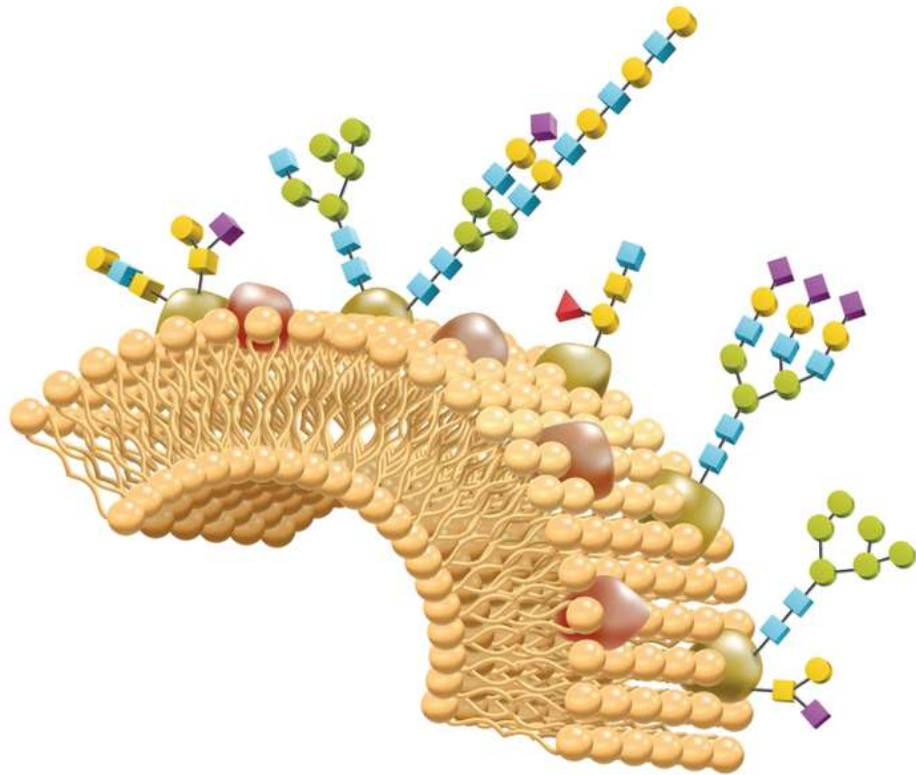# Enhancing Glycopeptide Detection, Identification, and Characterization through PGC-Incorporated LC-MS

Pittcon 2021

Graham Delafield
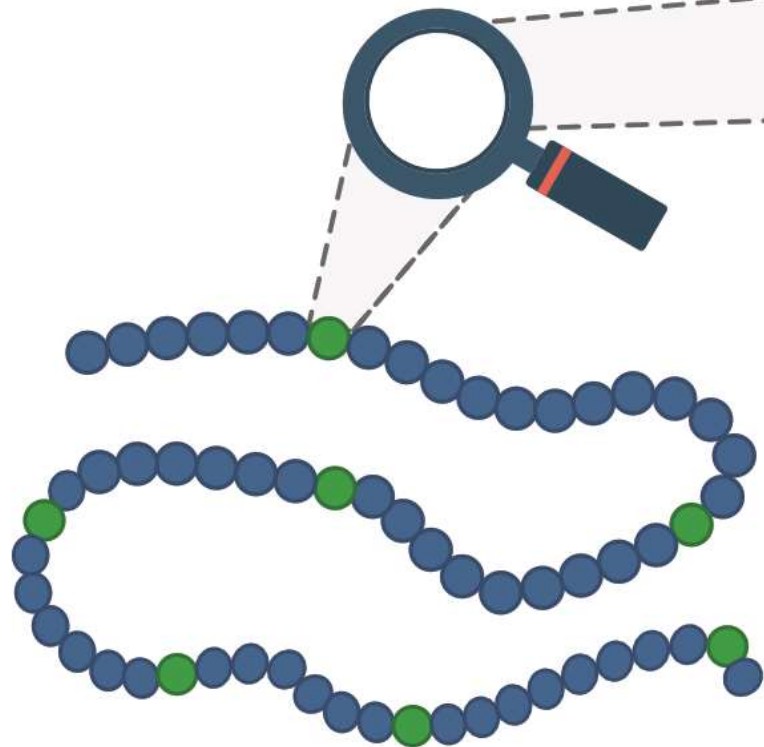University of Wisconsin - Madison

Introduction

Function

Cellular communication and immune response

Intrinsic/extrinsic signaling pathways
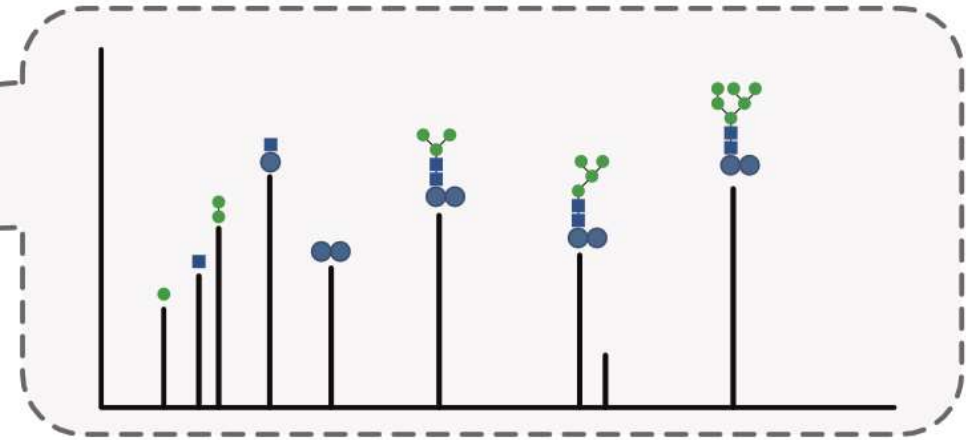
Protein folding and viability

Disease

Target for pathogen invasion

Altered expression during disease propagation

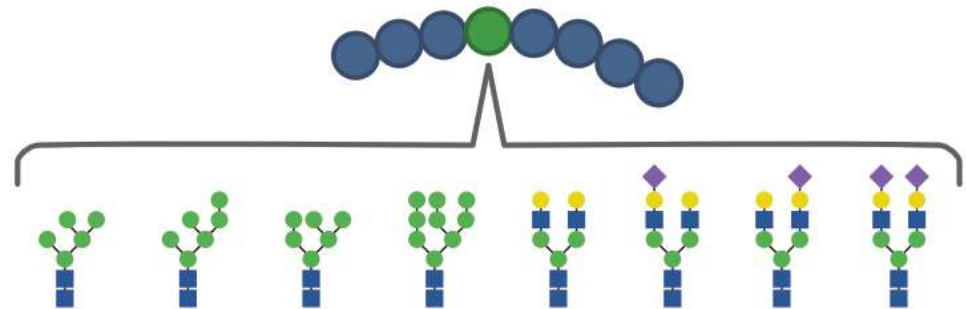Aberrant profiles across numerous disease
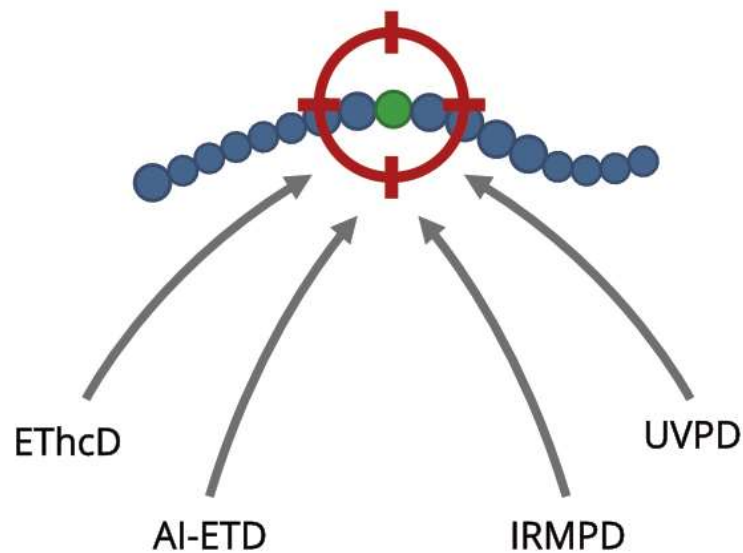
Glycoproteomic Need

Adequate Fragmentation

Glycosite Identification

Microheterogeneity Profiling

Areas of Interest

Enrichment

Dissociation

Spectral Scoring

EThcD

AI-ETD

IRMPD

UVPD
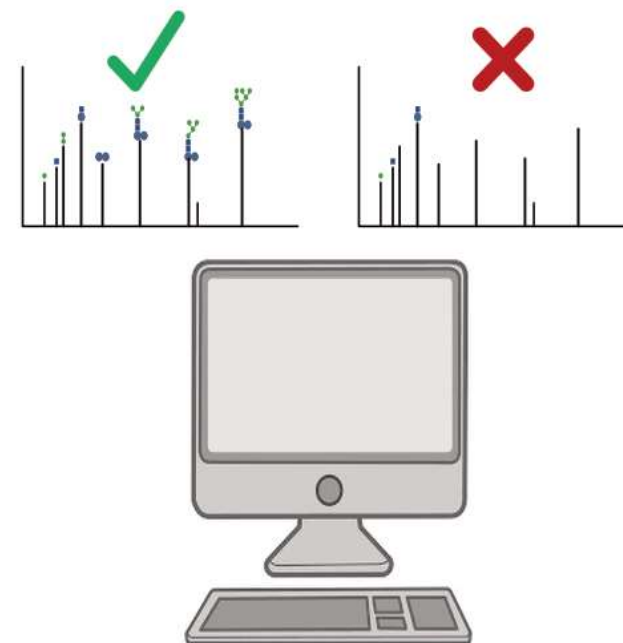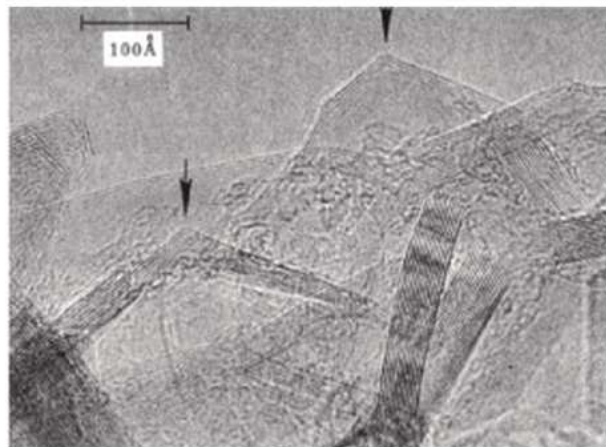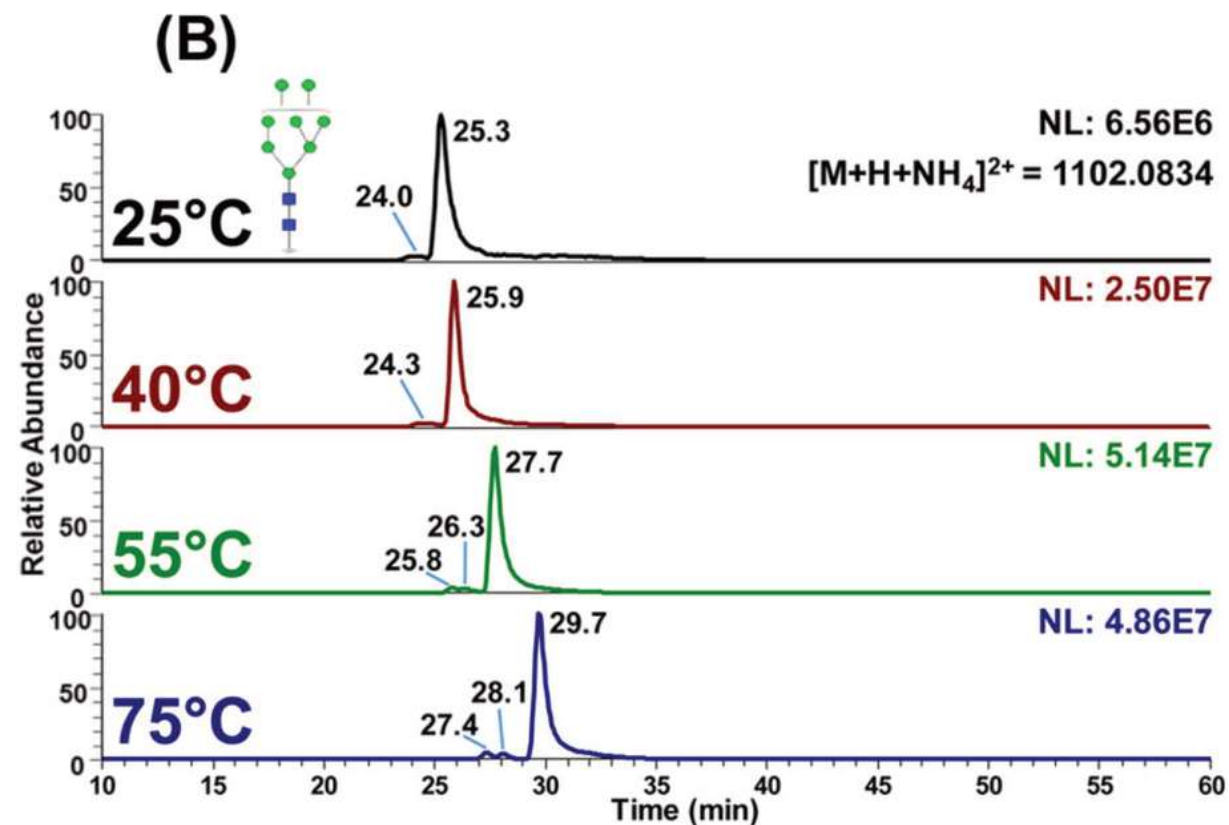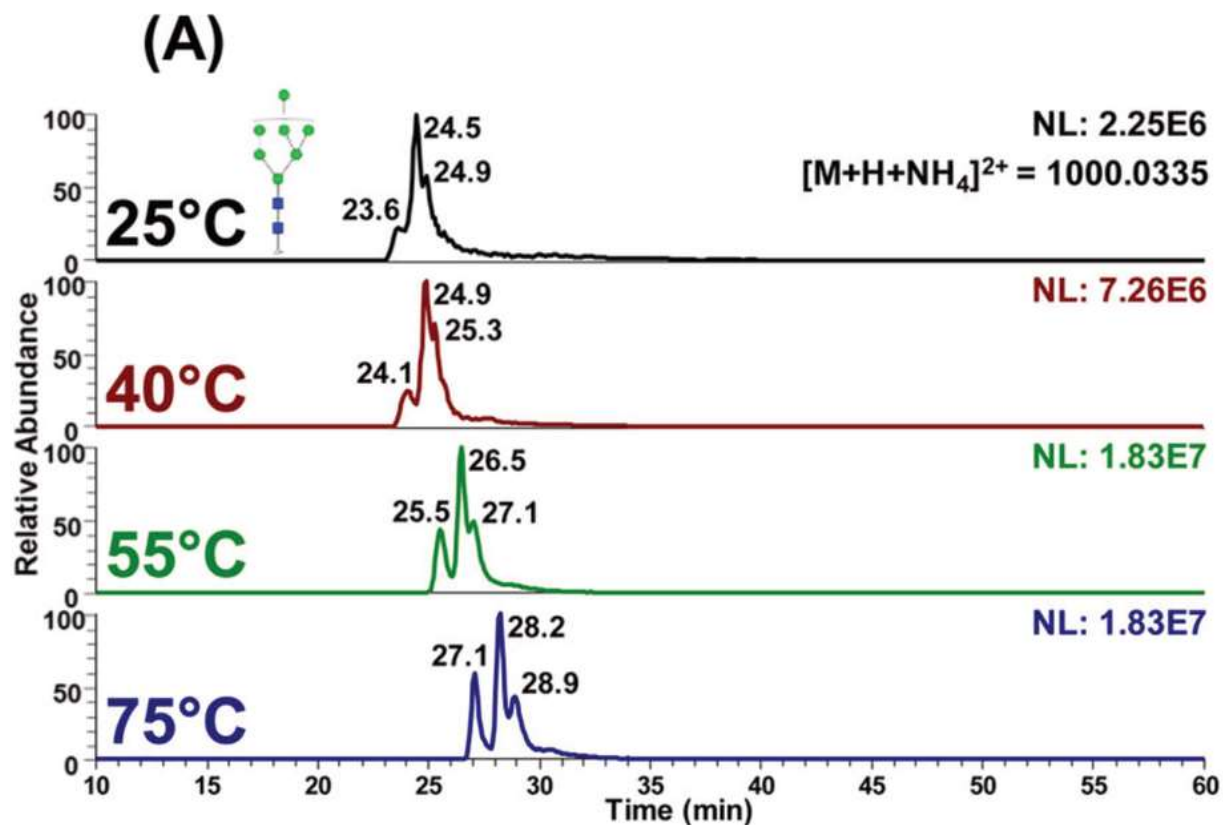
Alternative Separation

RPLC C18

PGC

Porous Graphitic Carbon (PGC)

1. Polar and electrostatic interactions

2. Solvent flexibility, compatible with traditional buffer systmes

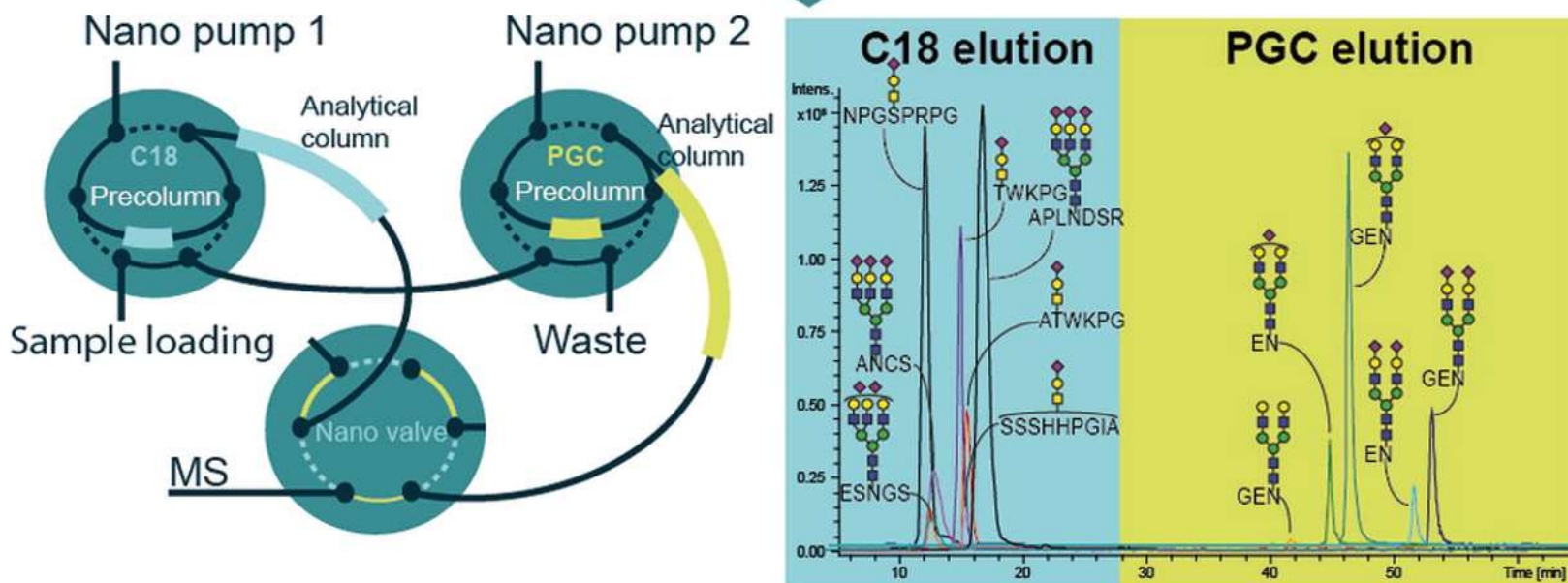3. Improved glycan retention and separation

Ali, A., et al. (2015). *Bulletin of the Korean Chemical Society* 36(6): 1733-1736.
West, C., et al. (2010). *Journal of Chromatography A* 1217(19): 3201-3216.

PGC Application

Zhou, S.; Huang, Y.; Dong, X.; Peng, W.; Veillon, L.; Kitagawa, D. A. S.; Aquino, A. J. A.; Mechref, Y., Isomeric Separation of Permethylated Glycans by Porous Graphitic Carbon (PGC)-LC-MS/MS at High Temperatures. *Analytical Chemistry* **2017,** *89* (12), 6590-6597.

PGC Application

Stavenhagen, K.; Plomp, R.; Wuhrer, M., *Analytical Chemistry* **2015,** *87* (23), 11691-11699.

Knowledge Gap

Searching for a compliment to traditional liquid phase separations that provides:

1. Access to proteome components traditionally missed in RPLC analyses
2. Expanded glycoproteome coverage
3. Improved liquid-phase separation of isomeric glycopeptides
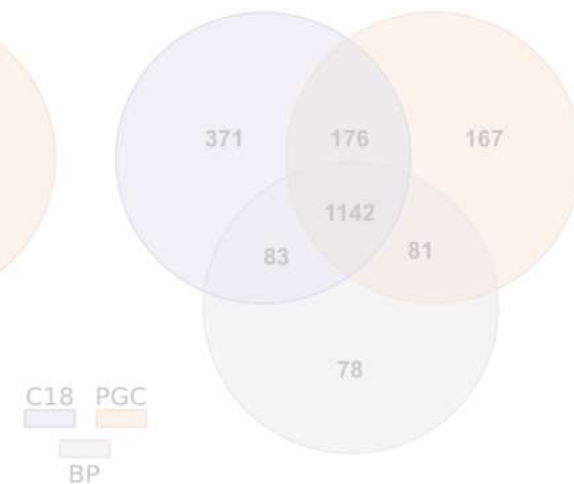4. Path toward facile characterization of disease-specific analytes

Methodology
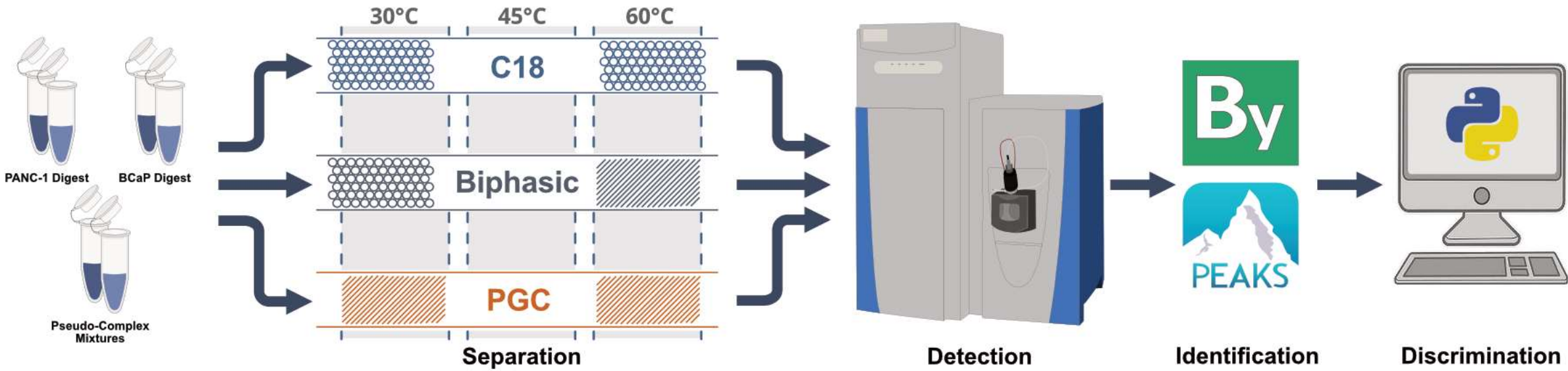
Methodology
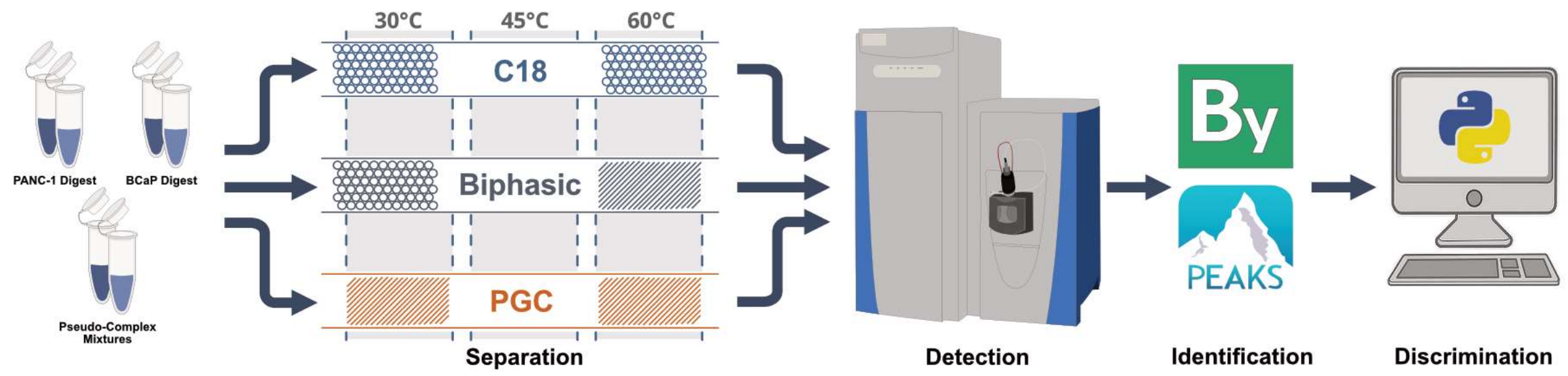
PANC-1 Digest   BCaP Digest
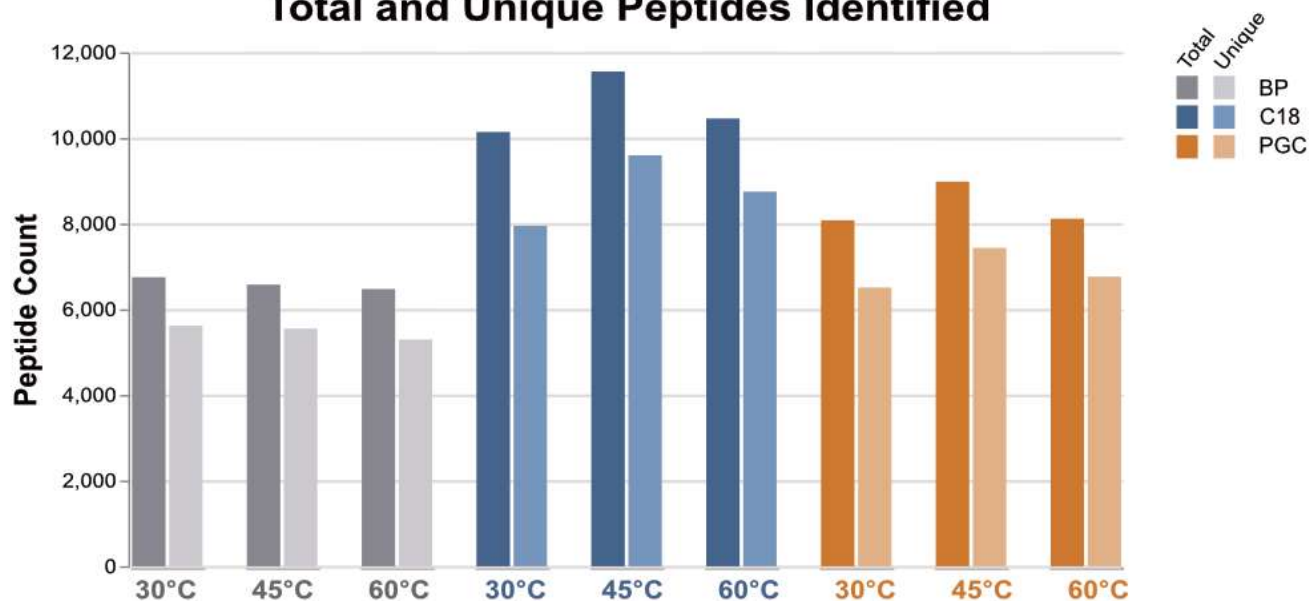
Pseudo-Complex
Mixtures

30°C   45°C   60°C

C18

Biphasic

PGC

Separation

Detection

By

PEAKS

Identification

Discrimination

Proteome Coverage

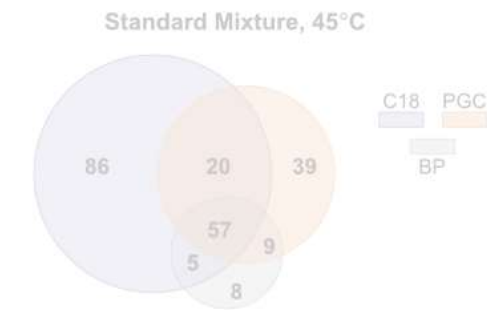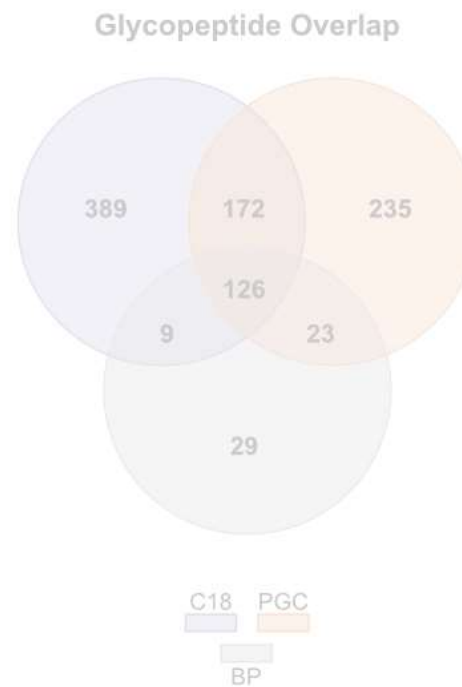# Proteome Coverage
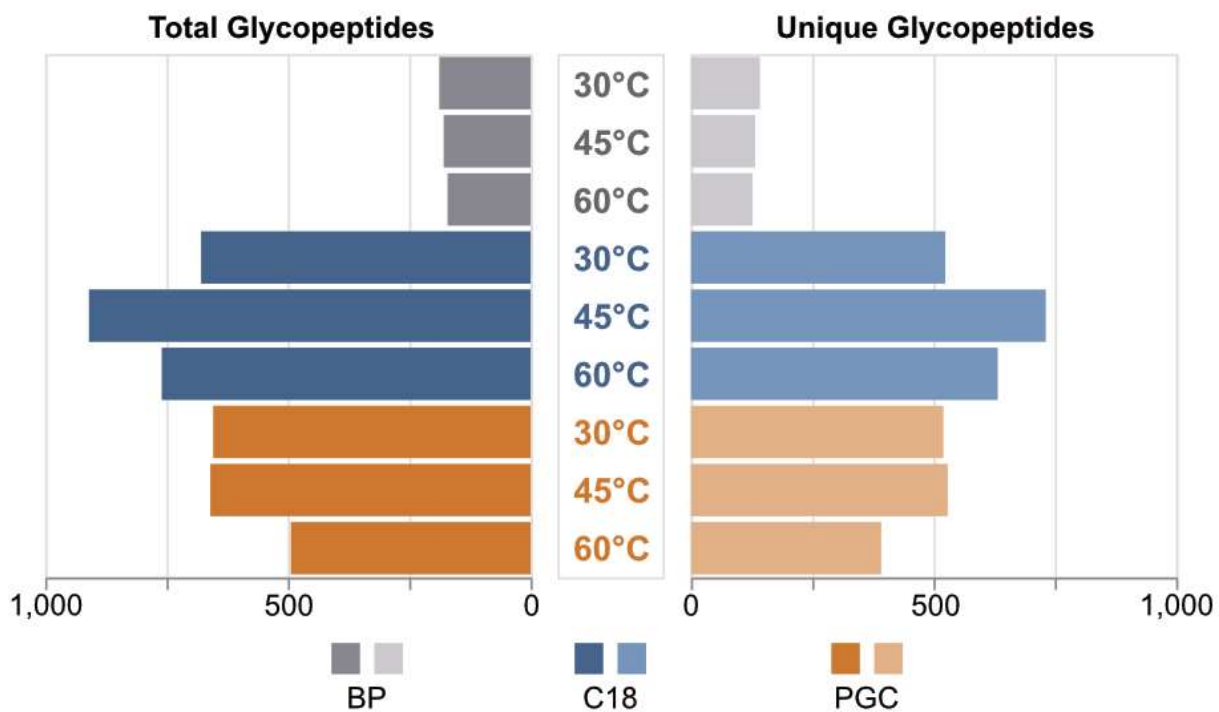
Peptide Character

Average peptide Length

GRAVY vs. Retention Time
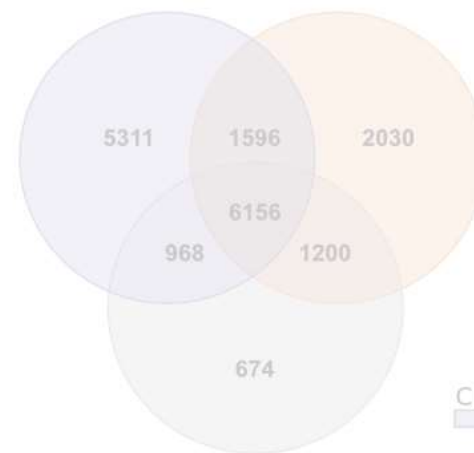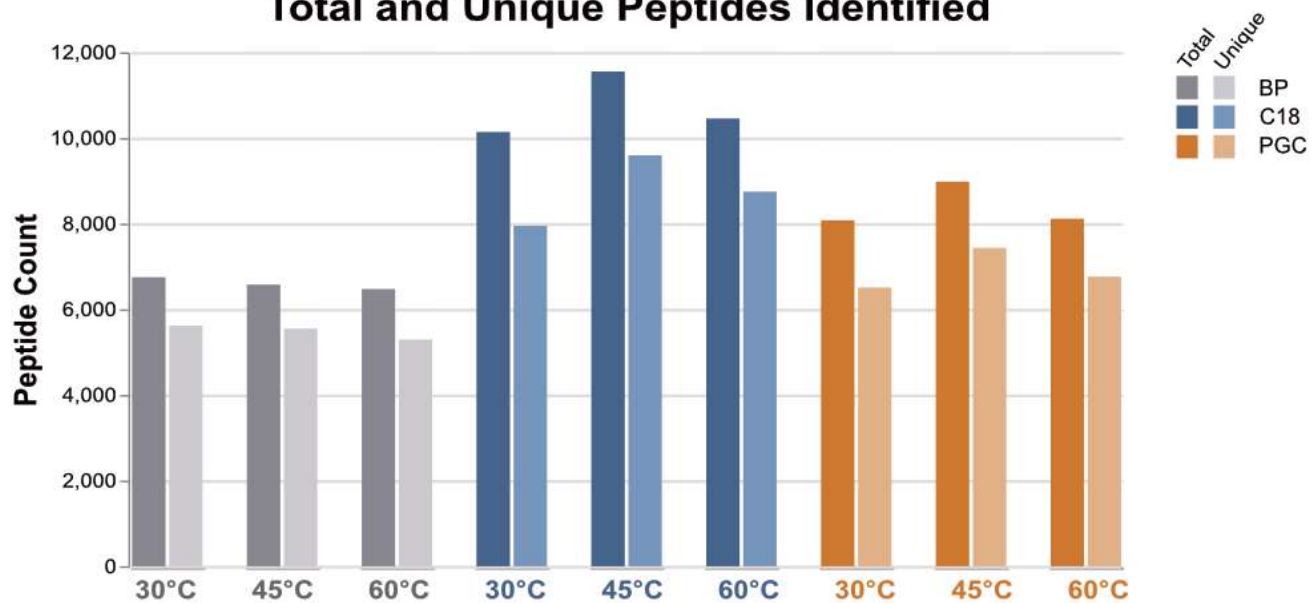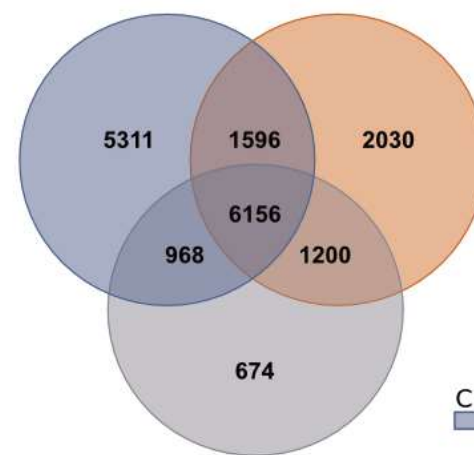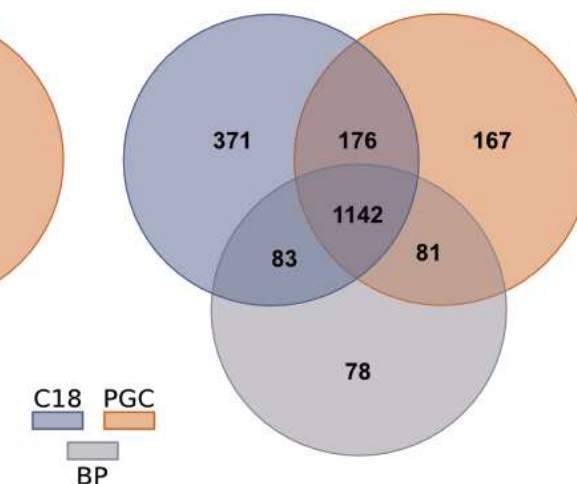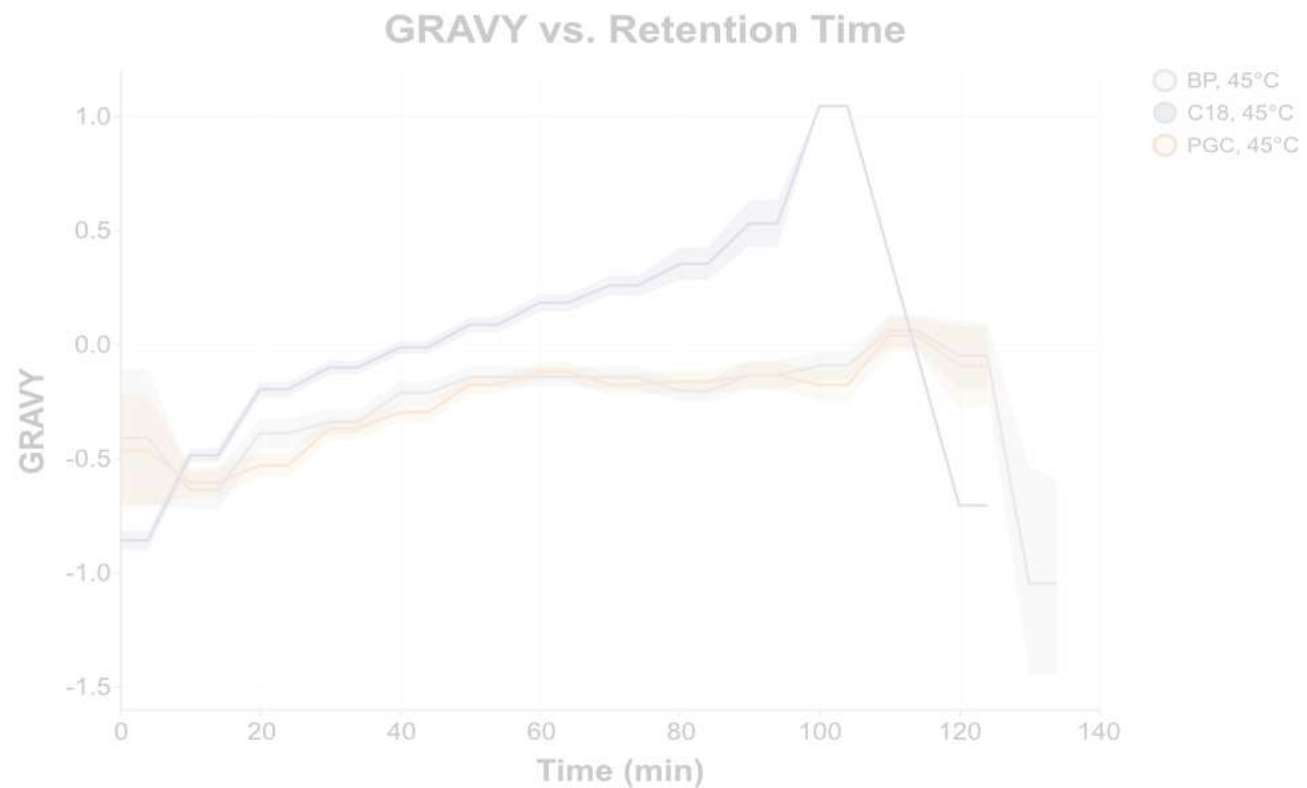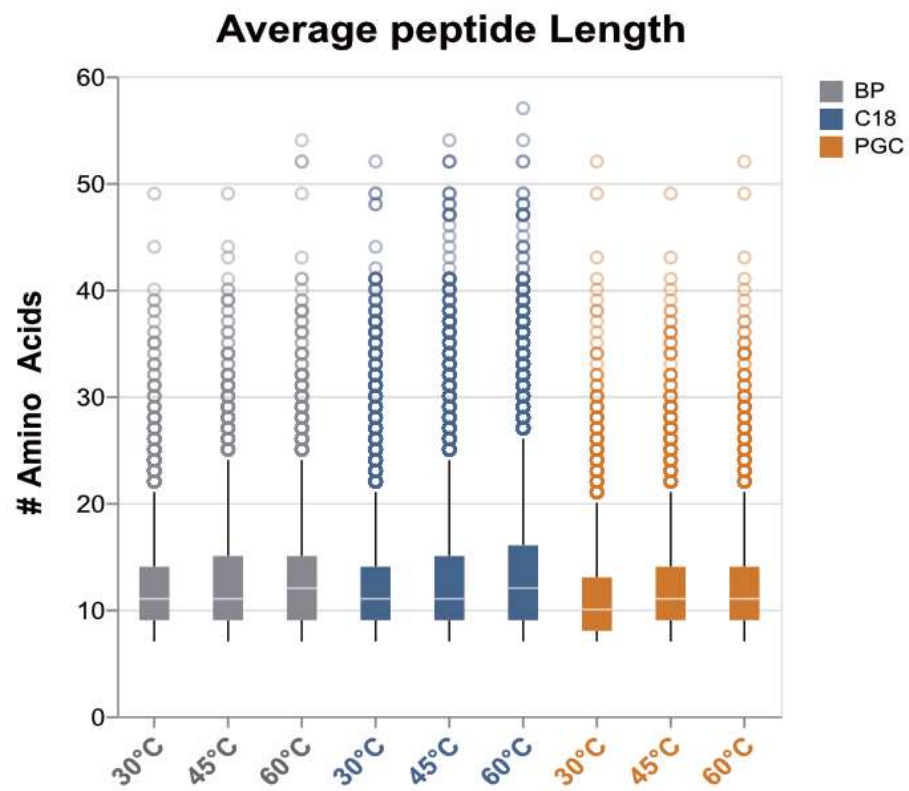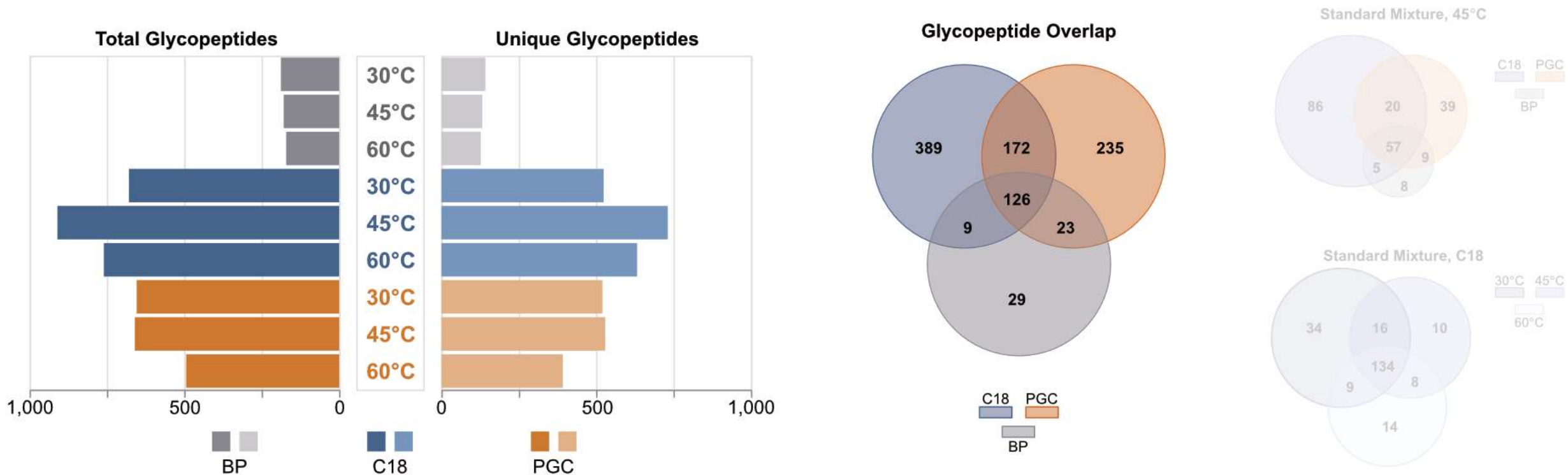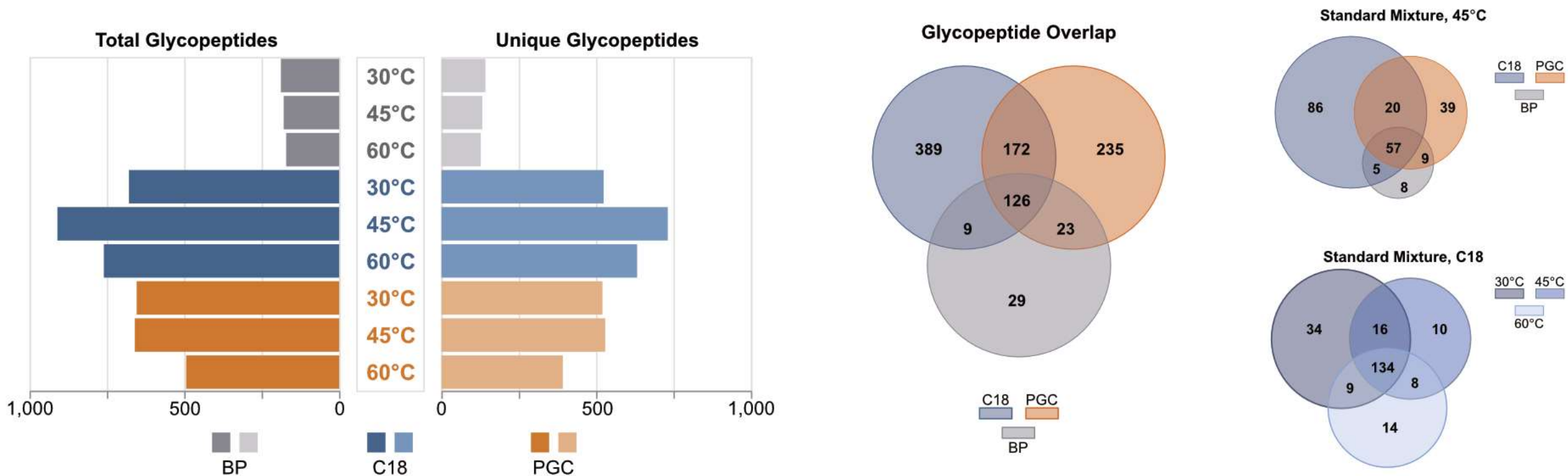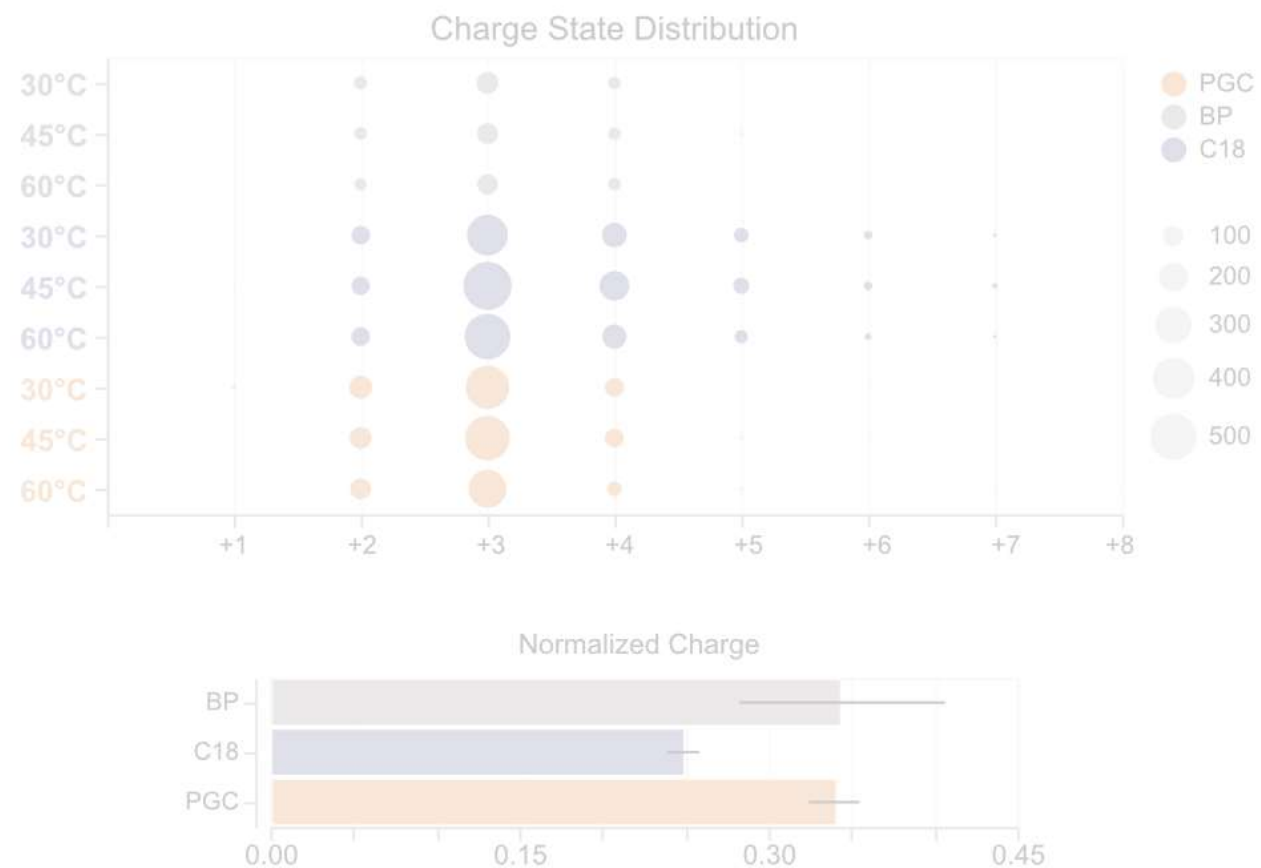
Peptide Character
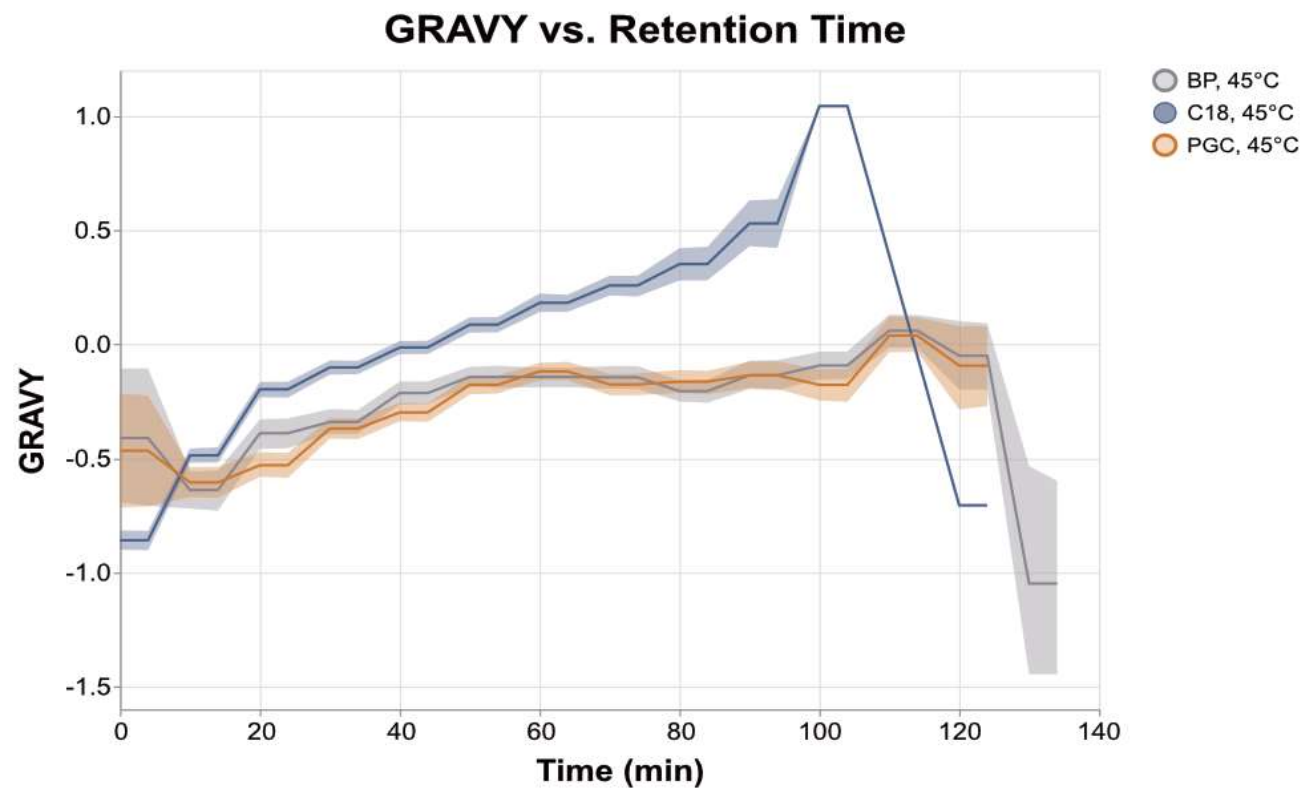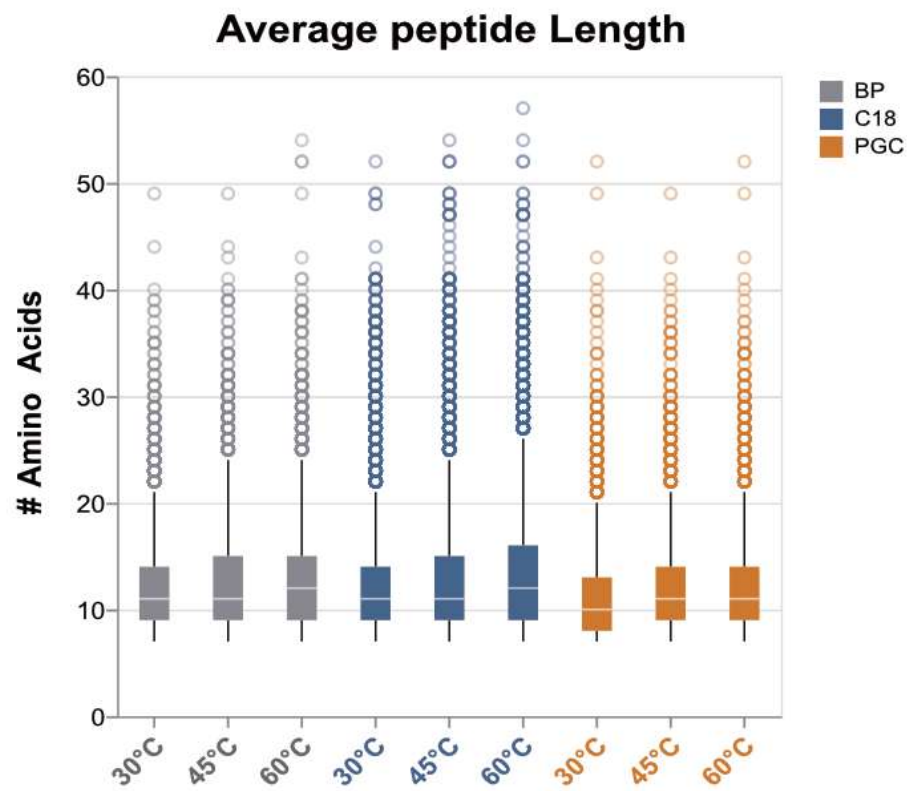
Glycopeptide Detection
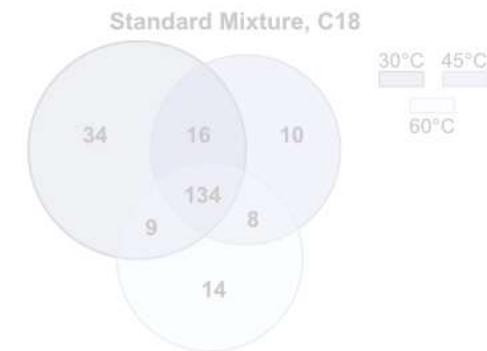
Glycopeptide Detection

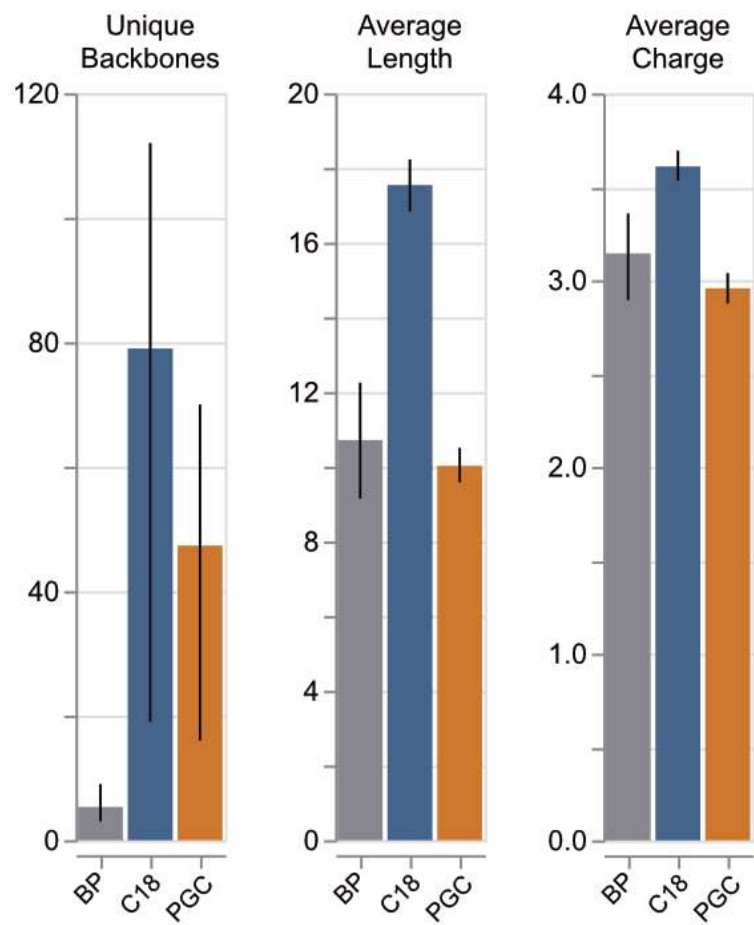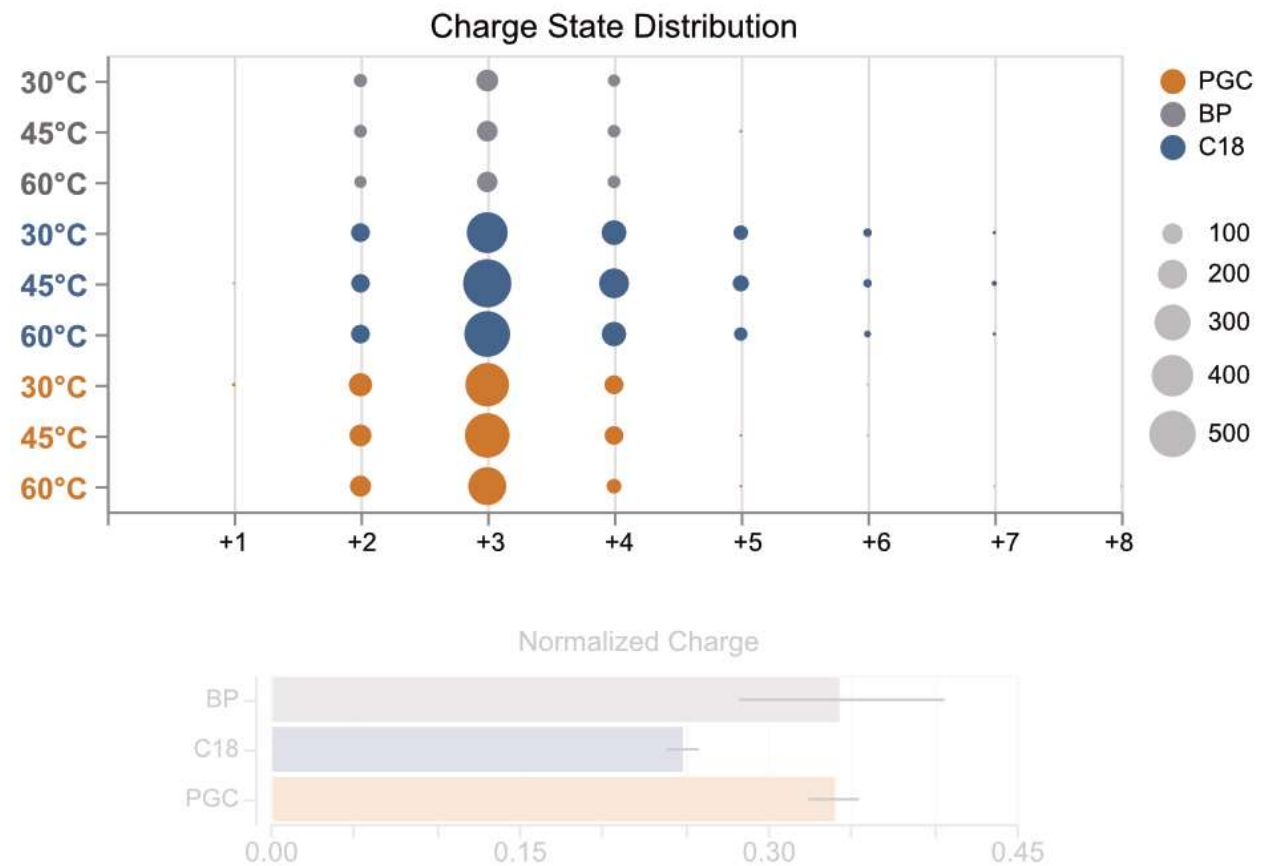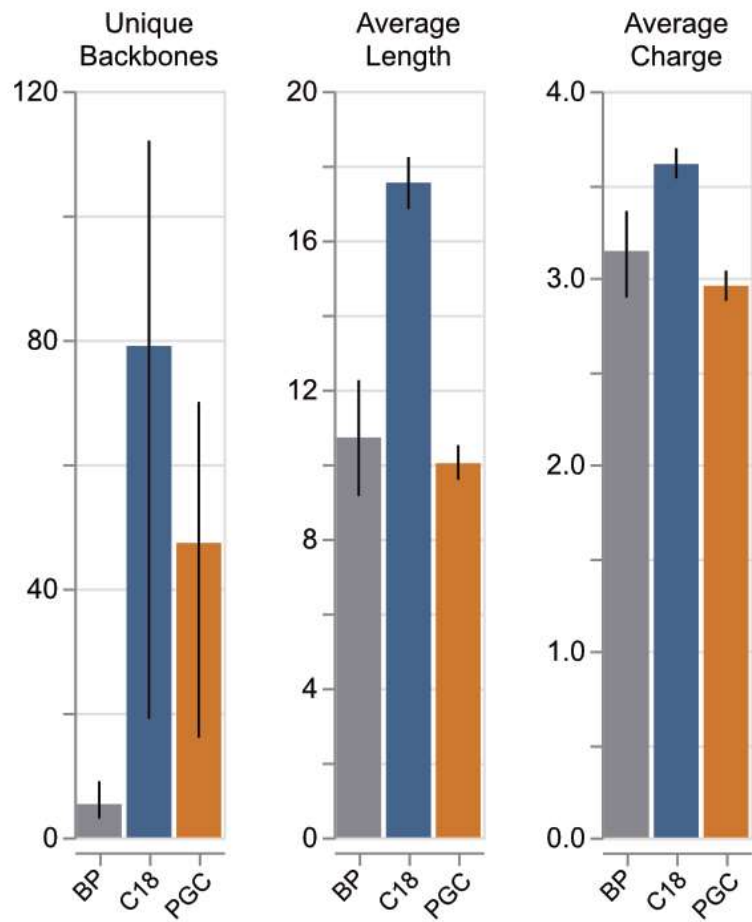# Glycopeptide Detection
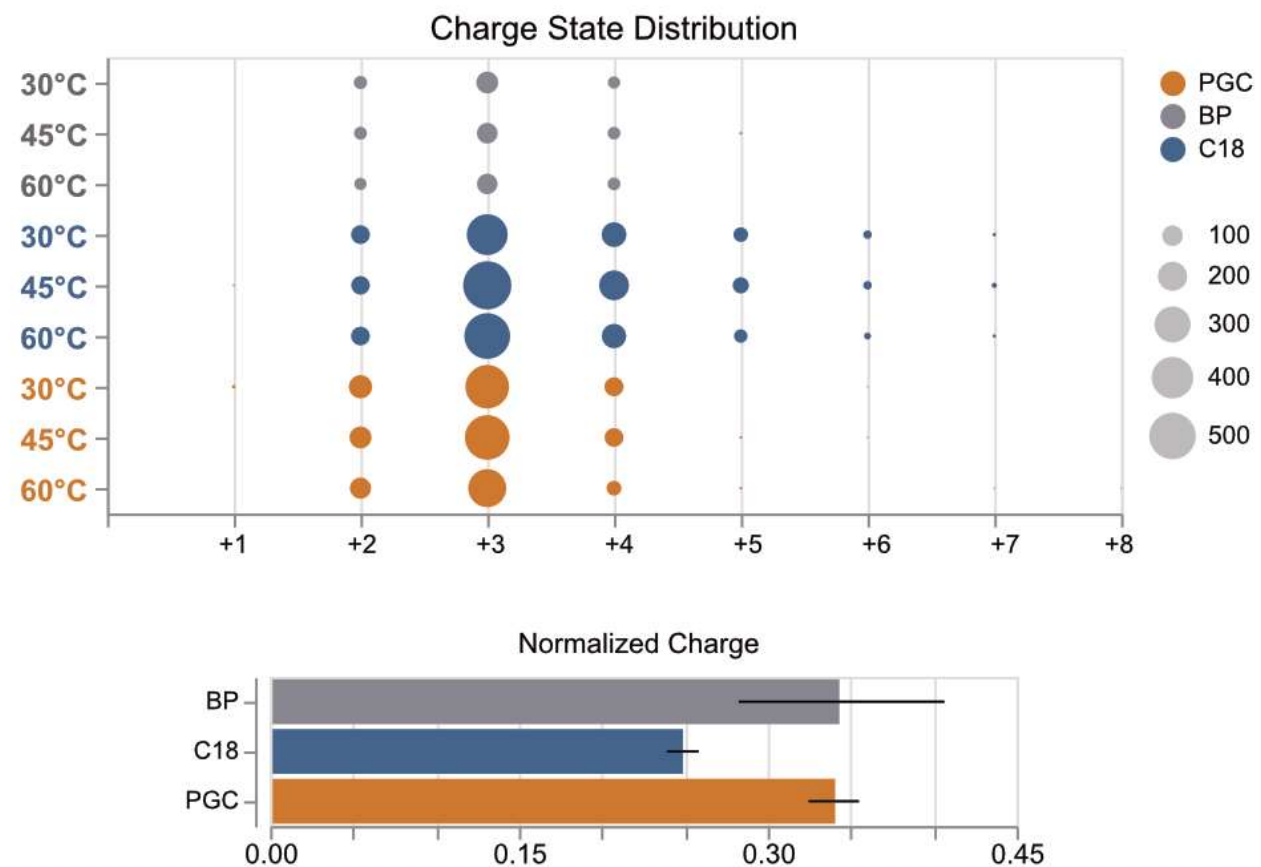
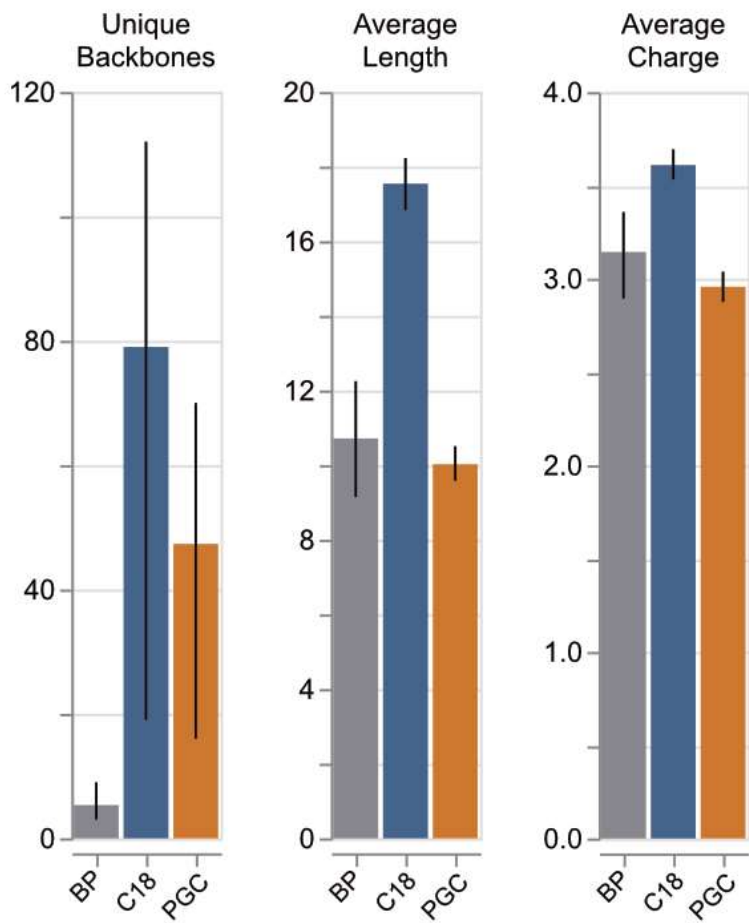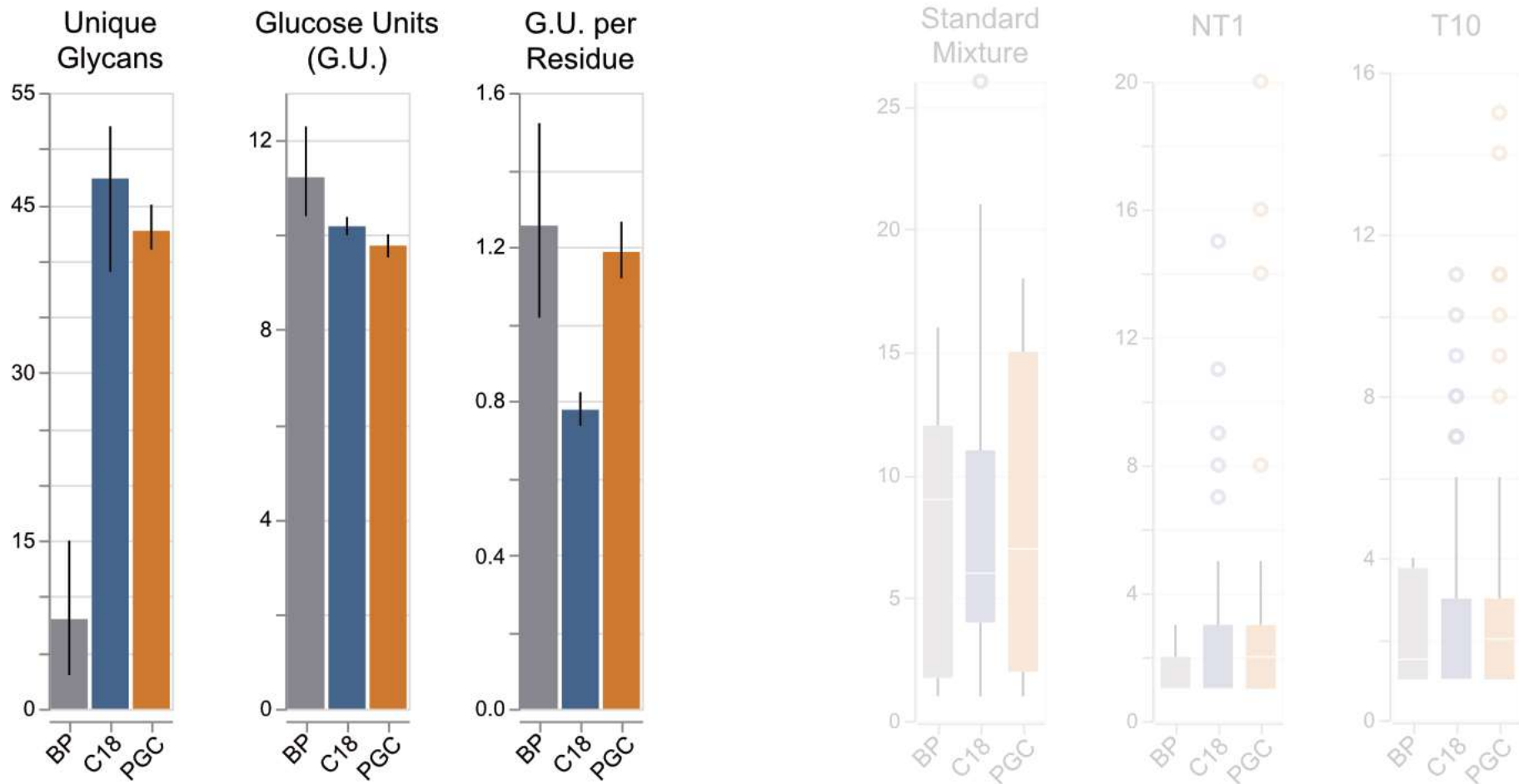# Peptide-level Differences

std. deviation, n=3

Peptide-level Differences

Peptide-level Differences
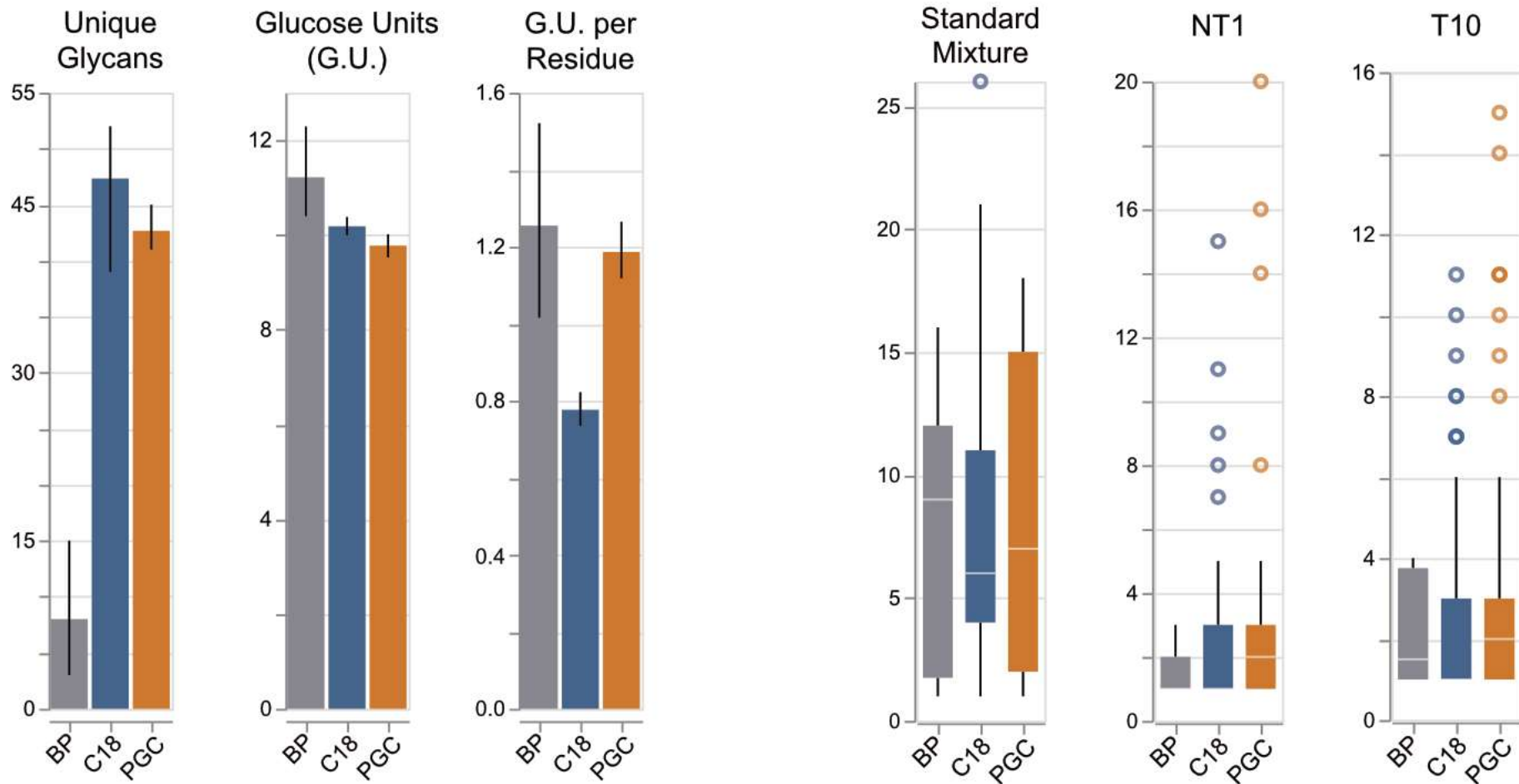
Glycan-level Differences
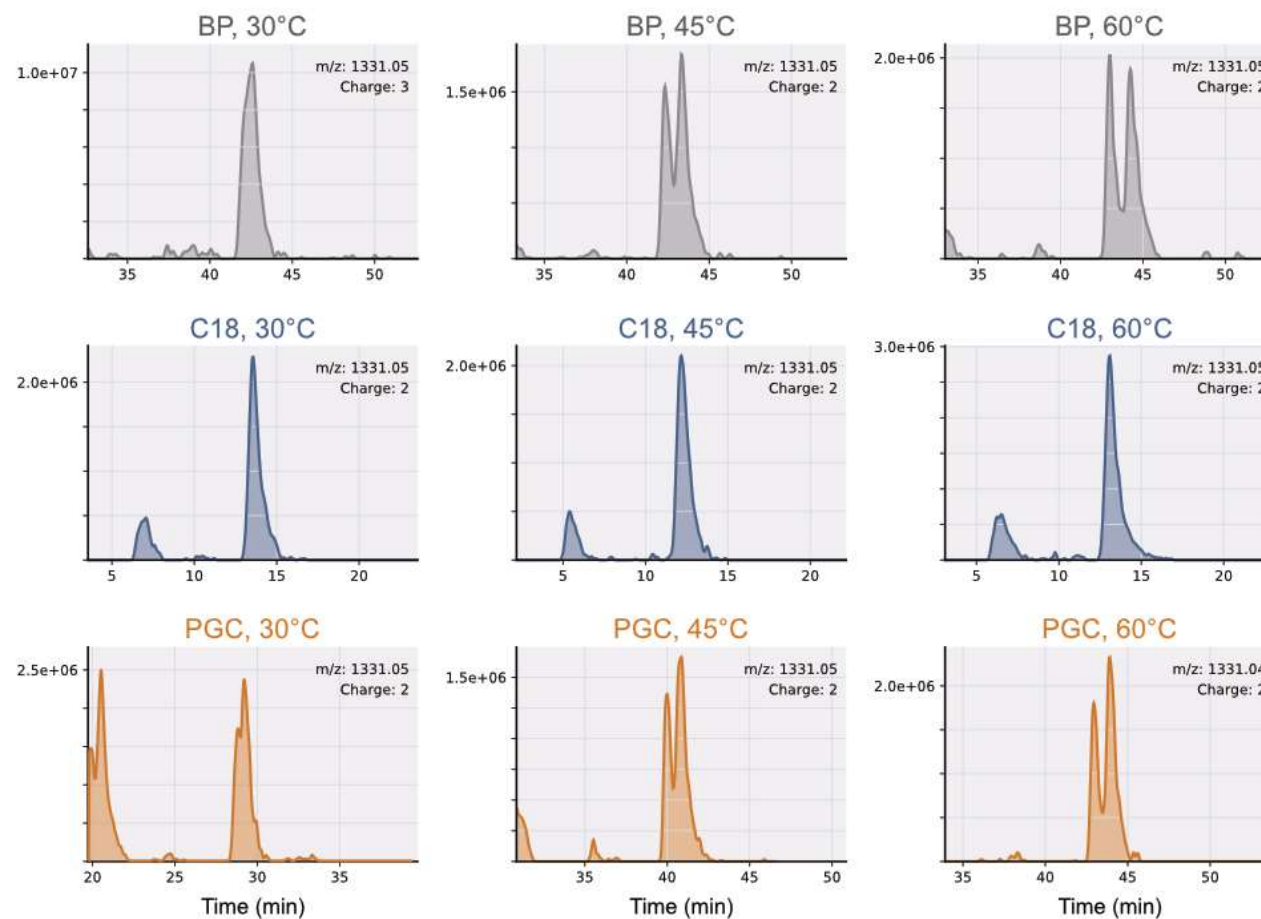

std. deviation, n=3

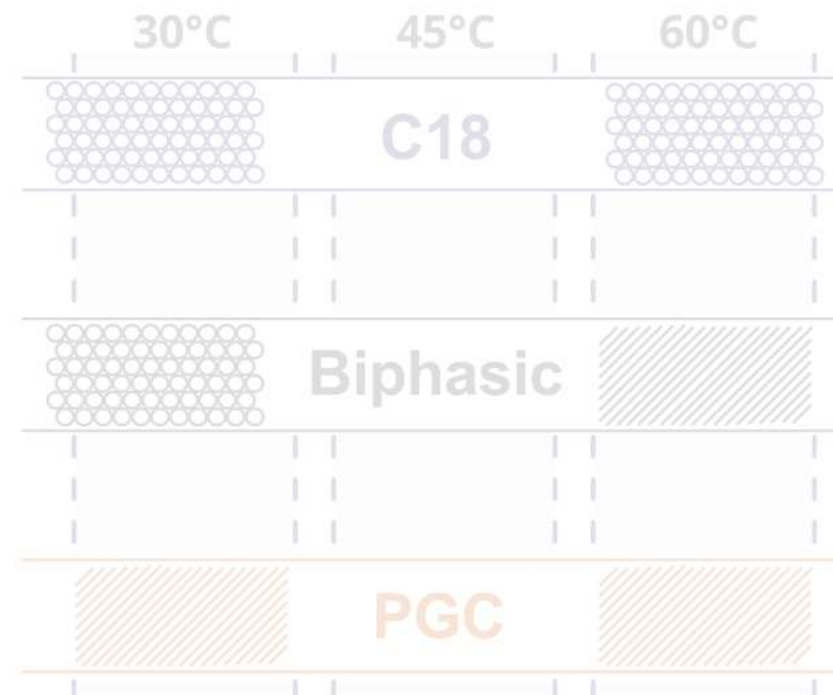# Glycan-level Differences
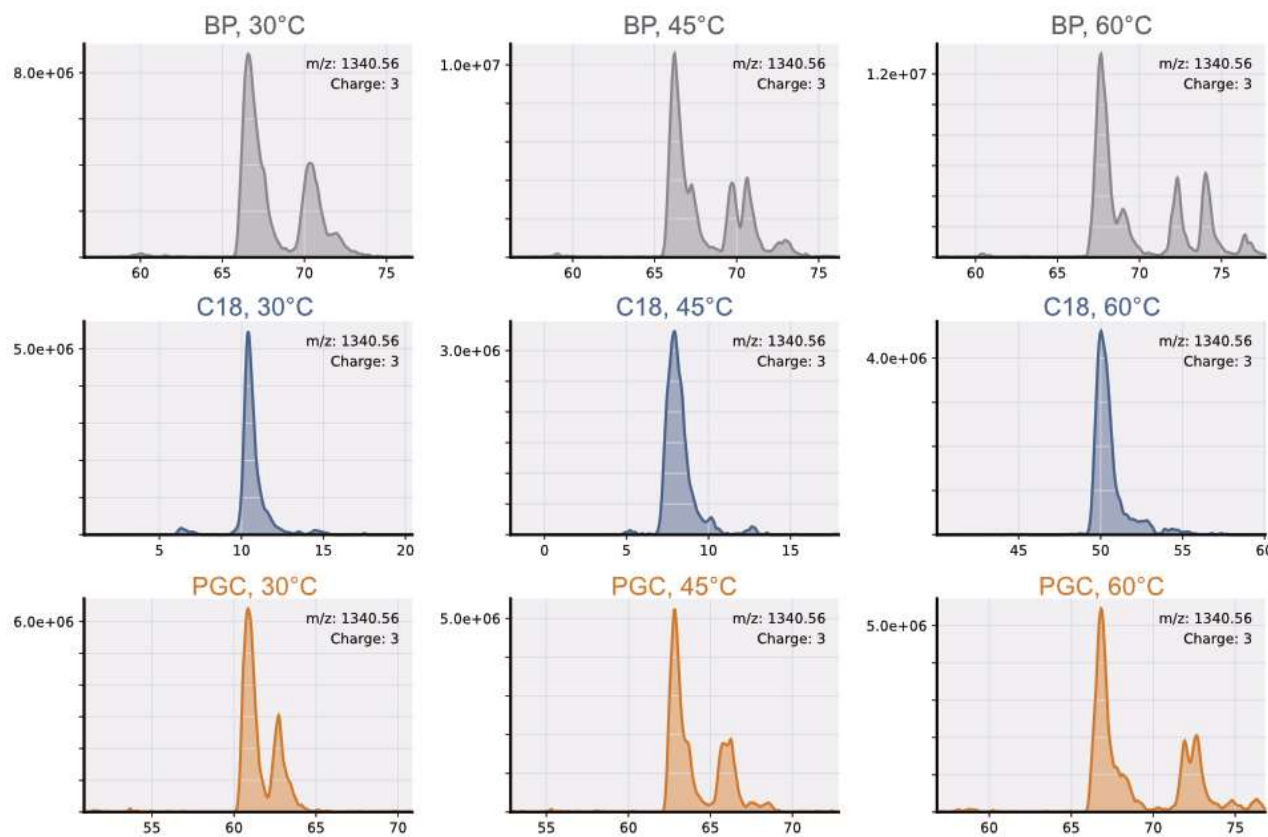

std. deviation, n=3

Liquid-phase Resolution

QNGTLSK + HexNAc(4)Hex(5)NeuAc(1)
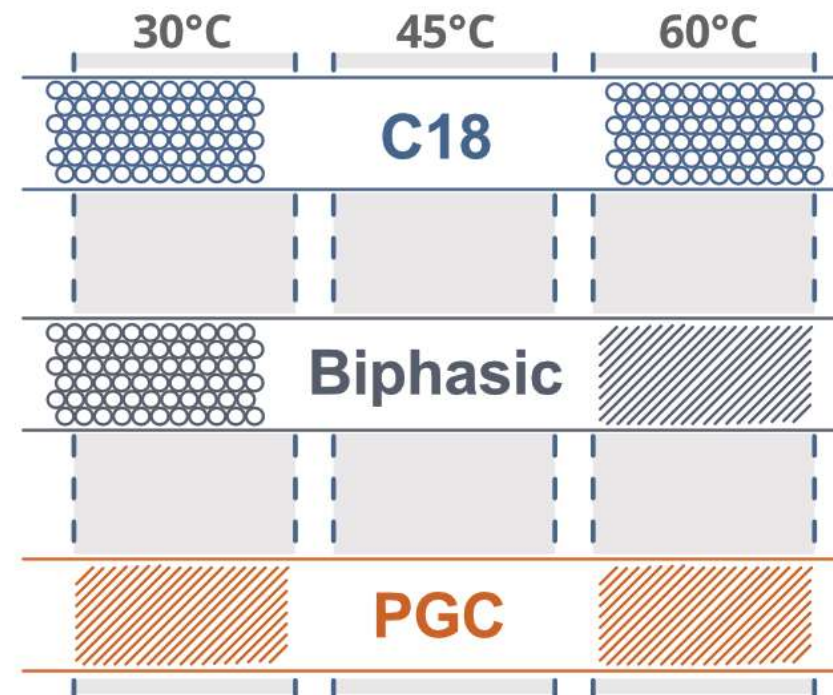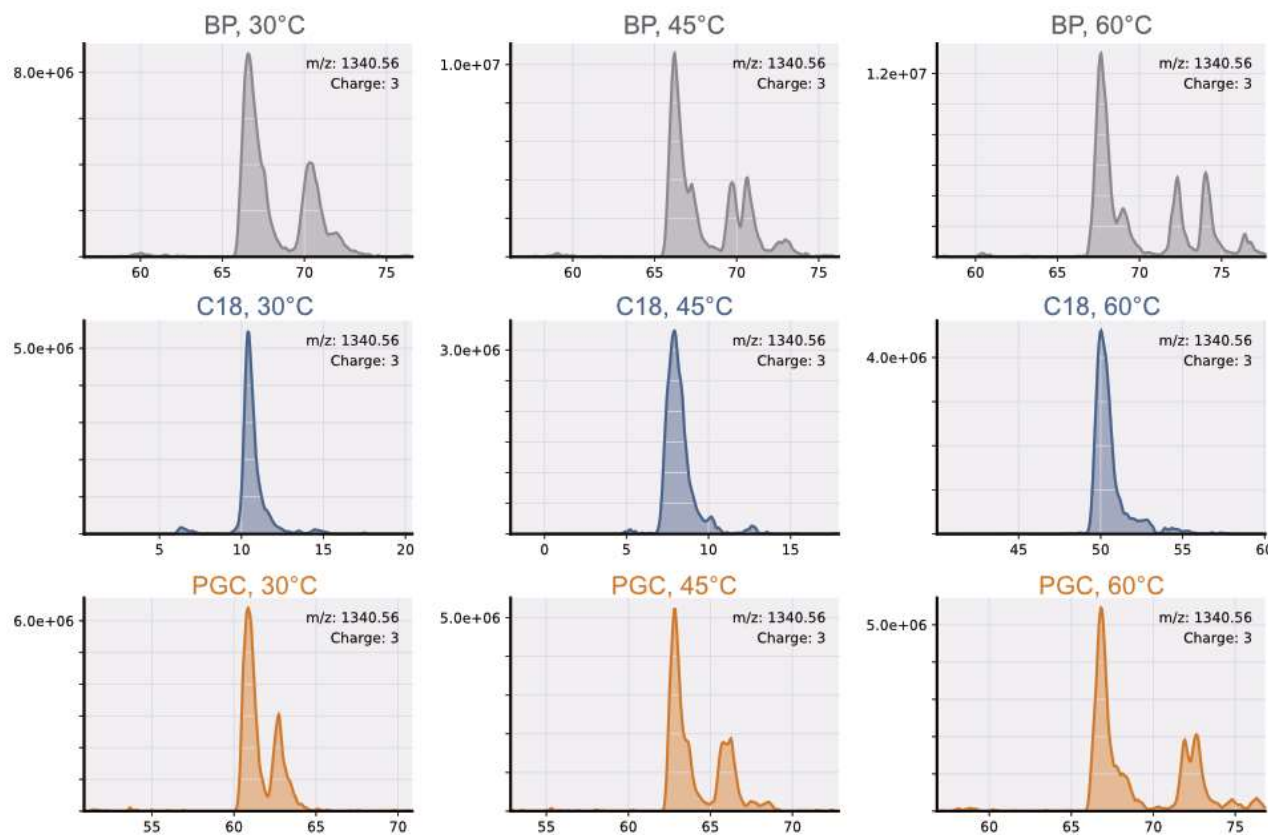
Liquid-phase Resolution

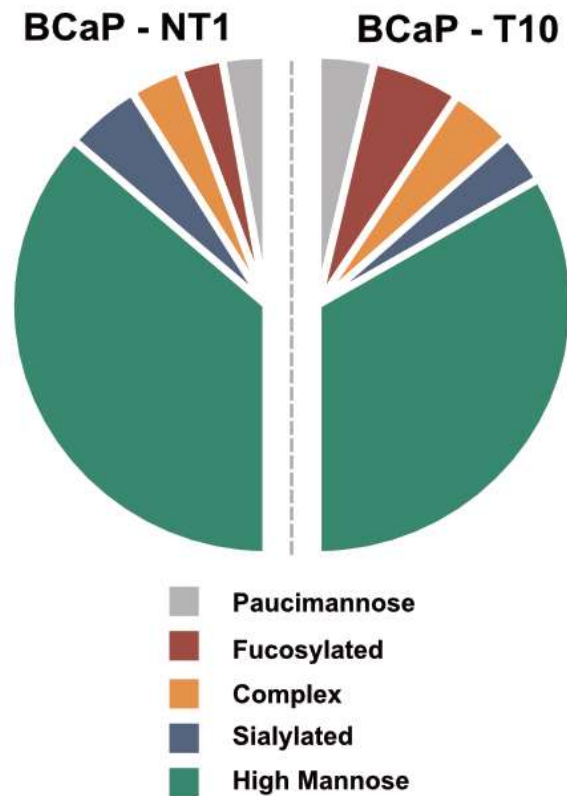LCPDCPLLAPLNDSR + HexNAc(5)Hex(6)NeuAc(1)

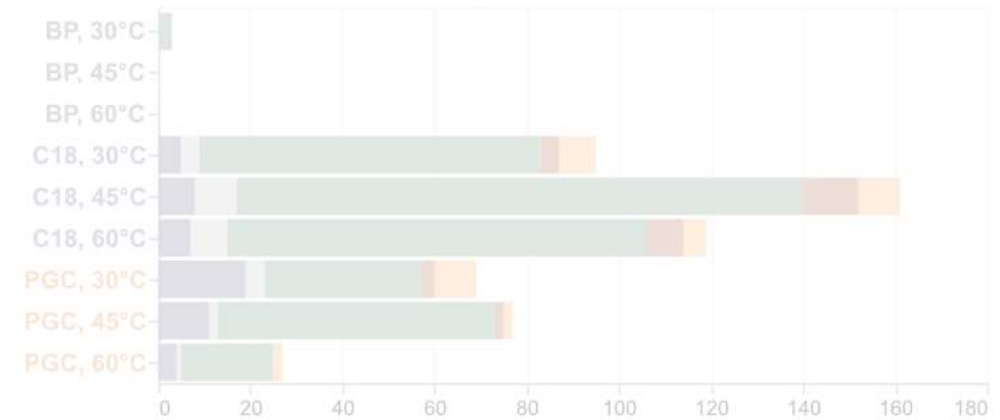# Liquid-phase Resolution

LCPDCPLLAPLNDSR + HexNAc(5)Hex(6)NeuAc(1)

1. Common for high mannose and complex glycans with mismatched antennae
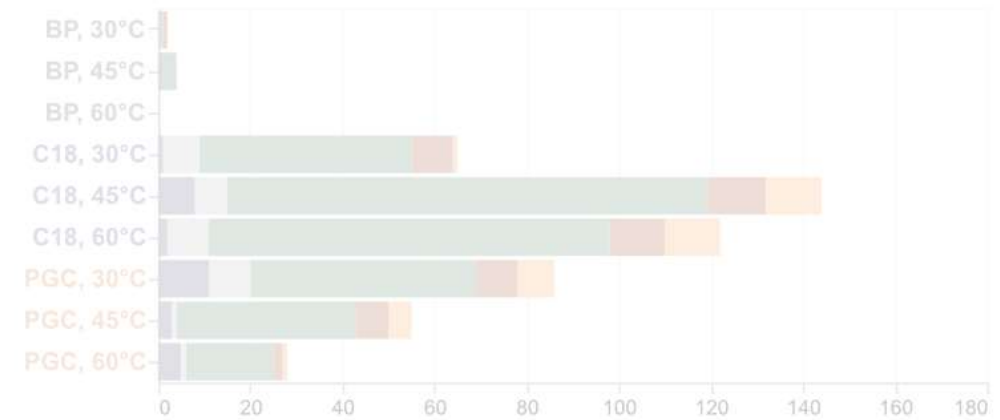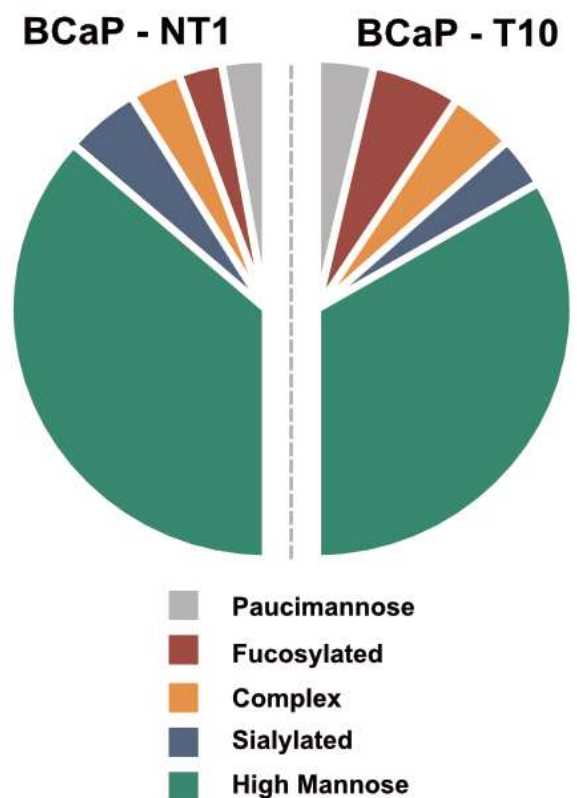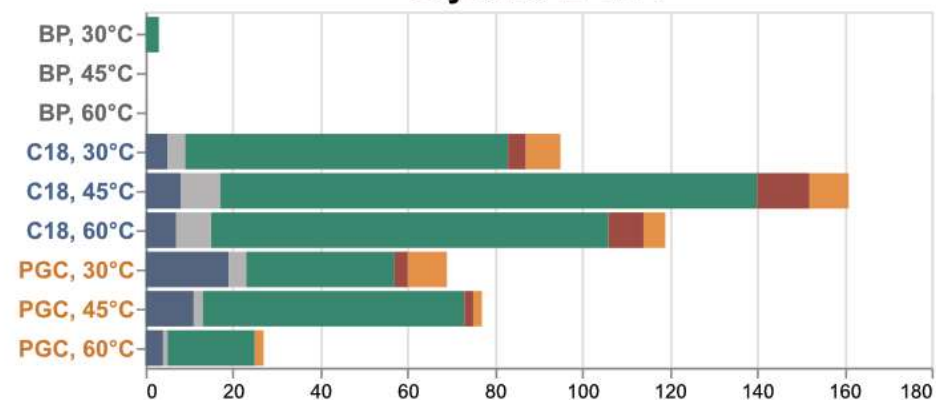2. Concentration-dependent

Glycoproteome Profiles

Glycoproteome Profiles
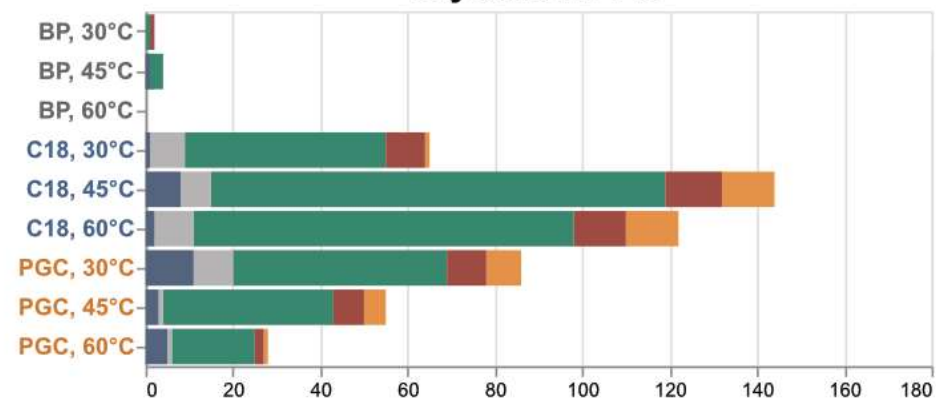
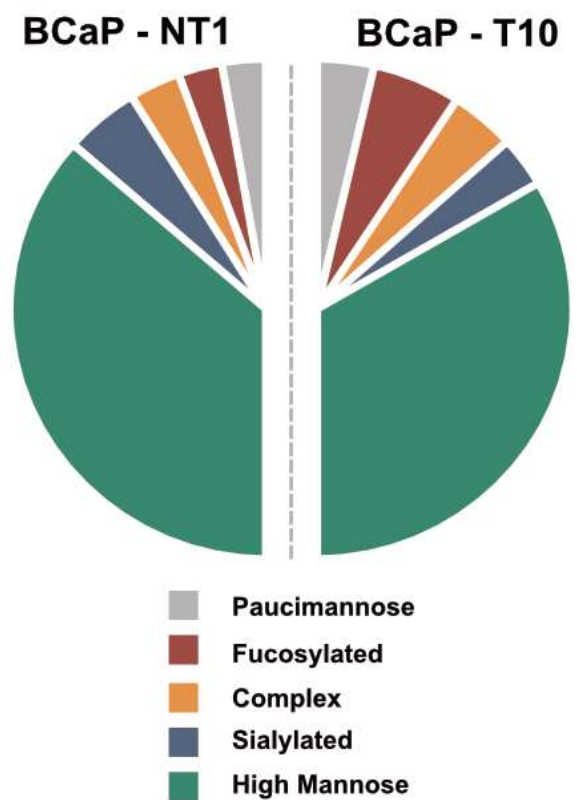# Peak Height
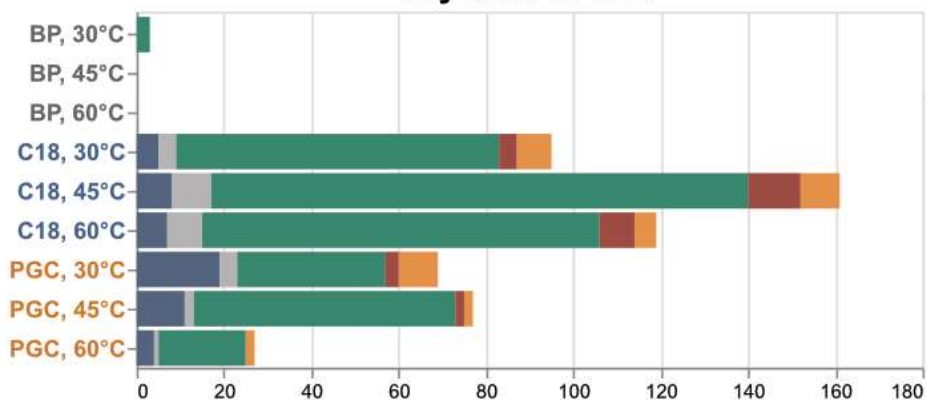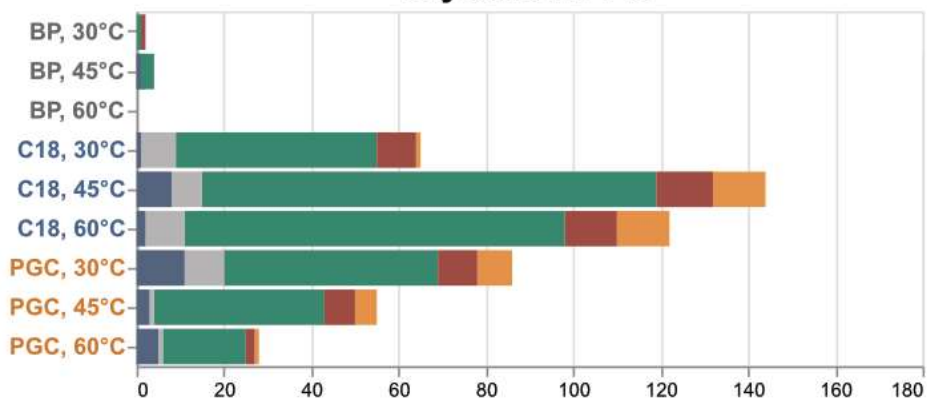

VWNSTFIEDYR + HexNAc(2)Hex(9)
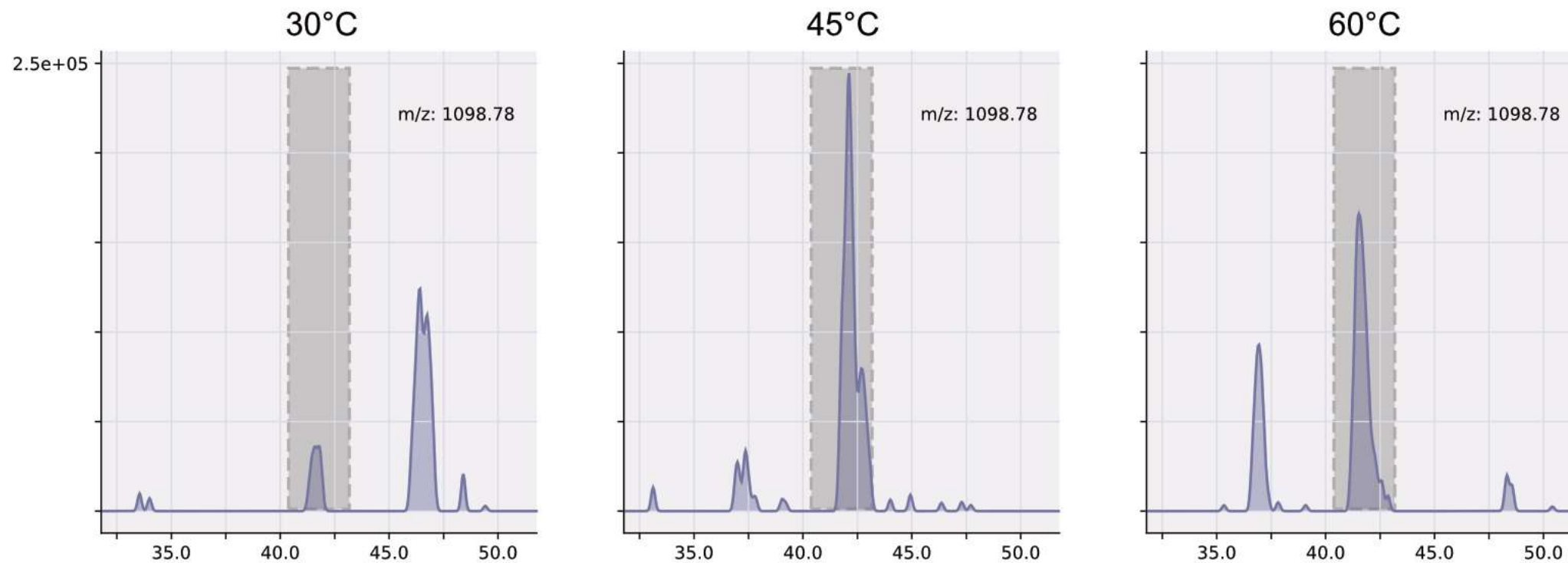
Glycoproteome Profiles

Conclusion

1. Tandem RPLC and PGC analysis provide complementary access to the human proteome

2. PGC demonstrates distinct peptide- and glycan-level differences in identified glycopeptides

3. Incorporation of PGC stationary phase is a facile avenue towards structural elucidation

4. PGC separations may provide advantages in investigative, targeted glycoproteomics applications

Acknowledgments

Dr. Lingjun Li
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Chris Sauer
Dylan Tabang