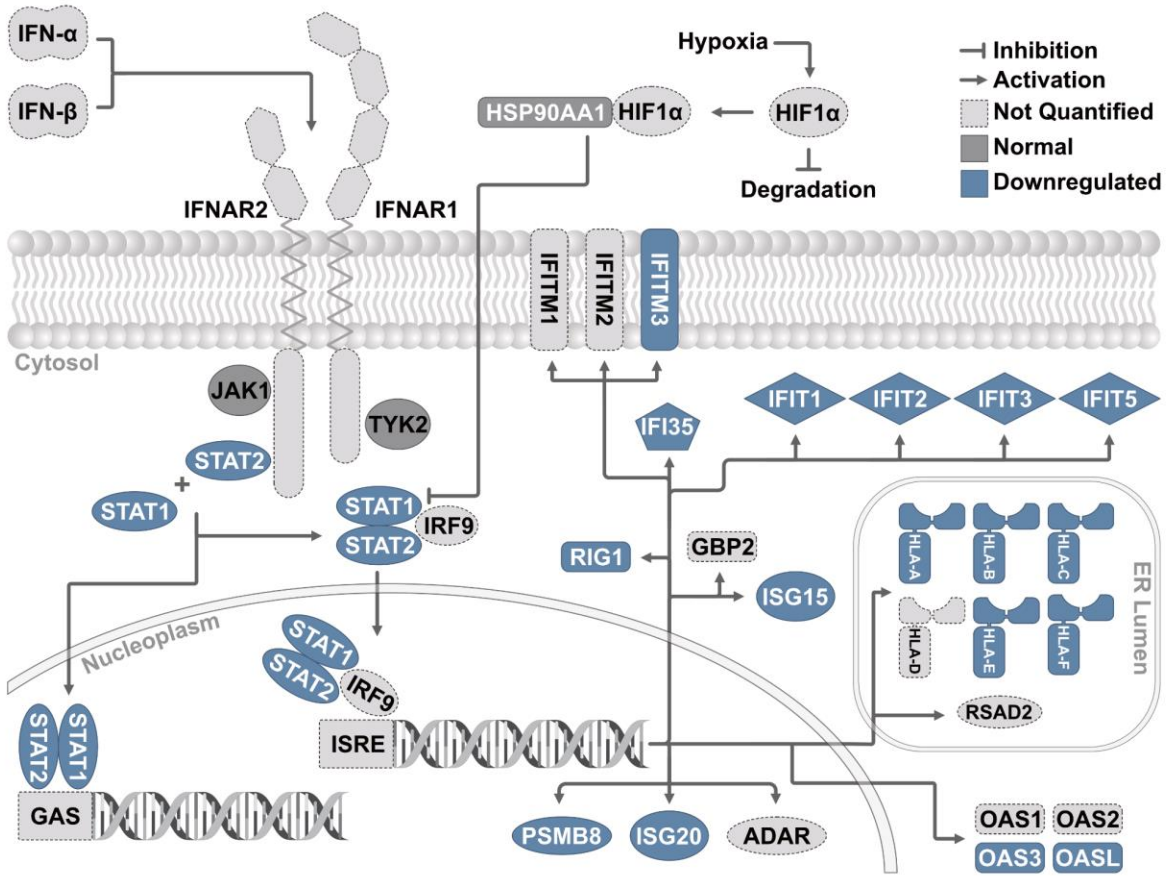
Illuminating Putative Biomarkers





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