GENOS

Global leader in high-throughput glycomics

Glycoprofiling catalogue

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Genos Technologies

UHPLC glycoprofiling

- 7 Waters UHPLC instruments with fluorescence detectors
- High-throughput glycoprofiling of up to 200 samples/day

CGE glycoprofiling

- 2 Applied Biosystems 3500 DNA sequencers adapted for glycan analysis
- High-throughput glycoprofiling of up to 300 samples/day

MS glycoprofiling

- 2 Bruker Compact Q-TOF coupled to Waters nanoUPLC systems
- Orbitrap Exploris 240 coupled to Dionex UltiMate 3000 RSLCnano system
- High-throughput glycoprofiling of up to 100 samples/day
- Structural characterization of glycans and glycosylation site occupancy

Genos Glycoprofiling

Glycoprofiling

- Released N-glycan analysis
- Site-specific glycosylation analysis
- Glycosylation site occupancy

Sample types

- Complex biological samples (e.g. human serum/plasma, cell lysates, tissues)
- Purified glycoproteins (e.g. human IgG, IgA, AGP, C3, transferrin, HDL, LDL, rodent IgG)
- Biopharmaceuticals (e.g. mAb)
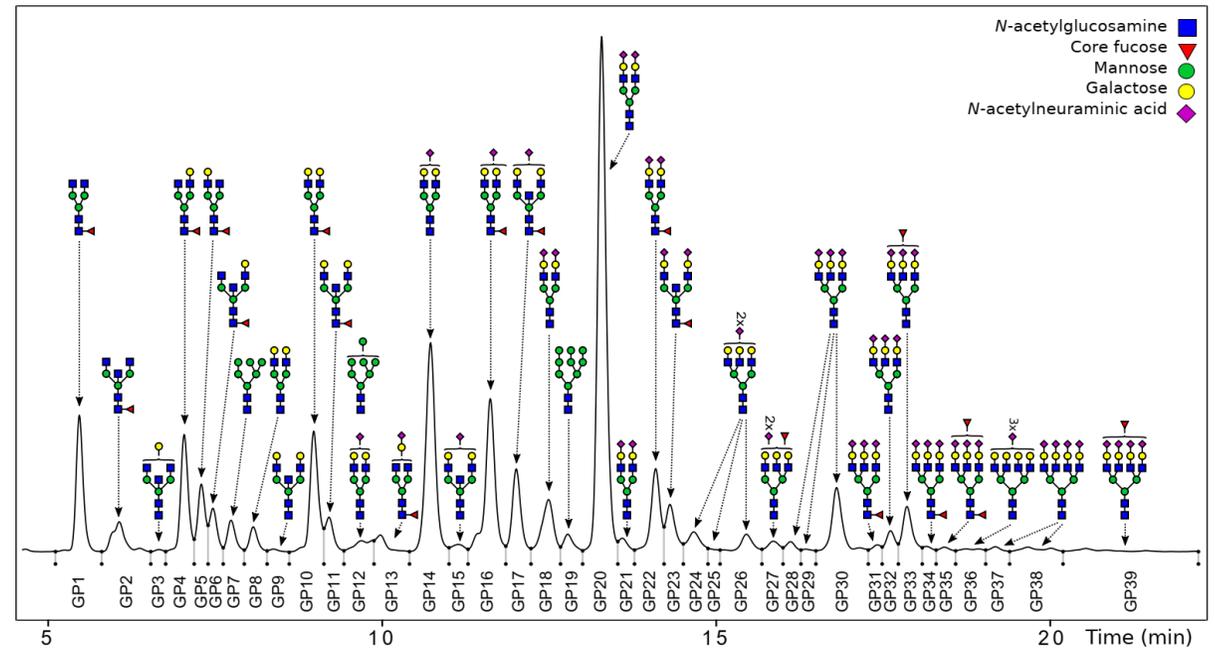
High-throughput analyses

- Analysis of total human serum/plasma N-glycans
- Analysis of total human IgG N-glycans
- Analysis of total human IgA N-glycans
- Analysis of subclass-specific human IgG N-glycopeptides
- Analysis of human AGP N-glycopeptides
- Analysis of human C3 N-glycopeptides
- Analysis of subclass-specific rodent IgG N-glycopeptides

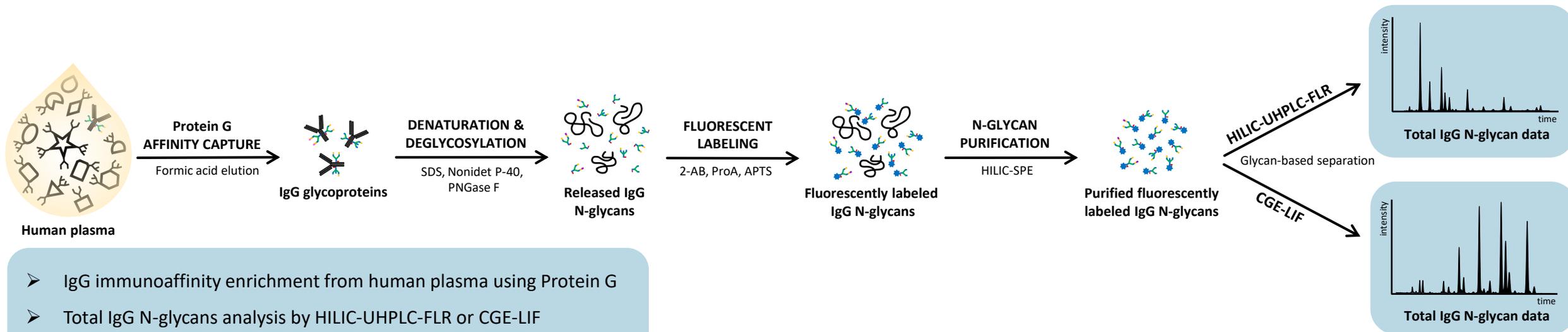# HT Glycoprofiling: Analysis of total human serum/plasma N-glycans
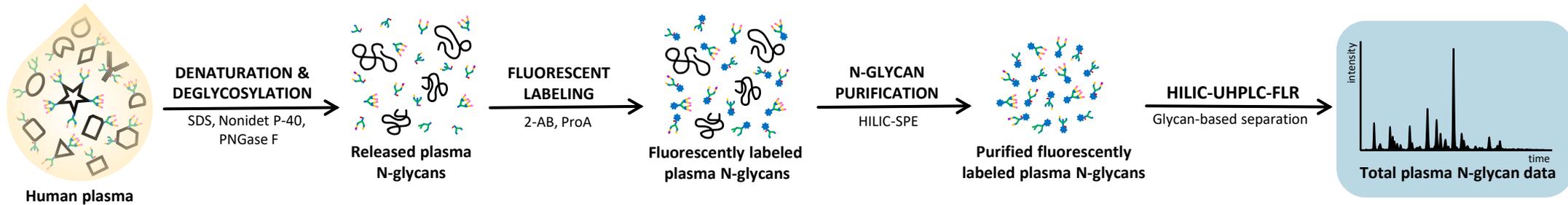
Representative chromatogram of **2-AB labeled plasma N-glycans**

> Total serum/plasma N-glycans analysis by HILIC-UHPLC-FLR

> Optimized workflow for high-throughput analysis

Akmačić, I Trbojević et al. "High-throughput glycomics: optimization of sample preparation." *Biochemistry. Biokhimiia* vol. 80,7 (2015): 934-42. doi:10.1134/S0006297915070123
Zaytseva, Olga O et al. "Heritability of Human Plasma *N*-Glycome." *Journal of proteome research* vol. 19,1 (2020): 85-91. doi:10.1021/acs.jproteome.9b00348

HT Glycoprofiling: Analysis of total human IgG N-glycans

- IgG immunoaffinity enrichment from human plasma using Protein G
- Total IgG N-glycans analysis by HILIC-UHPLC-FLR or CGE-LIF
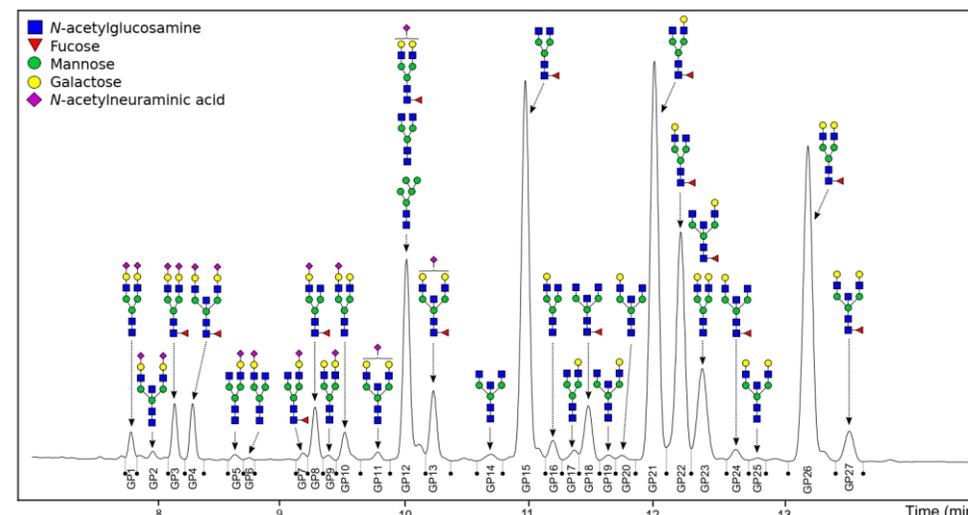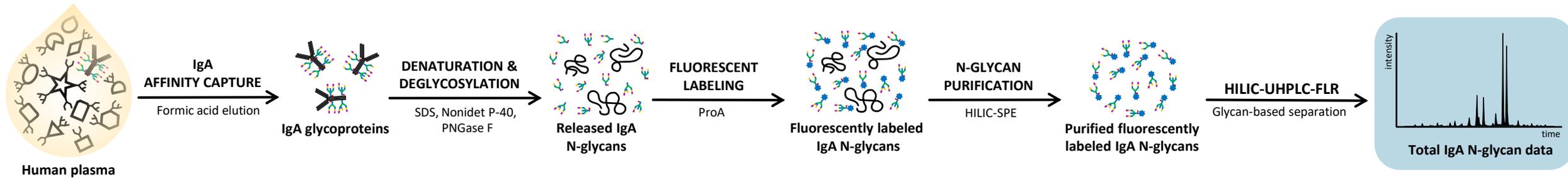- Optimized workflows for high-throughput analysis
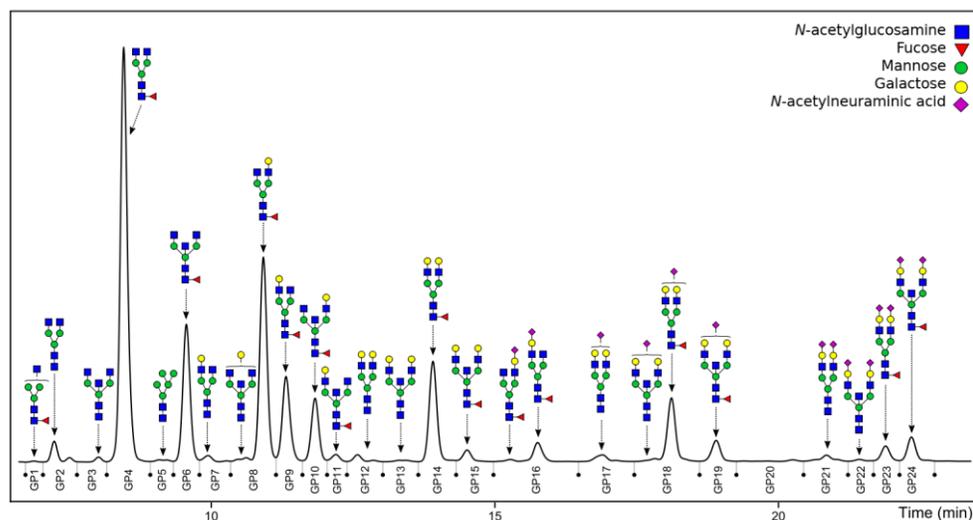
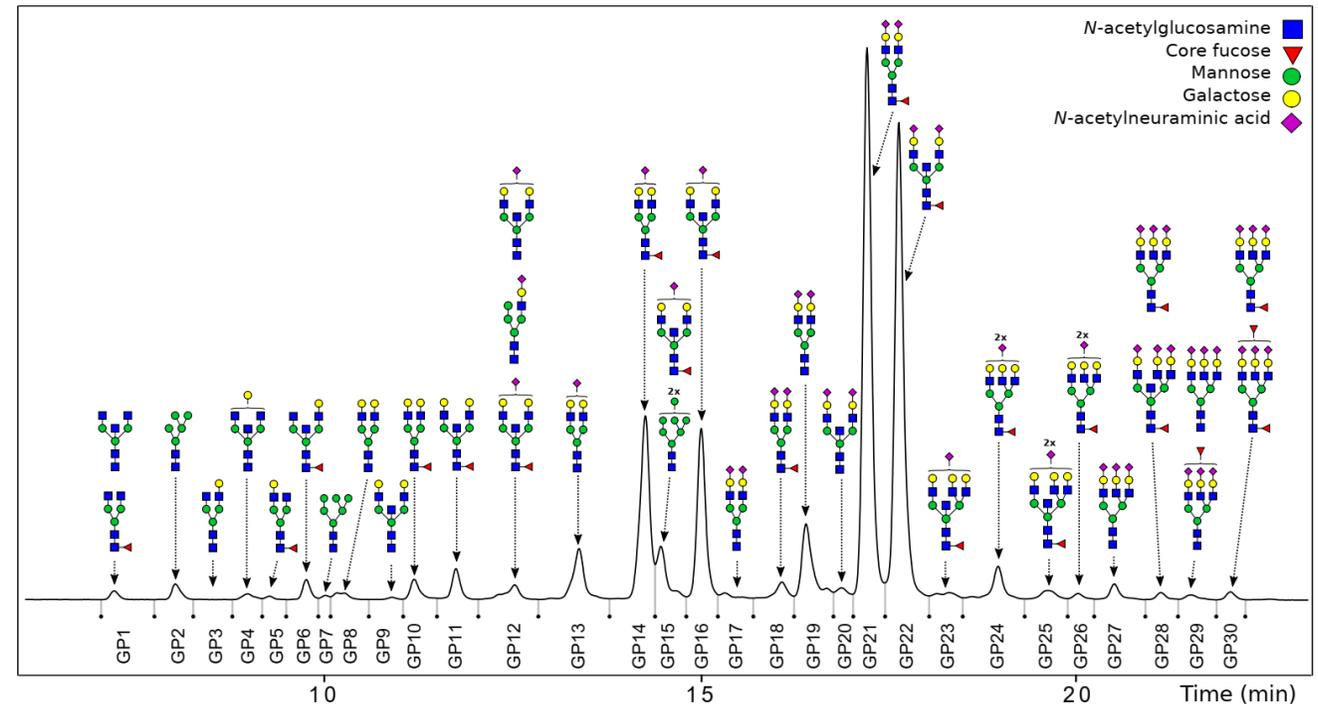*Representative chromatogram of **2-AB labeled IgG N-glycans***

*Representative electropherogram of **APTS labeled IgG N-glycans***

Pučić, Maja et al. "High throughput isolation and glycosylation analysis of IgG-variability and heritability of the IgG glycome in three isolated human populations." *Molecular & cellular proteomics : MCP* vol. 10,10 (2011): M111.010090. doi:10.1074/mcp.M111.010090
Pučić-Baković, Maja. "High-Throughput Analysis of the IgG N-Glycome by UPLC-FLR." *Methods in molecular biology (Clifton, N.J.)* vol. 1503 (2017): 21-29. doi:10.1007/978-1-4939-6493-2_3
Hanić, Maja et al. "N-Glycan Analysis by Ultra-Performance Liquid Chromatography and Capillary Gel Electrophoresis with Fluorescent Labeling." *Current protocols in protein science* vol. 97,1 (2019): e95. doi:10.1002/cpps.95

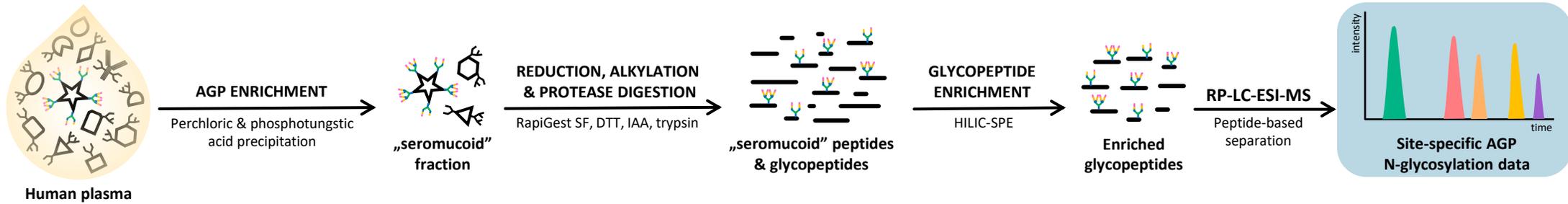# HT Glycoprofiling: Analysis of total human IgA N-glycans

- IgA immunoaffinity enrichment from human plasma using CaptureSelect™ IgA affinity matrix
- Total IgA N-glycans analysis by HILIC-UHPLC-FLR
- Optimized workflow for high-throughput analysis

*Representative chromatogram of **ProA labeled IgA N-glycans***

Štambuk, Tamara et al. „Associations between plasma protein, IgG and IgA N-glycosylation and metabolic health markers in pregnancy and gestational diabetes". *In Review.* https://www.researchsquare.com/article/rs-344452/v1

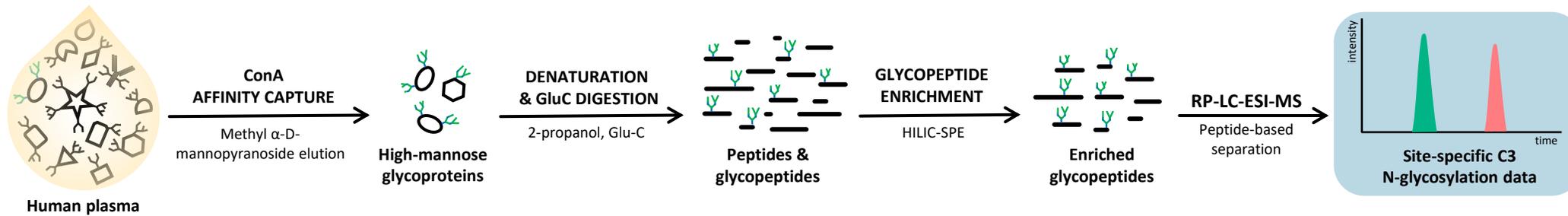# HT Glycoprofiling: Analysis of human AGP N-glycopeptides

- AGP enrichment by precipitation from human plasma „seromucoid" fraction
- AGP (AGP1 and AGP2 isoforms) site-specific glycoprofiling by nano-LC-MS
- AGP Asn-33, Asn-56, Asn-72, Asn-93, and Asn-103 N-glycosylation profiles
- Optimized workflow for high-throughput analysis
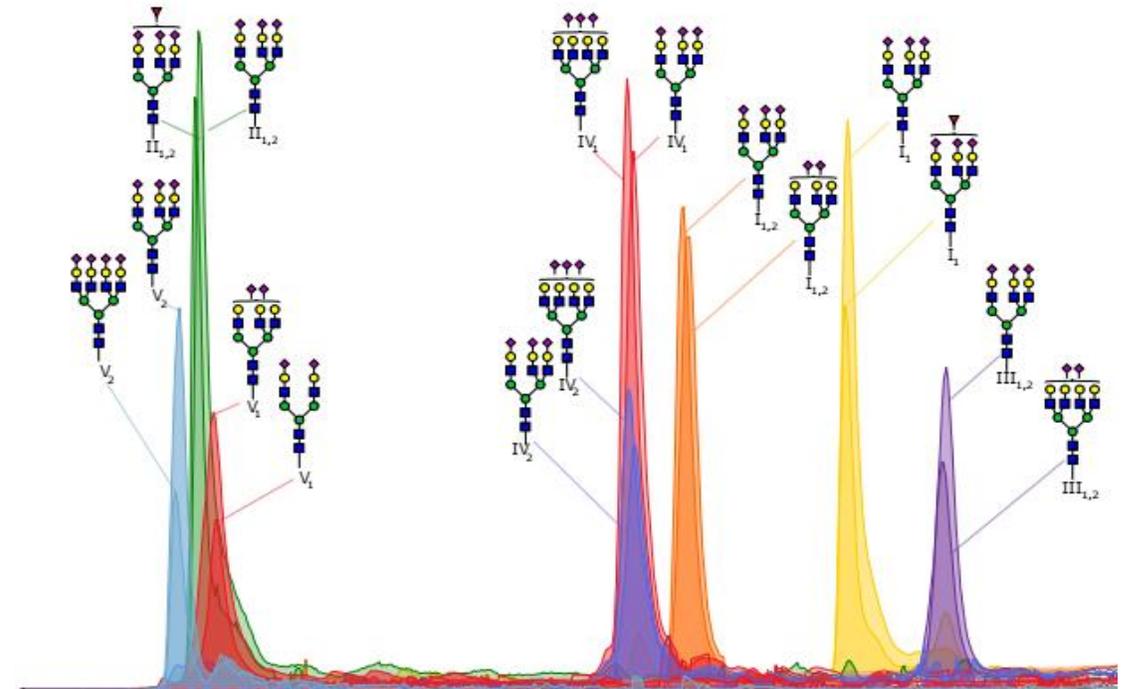
Representative extracted ion chromatograms of the most abundant AGP N-glycopeptides

Keser, Toma et al. "High-Throughput and Site-Specific N-Glycosylation Analysis of Human Alpha-1-Acid Glycoprotein Offers a Great Potential for New Biomarker Discovery." *Molecular & cellular proteomics : MCP* vol. 20 (2021): 100044. doi:10.1074/mcp.RA120.002433

HT Glycoprofiling: Analysis of human C3 N-glycopeptides

- ➤ C3 affinity enrichment from human plasma using ConA
- ➤ C3 site-specific glycoprofiling by nano-LC-MS
- ➤ C3 Asn-85 and Asn-939 N-glycosylation profiles
- ➤ Optimized workflow for high-throughput analysis

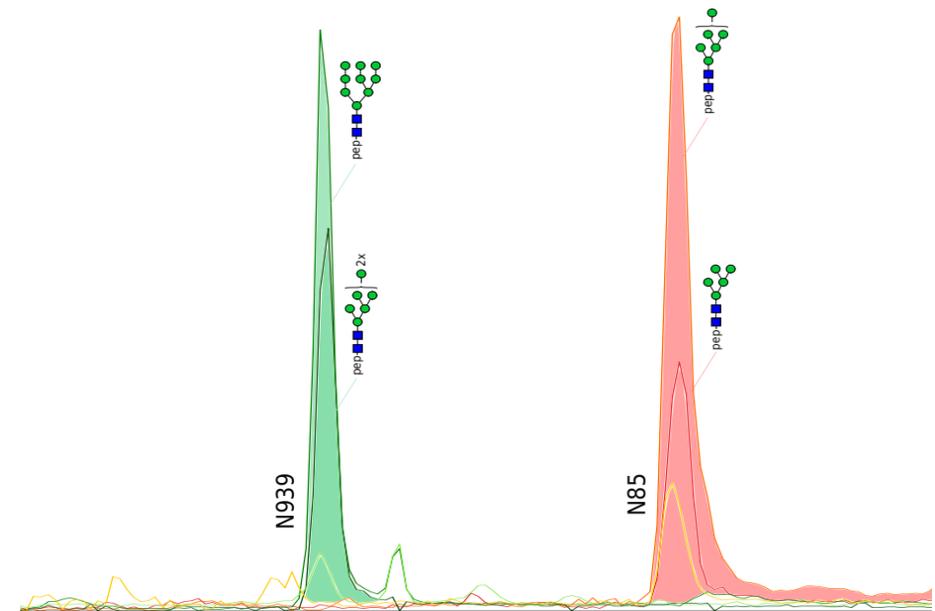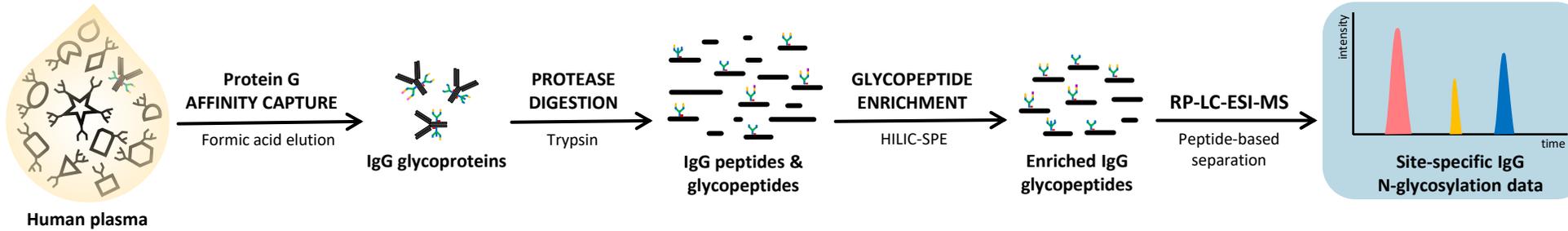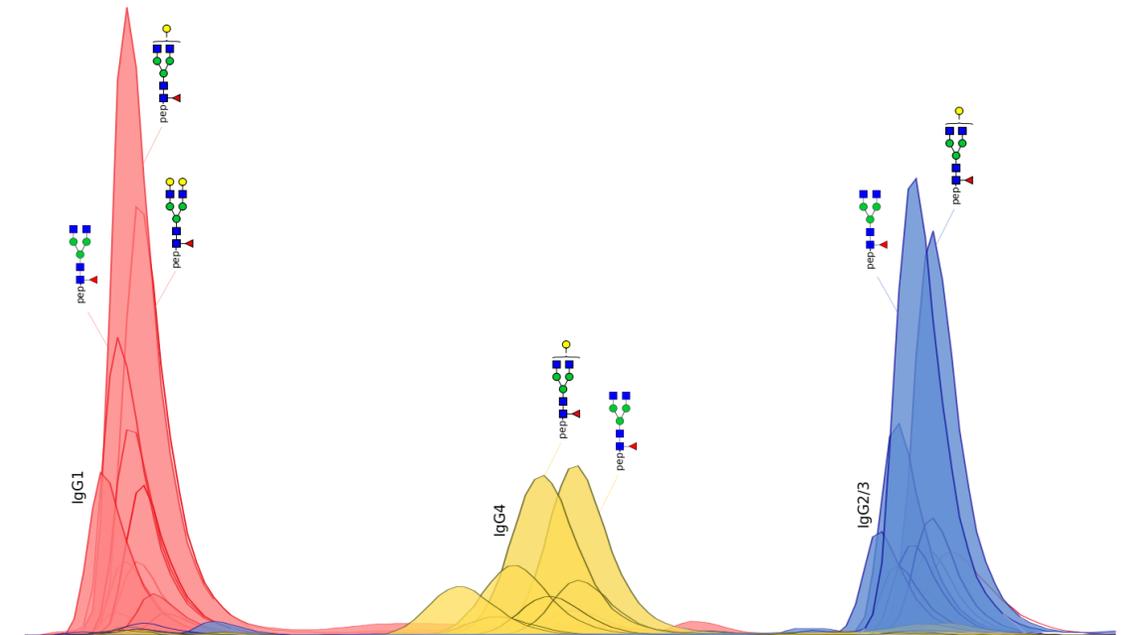*Representaive extracted ion chromatograms of **C3 N-glycopeptides***

HT Glycoprofiling: Analysis of human IgG N-glycopeptides

- IgG immunoaffinity enrichment from human plasma using Protein G
- IgG site- and subclass-specific glycoprofiling by nano-LC-MS
- IgG fragment crystallizable (Fc) Asn-297 N-glycosylation profiles
- Optimized workflow for high-throughput analysis

*Representative extracted ion chromatograms of the most abundant **human IgG N-glycopeptides***

Štambuk, Jerko et al. "Global variability of the human IgG glycome." *Aging* vol. 12,15 (2020): 15222-15259. doi:10.18632/aging.103884

HT Glycoprofiling: Analysis of rodent IgG N-glycopeptides

Mouse/rat plasma

Protein G/L
AFFINITY CAPTURE
Formic acid elution

IgG glycoproteins

PROTEASE
DIGESTION
Trypsin

IgG peptides &
glycopeptides

GLYCOPEPTIDE
ENRICHMENT
C18/HILIC-SPE

Enriched IgG
glycopeptides

RP-LC-ESI-MS

Peptide-based separation

RP-LC-ESI-MS

intensity — time

Site-specific mouse IgG
N-glycosylation data

intensity — time

Site-specific rat IgG
N-glycosylation data

Example of extracted ion chromatograms of **mouse IgG N-glycopeptides**

Example of extracted ion chromatograms of **rat IgG N-glycopeptides**

➢ IgG immunoaffinity enrichment from mouse or rat plasma using Protein G or Protein L

➢ IgG site- and subclass-specific glycoprofiling by nano-LC-MS

➢ IgG fragment crystallizable (Fc) Asn-297 N-glycosylation profiles

➢ Optimized workflows for high-throughput analysis

Zaytseva, Olga O et al. "MIgGGly (mouse IgG glycosylation analysis) - a high-throughput method for studying Fc-linked IgG N-glycosylation in mice with nanoUPLC-ESI-MS." *Scientific reports* vol. 8,1 13688. 12 Sep. 2018, doi:10.1038/s41598-018-31844-1
Habazin, Siniša et al. "High-throughput rat immunoglobulin G N-glycosylation profiling revealed subclass-specific changes associated with chronic stress." *Journal of proteomics* vol. 245 (2021): 104293. doi:10.1016/j.jprot.2021.104293