

LudgerTag™ Procainamide Glycan Labelling System

Sensitive profiling of glycan samples using
(U)HPLC, ESI-MS, and LC-ESI-MS



In this presentation you will see how:

Procainamide

*is applicable to both (U)HPLC, ESI-MS,
and LC-ESI-MS analysis*

*Procainamide labelling is
suitable for 25pmol – 25nmol
quantity of sample and can
identify minor glycan species*

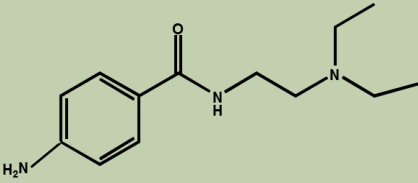
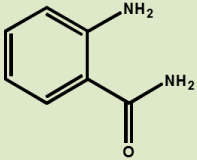
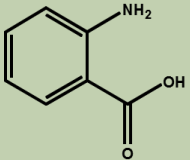
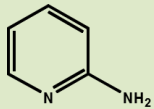


*The validation of the
LudgerTag procainamide
labeling system makes it
suitable to GMP work*

*The incorporation of 2PB
reductant makes the method
easy/safer to use than sodium
cyanoborohydride*

*This technology can be
used for biopharma studies
(e.g. IgG and EPO, N and O- glycosylation)
and different biological samples
(e.g. saliva and blood)*

Fluorescent labels and their suitability to (U)HPLC, MS

| Fluorophore | Structure | (U)HPLC | MS | Comments |
|----------------------------|---|---------|-------|---|
| Procainamide (PROC) |  | ***** | ***** | Enhances MS ionization efficiency. Suitable for LC-MS systems, HPLC (HILIC, RP, WAX) and ESI-MS Low sample amount required Improves identification of very low abundance glycans |
| 2-aminobenzamide (2-AB) |  | **** | **** | The most widely used fluorescent tag. Suitable for HPLC (HILIC, RP, WAX) and MS Higher sample amount required for ESI-MS |
| 2-aminobenzoic acid (2-AA) |  | **** | **** | Suitable for HPLC (HILIC, RP) and MS Higher sample amount required for ESI-MS |
| 2-aminopyridine (2-AP) |  | *** | **** | Suitable for HPLC and MS Higher sample amount required for HPLC and MS analysis |

5 stars = excellent, 4 stars = good, 3 stars = fair, 1 - 2 stars = poor

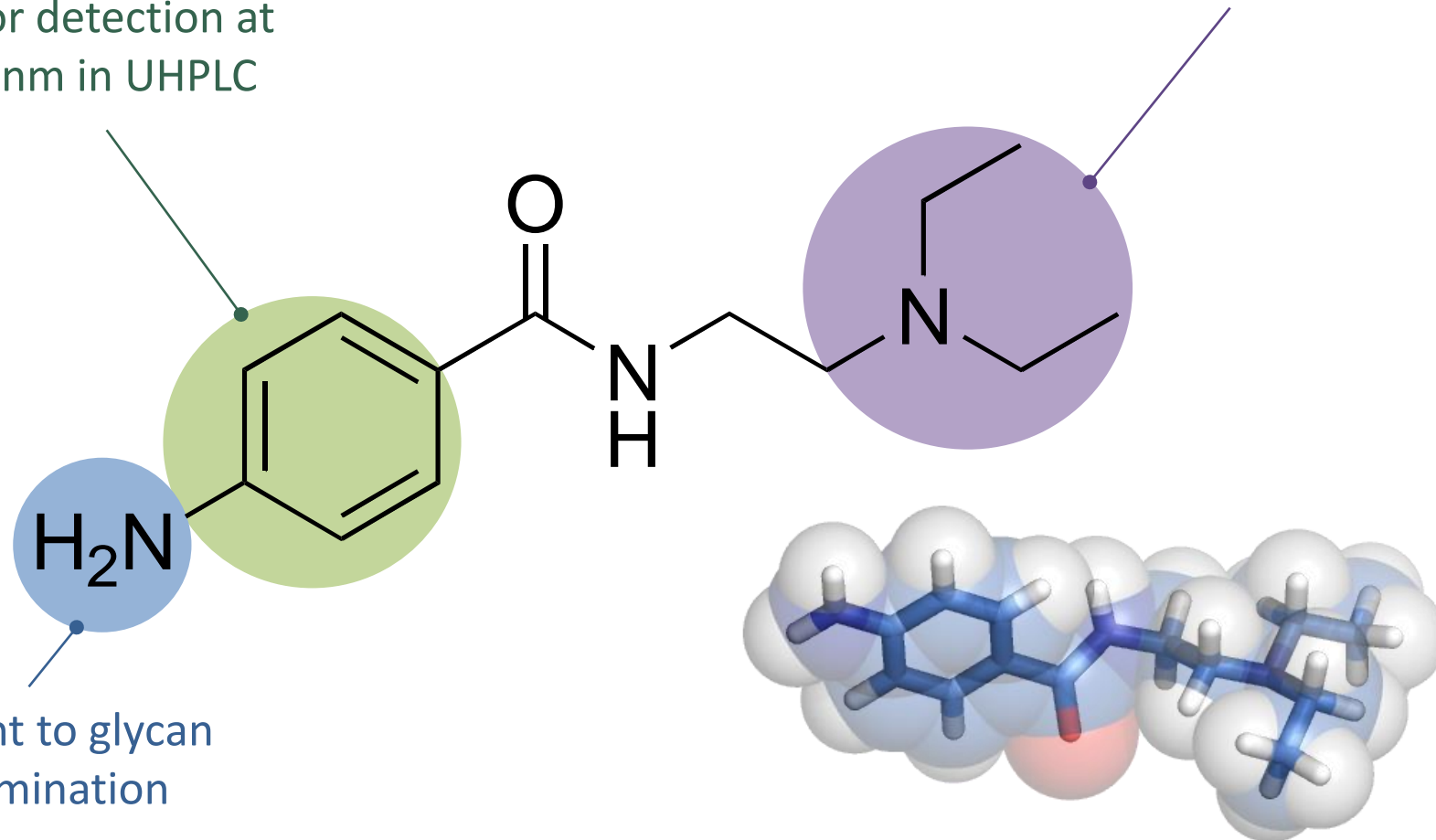
The anatomy of procainamide

4-amino-N-(2-diethylaminoethyl) benzamide

2-(diethylamino)ethyl group enhances the ionization efficiency in the positive ESI mode

Fluorescent group for detection at Ex 310 and Em 370 nm in UHPLC

Added mass
219.172 Da



Site of attachment to glycan
via reductive amination

LudgerTag Procainamide Labelling Kits

Our procainamide labelling technology uses the same *reductive amination* labelling method that has been used for 2AB & 2AA. The following procainamide labelling kits are available:

| Kit name | Reductant | Kit size |
|---------------|-------------------------|------------|
| LT-KPROC-24 | Sodium cyanoborohydride | 24 samples |
| LT-KPROC-96 | | 96 samples |
| LT-KPROC-VP24 | 2-picoline borane | 24 samples |

Sodium cyanoborohydride is a gold standard reductant used in glycan labelling. Best practice is to perform the labelling in a fume cupboard.

2-picoline borane (2-PB) is less toxic than sodium cyanoborohydride and can be used 'on a laboratory bench'.



Standards for procainamide workflow



Process controls:

Glycoproteins – IgG, Fetuin

Unlabelled glycans – including:

- N- and O-glycans
- Purified di-, tri-, tetraantennary glycans
- High mannose glycans
- Glycan libraries

| Ludger System Suitability Standards and Controls | | Process controls (control versus starting sample) | | Sample Process (control) | | Ludger Process (control) | | MS/MS Suitability | | Ludger System Suitability | | MS/MS System Suitability | | System Identification by accurate mass or MS/MS matching | | Quantification | | Endorsement level | | |
|--|-----------------------|---|-----------------------------------|--------------------------|-------|--------------------------|-------|-------------------|-------|---------------------------|-------|--------------------------|-------|--|-------|----------------|-------|-------------------|-------|---|
| Product Category | Ludger Code | Product Description | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | MS/MS | |
| Glycoproteins/Glycosylated | IGP-IGG | IgG Glycoprotein | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | IGP-FET | Fetuin Glycoprotein | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | IGP-IPR-AN232 | IPR-AN232 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Monosaccharide and Sialic Acid standards | CM-SP | SP Sialic Acid Reference Panel | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CM-NEU-AC | Neu5Ac | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CM-NEU-6C | Neu6C | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CM-NEU-5AC2 | Neu5Ac2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CM-MON-6AX | Mono6A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Unlabelled N-Glycans | CM-XYL | Xylose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CM-X | BL, T1 and Tera antennary N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CM-Man-x | High Mannose N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | BO-CHITROBIOS | Chitinose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | BO-CH-MANN | Mann | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CLIM-IGG | IgG N-glycan library | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | SL-MAN6 | Man 6 glycan ref panel | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CLIM-FETUIN | Fetuin N-glycan library | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | CLIM-FETUIN | Fetuin O-glycan library | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| | 2-AB labelled glycans | CAB-GHP | 2-AB labelled Glucose Homopolymer | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| BO-CAB-CH | | 2-AB labelled Chitinose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| CAB-IGG | | 2-AB labelled IgG N-glycan library | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| CAB-x | | BL, T1 and Tera antennary N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| CAB-Man-x | | High Mannose N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| 2-AA labelled glycans | CAB-Glc-x | O-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CAB-AlphaGal | Alpha-Gal standard | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CAA-GHP | 2-AA labelled Glucose Homopolymer | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | BO-CAA-CH | 2-AA labelled Chitinose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| APTS labelled glycans | CAA-Man-x | High Mannose N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CAA-AlphaGal | Alpha-Gal standard | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CAPT5-IGG | APTS labelled IgG N-glycan library | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CAPT5-x | APTS labelled N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| PROC labelled glycans | CAPT5-IGG | APTS labelled IgG N-glycan library | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CAPT5-x | APTS labelled N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CPROG-GHP | PROC labelled Glucose Homopolymer | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CPROG-IGG | Proc labelled IgG N-glycan library | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Permethyated Glycan Standards | CPROG-x | BL and Tera antennary N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CPROG-Man-x | High Mannose N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CPM-IGG | Permethyated IgG N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| | CPM-CL3-IGG | Permethyated 13C IgG N-glycans | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |

For full list of standards check comparison table:
<https://www.ludger.com/system-suitability-standards>

System suitability standards:

Labelled glycans:

- Purified di-, tri-, tetraantennary glycans
- High mannose glycans
- Glycan libraries

Labelled Glucose Homopolymer (GHP)

LudgerTag Procainamide Workflow

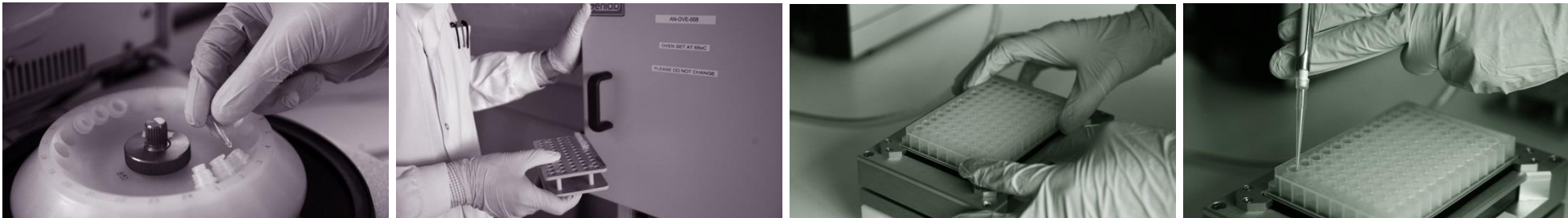
Released Glycans



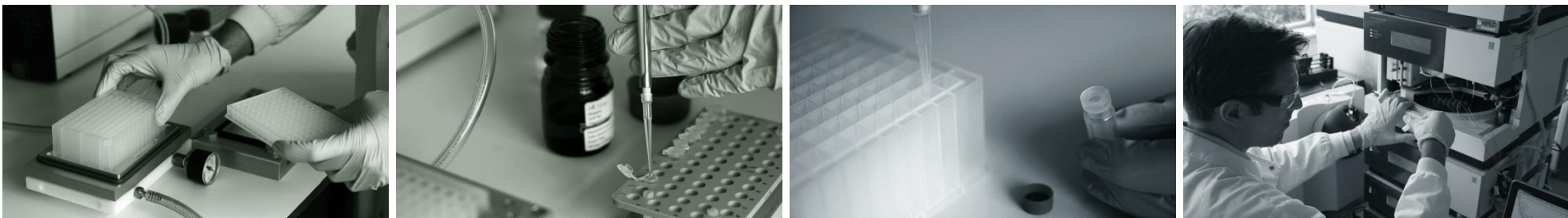
Procainamide Labelling



Enrichment (Purification) and Clean up



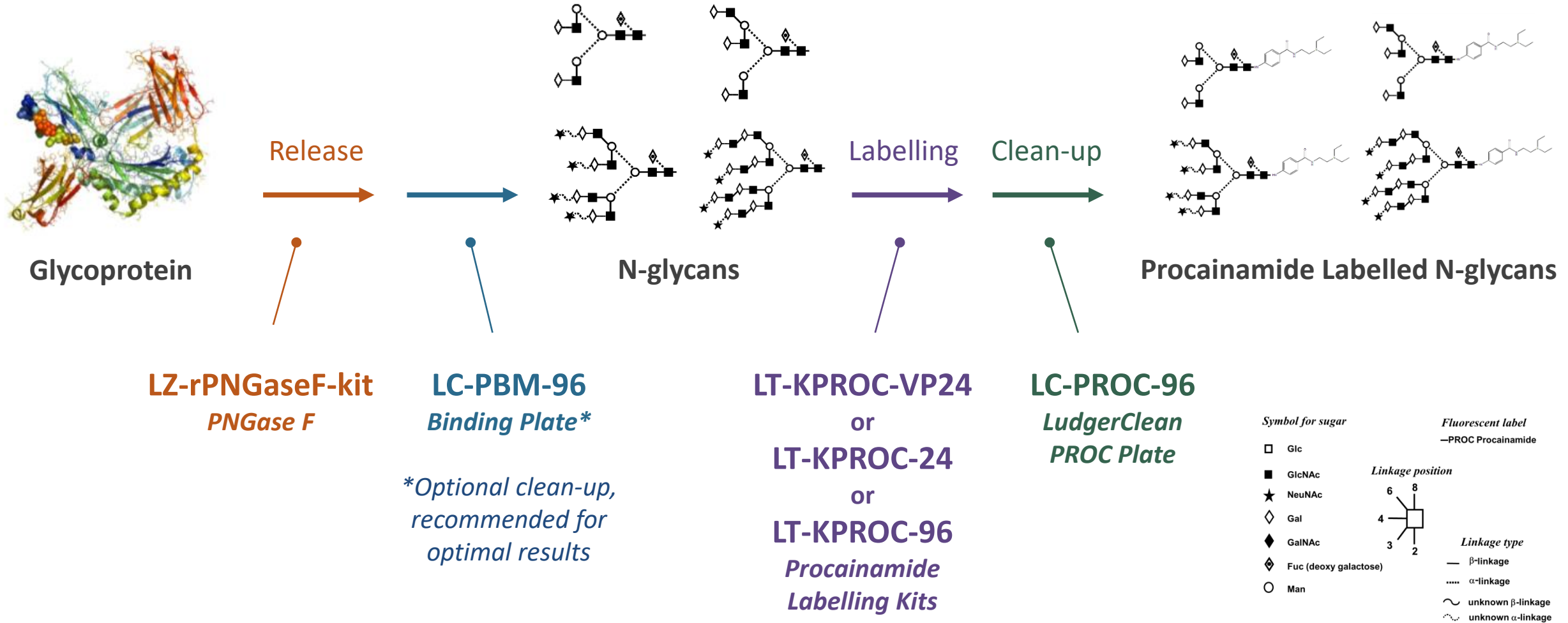
Ready for Analysis



How we are using the
LudgerTag Procainamide
Labelling System
- for N-glycans

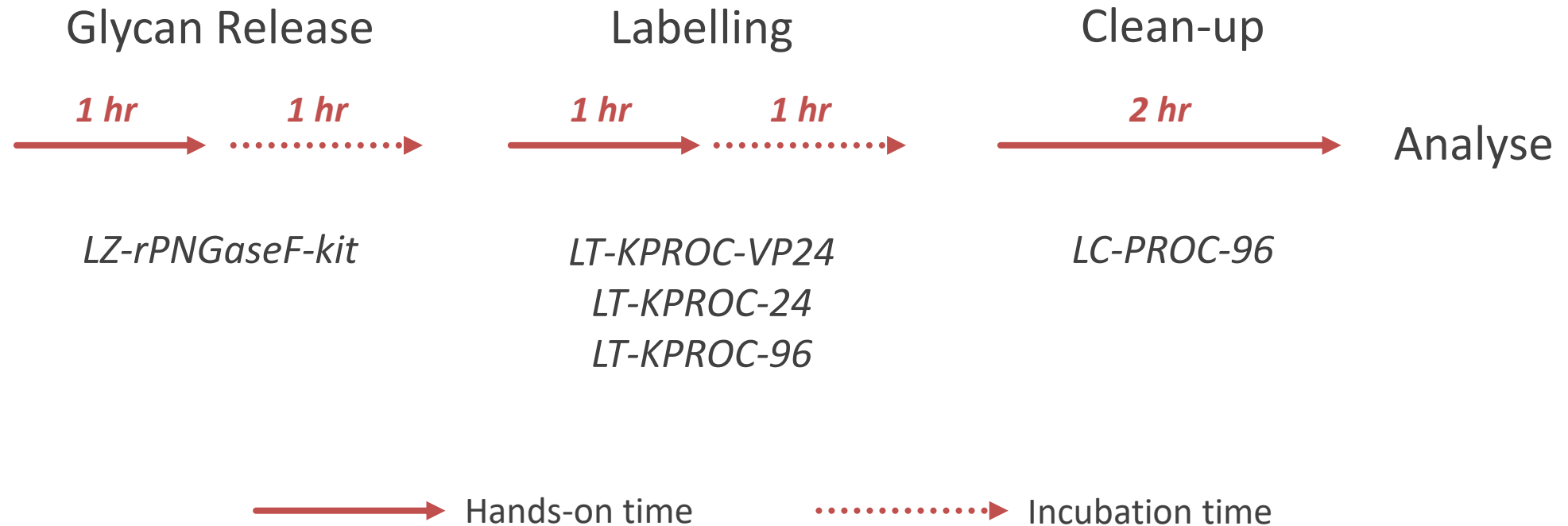


Workflow - LudgerTag Procainamide for N-glycan profiling and identification



Sample preparation within 1 day – N-glycans

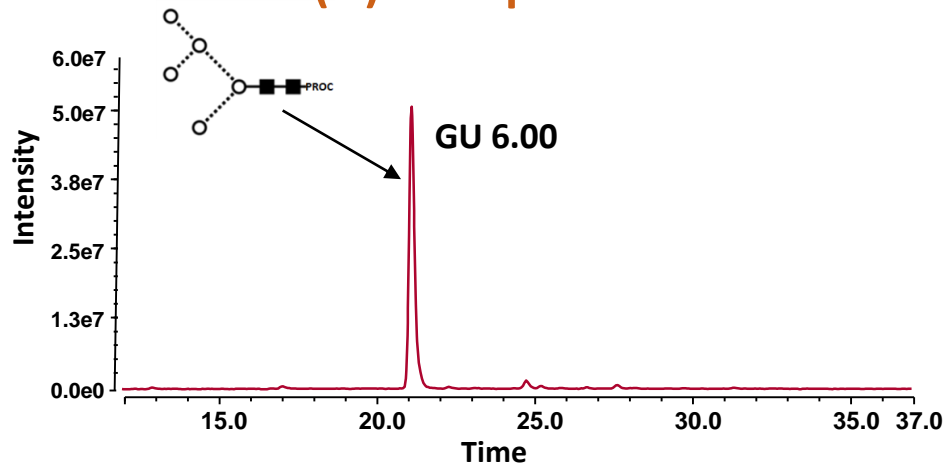
Timing for processing of 96 samples



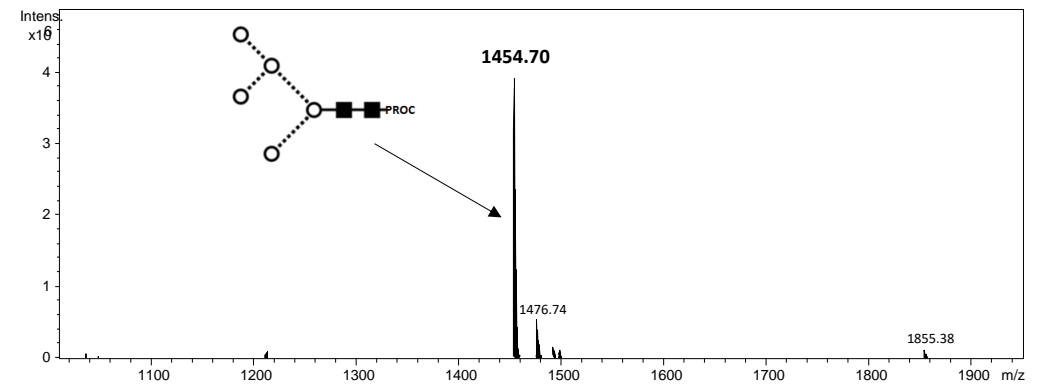
Typical Data



(U)HPLC profile

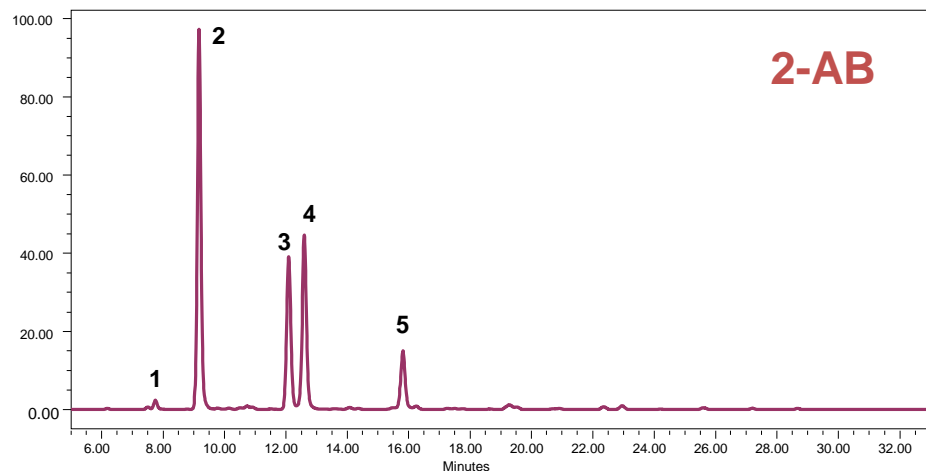


ESI-MS profile

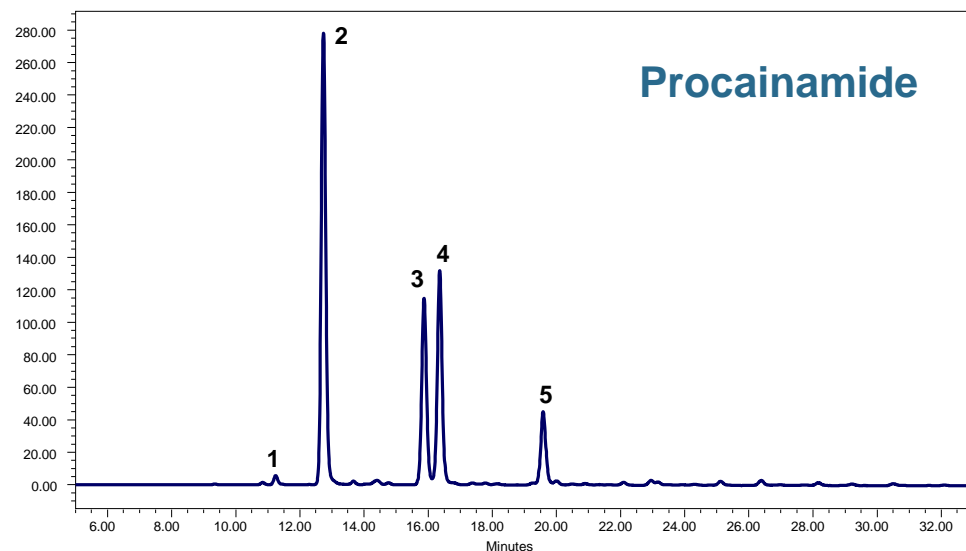


Comparison of 2-AB and Procainamide Glycoprofiling by (U)HPLC

Procainamide labelling of glycans gives comparable results to the gold standard 2-AB labelling of glycans



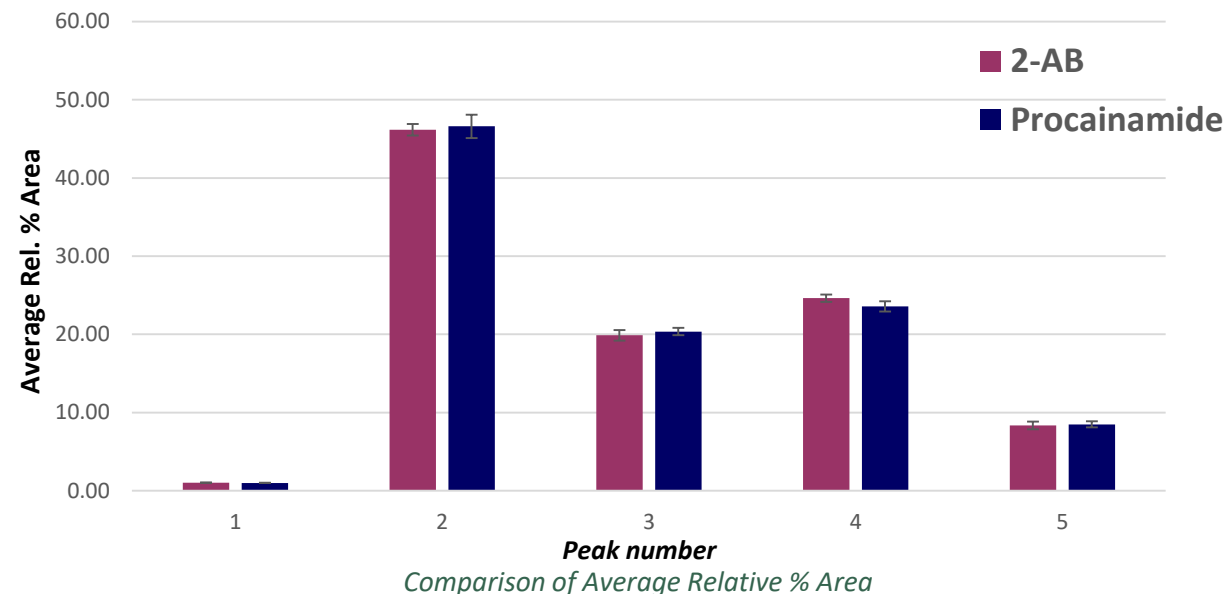
UPLC Chromatogram of 2-AB labeled glycans from mAb



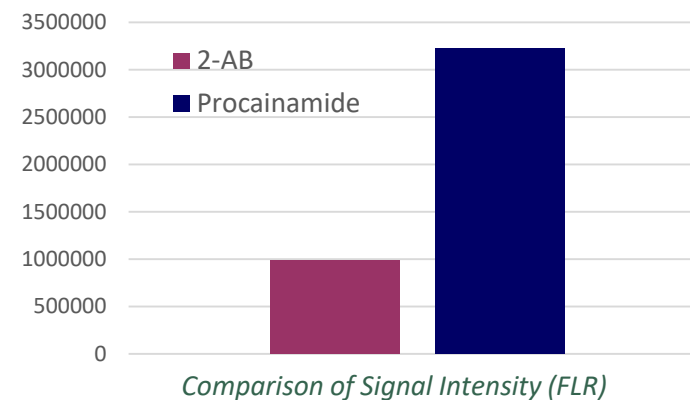
UPLC Chromatogram of Procainamide labelled glycans from mAb



Comparable in quantitation and in reliability, using a Waters ACQUITY UPLC Glycan BEH Amide Column (HILIC)

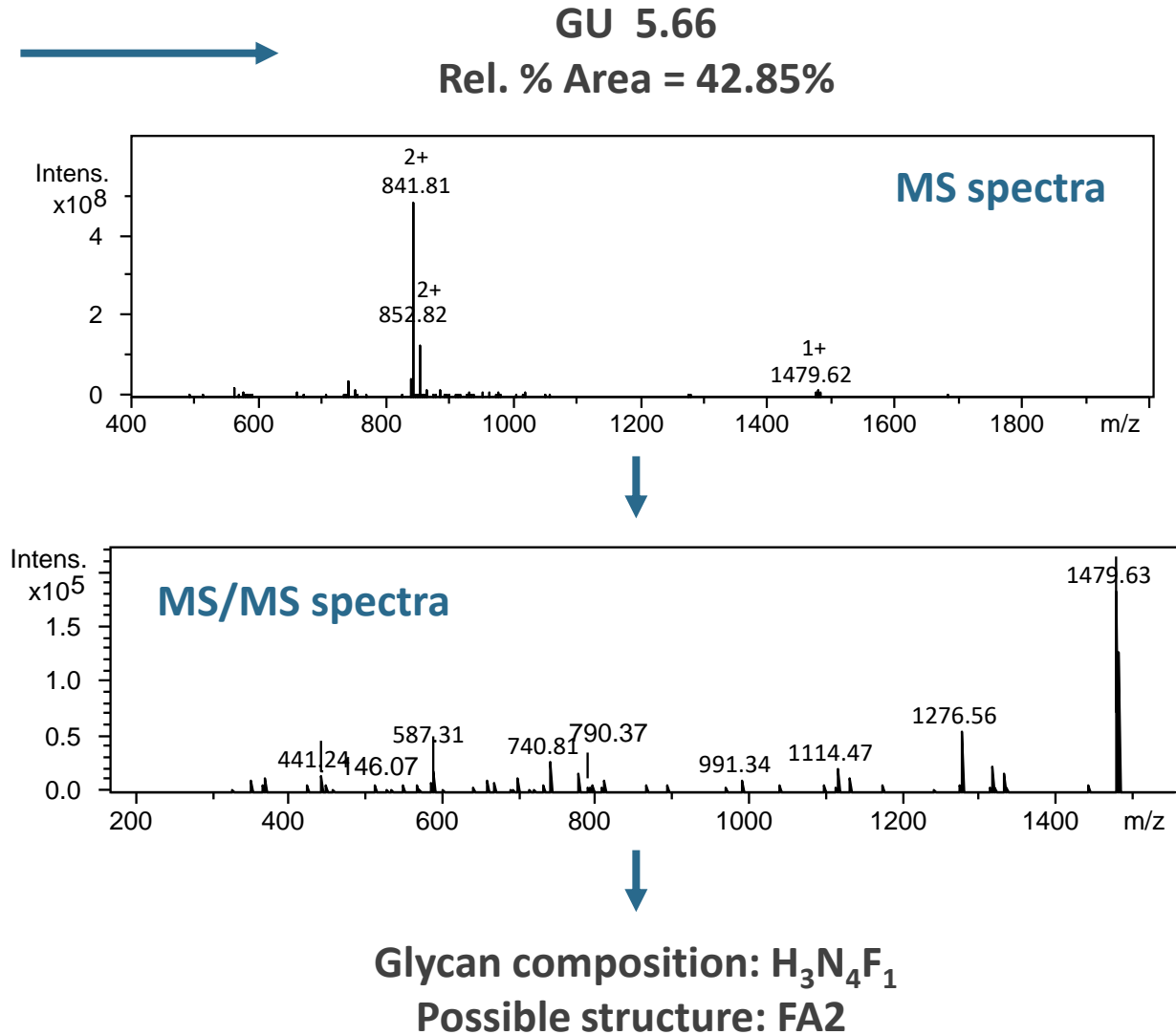
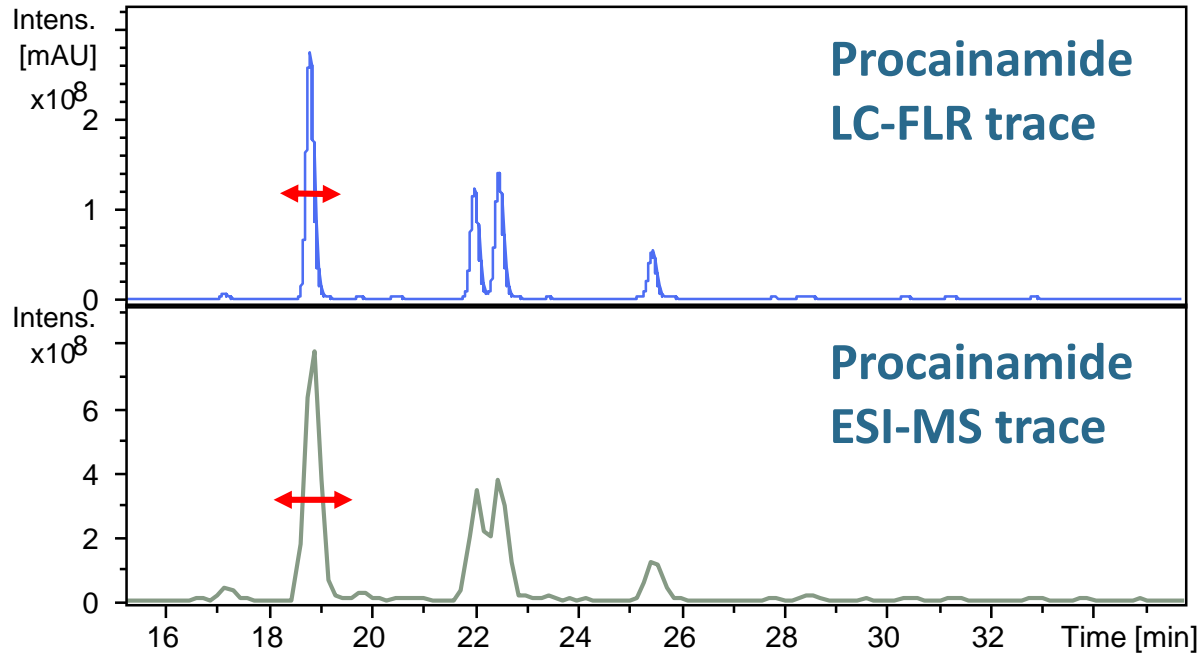


Comparison of Average Relative % Area

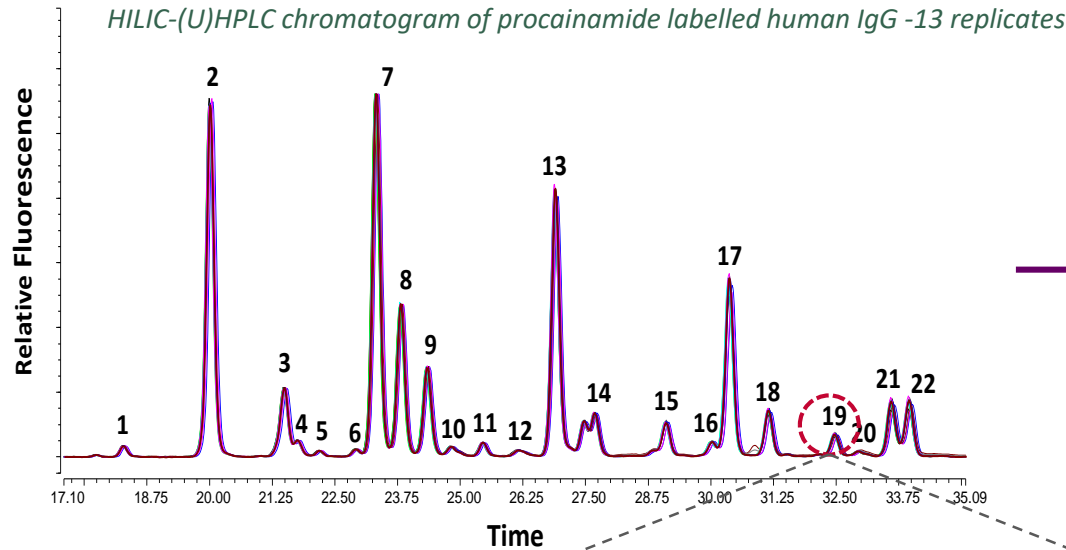


Comparison of Signal Intensity (FLR)

Procainamide Glycoprofiling by LC-MS

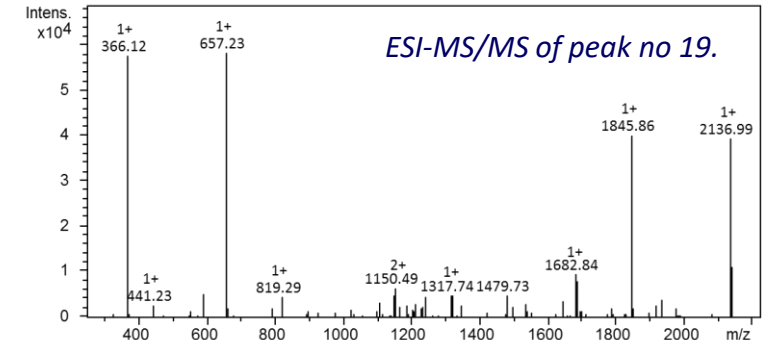
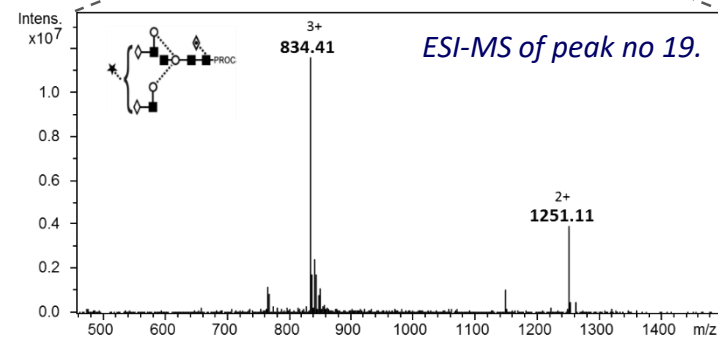


LC-MS analysis of human IgG



| Peak Id | GU | % Area | | | | | | | | | | | | | Average | STDev | CV |
|---------|------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|---------|-------|-------|
| | | Rep1 | Rep2 | Rep3 | Rep4 | Rep5 | Rep6 | Rep7 | Rep8 | Rep9 | Rep10 | Rep11 | Rep12 | Rep13 | | | |
| 1 | 5.26 | 0.59 | 0.51 | 0.50 | 0.57 | 0.54 | 0.55 | 0.49 | 0.55 | 0.56 | 0.58 | 0.57 | 0.52 | 0.54 | 0.54 | 0.03 | 5.74 |
| 2 | 5.65 | 18.49 | 17.99 | 18.46 | 17.74 | 17.65 | 17.75 | 17.38 | 17.80 | 17.61 | 17.69 | 17.42 | 17.65 | 17.80 | 17.80 | 0.34 | 1.90 |
| 3 | 5.99 | 4.12 | 4.03 | 4.10 | 3.94 | 3.92 | 3.92 | 3.90 | 3.95 | 3.90 | 3.95 | 3.80 | 3.89 | 3.95 | 3.95 | 0.09 | 2.20 |
| 4 | 6.05 | 0.93 | 0.89 | 0.92 | 0.92 | 0.92 | 0.89 | 0.91 | 0.93 | 0.90 | 0.91 | 0.93 | 0.91 | 0.91 | 0.91 | 0.01 | 1.51 |
| 5 | 6.16 | 0.47 | 0.41 | 0.42 | 0.43 | 0.48 | 0.42 | 0.42 | 0.48 | 0.41 | 0.44 | 0.47 | 0.39 | 0.44 | 0.44 | 0.03 | 6.76 |
| 6 | 6.33 | 0.49 | 0.49 | 0.50 | 0.49 | 0.49 | 0.45 | 0.47 | 0.49 | 0.49 | 0.46 | 0.48 | 0.49 | 0.48 | 0.48 | 0.01 | 2.95 |
| 7 | 6.43 | 18.76 | 18.51 | 18.81 | 18.08 | 18.22 | 18.26 | 17.91 | 18.22 | 18.15 | 18.05 | 18.06 | 18.27 | 18.28 | 18.28 | 0.27 | 1.47 |
| 8 | 6.55 | 8.11 | 7.95 | 8.13 | 7.85 | 7.87 | 7.90 | 7.80 | 7.88 | 7.88 | 7.87 | 7.86 | 7.94 | 7.92 | 7.92 | 0.10 | 1.23 |
| 9 | 6.68 | 5.01 | 4.92 | 4.99 | 4.84 | 4.89 | 4.85 | 4.81 | 4.85 | 4.82 | 4.84 | 4.86 | 4.88 | 4.88 | 4.88 | 0.06 | 1.30 |
| 10 | 6.8 | 0.91 | 0.91 | 0.89 | 0.95 | 0.90 | 0.91 | 0.93 | 0.93 | 0.88 | 0.93 | 0.85 | 0.89 | 0.91 | 0.91 | 0.03 | 2.86 |
| 11 | 6.96 | 0.95 | 0.89 | 0.97 | 0.95 | 0.98 | 0.92 | 0.97 | 0.91 | 0.94 | 0.97 | 0.98 | 0.92 | 0.95 | 0.95 | 0.03 | 3.04 |
| 12 | 7.13 | 0.81 | 0.77 | 0.73 | 0.78 | 0.76 | 0.73 | 0.76 | 0.73 | 0.75 | 0.77 | 0.72 | 0.78 | 0.76 | 0.76 | 0.03 | 3.37 |
| 13 | 7.33 | 13.89 | 13.92 | 13.97 | 13.74 | 13.97 | 14.11 | 13.89 | 13.94 | 14.05 | 13.93 | 14.11 | 14.13 | 13.97 | 13.97 | 0.11 | 0.78 |
| 14 | 7.54 | 4.28 | 4.30 | 4.25 | 4.26 | 4.34 | 4.31 | 4.32 | 4.30 | 4.30 | 4.32 | 4.31 | 4.32 | 4.30 | 4.30 | 0.02 | 0.58 |
| 15 | 7.93 | 2.31 | 2.58 | 2.33 | 2.62 | 2.43 | 2.38 | 2.88 | 2.61 | 2.60 | 2.61 | 2.55 | 2.52 | 2.54 | 2.54 | 0.15 | 5.93 |
| 16 | 8.19 | 0.88 | 0.96 | 0.91 | 0.97 | 0.97 | 0.91 | 1.03 | 0.96 | 0.95 | 0.97 | 0.94 | 0.93 | 0.95 | 0.95 | 0.04 | 3.89 |
| 17 | 8.3 | 9.01 | 9.36 | 9.13 | 9.21 | 9.54 | 9.68 | 9.56 | 9.54 | 9.70 | 9.45 | 9.80 | 9.66 | 9.47 | 9.47 | 0.24 | 2.49 |
| 18 | 8.52 | 2.47 | 2.68 | 2.50 | 2.61 | 2.72 | 2.74 | 2.66 | 2.62 | 2.70 | 2.62 | 2.69 | 2.63 | 2.64 | 2.64 | 0.08 | 2.99 |
| 19 | 8.92 | 1.42 | 1.34 | 1.25 | 1.39 | 1.37 | 1.34 | 1.49 | 1.39 | 1.39 | 1.38 | 1.38 | 1.35 | 1.37 | 1.37 | 0.05 | 3.91 |
| 20 | 9.07 | 0.51 | 0.56 | 0.52 | 0.77 | 0.62 | 0.56 | 0.69 | 0.59 | 0.56 | 0.60 | 0.60 | 0.58 | 0.60 | 0.60 | 0.07 | 11.55 |
| 21 | 9.28 | 2.76 | 2.99 | 2.83 | 3.05 | 3.15 | 3.19 | 3.29 | 3.10 | 3.22 | 3.18 | 3.27 | 3.13 | 3.10 | 3.10 | 0.16 | 5.11 |
| 22 | 9.39 | 2.83 | 3.04 | 2.87 | 3.21 | 3.27 | 3.23 | 3.42 | 3.23 | 3.25 | 3.24 | 3.39 | 3.21 | 3.18 | 3.18 | 0.17 | 5.47 |

HILIC-(U)HPLC data for procainamide labelled human IgG -13 replicates



| Peak no 19 | | | | | | | | | | [M/Z] characteristic fragment ions (composition) | | | |
|-------------|------------|---------|------------|--------------------|---------------------|---------------------|--------------------|---------------------|---------------------|--|-------------------------|-----------------------|--------------------|
| Composition | | | | Calculated | | | Registered | | | | | | |
| Hex (H) | HexNAc (N) | Fuc (F) | Neu5Ac (S) | [M/Z] ⁺ | [M/Z] ²⁺ | [M/Z] ³⁺ | [M/Z] ⁺ | [M/Z] ²⁺ | [M/Z] ³⁺ | | | | |
| 5 | 5 | 1 | 1 | 2501.01 | 1251.01 | 864.34 | nd | 1251.11 | 834.41 | 2136.01 (H4N4F1S1-PROC) | 1770.38 (H3N3F1S1-PROC) | 1479.73 (H3N3F1-PROC) | 790.40 (N2F1-PROC) |
| | | | | | | | | | | 1989.88 (H4N4S1-PROC) | 1712.63 (H5N3S1) | 1317.74 (H2N3F1-PROC) | 657.23 (H1N1S1) |
| | | | | | | | | | | 1974.88 (H3N4F1S1-PROC) | 1682.84 (H3N4F1-PROC) | 1277.69 (H3N2F1-PROC) | 587.38 (N1F1-PROC) |
| | | | | | | | | | | 1932.84 (H4N3F1S1-PROC) | 1642.77 (H4N3F1-PROC) | 1114.59 (H2N2F1-PROC) | 441.23 (N1-PROC) |
| | | | | | | | | | | 1844.87 (H4N4F1-PROC) | 1536.57 (H3N4-PROC) | 819.29 (H2N1S1) | 366.12 (H1N1) |

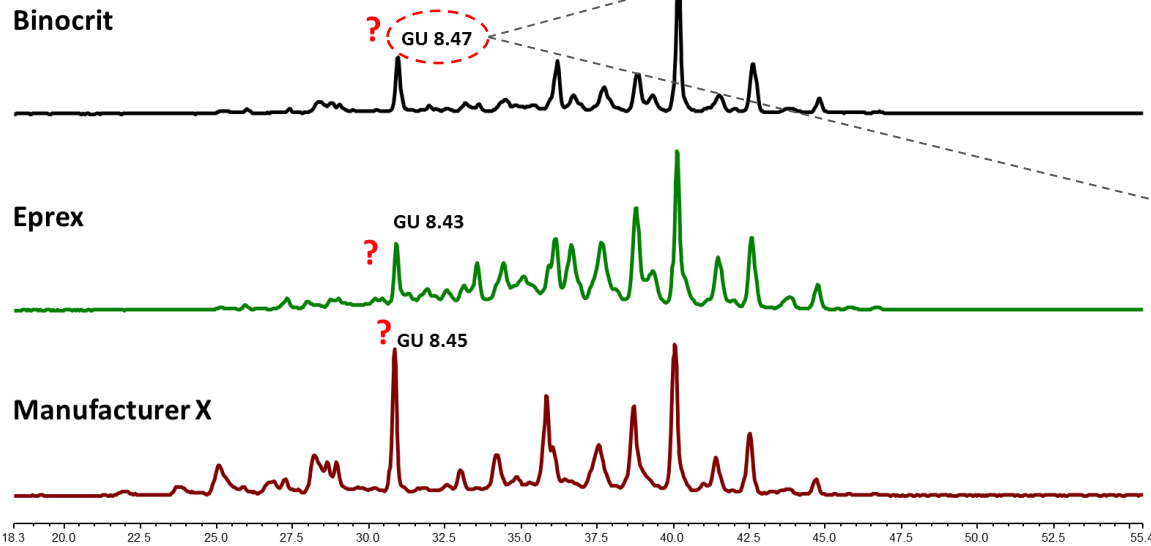
ESI-MS and MS/MD data for HILIC peak 19

- Repeatabile and robust
- One system for relative quantitation and identification of glycans (HILIC-(U)HPLC fitted with online MS)

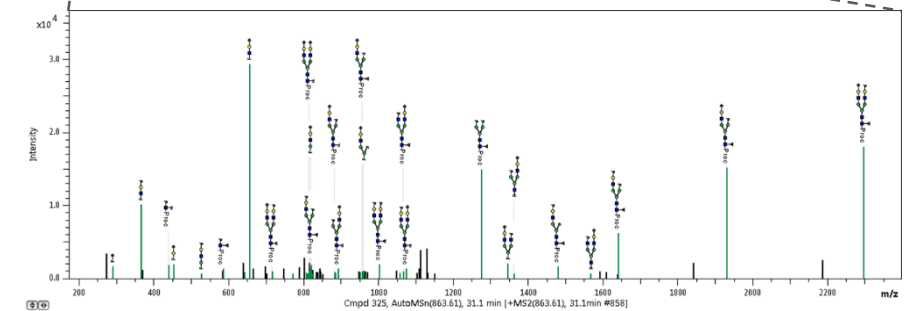
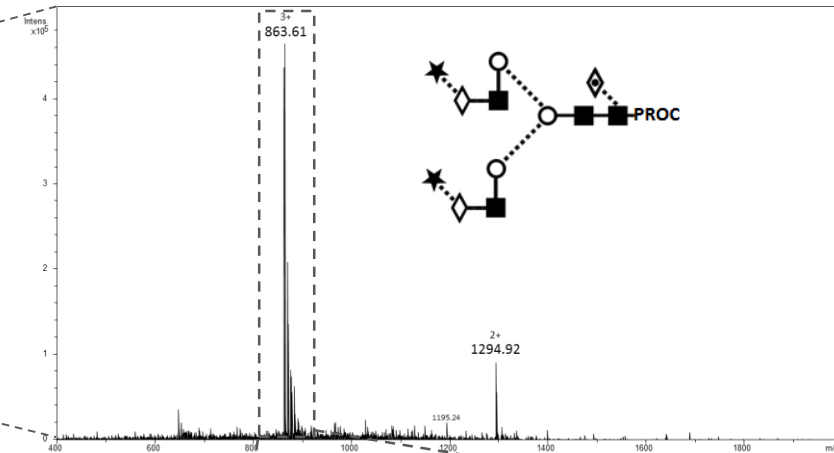
LC-MS analysis of biosimilars

erythropoietin, EPO

HILIC-(U)HPLC chromatograms of procainamide labelled N-glycans released from EPO (3 different manufacturers)



ESI-MS of selected (GU 8.47) procainamide labelled peak



ESI-MS/MS of selected (GU 8.47) procainamide labelled peak

LC-MS analysis of complex biological samples

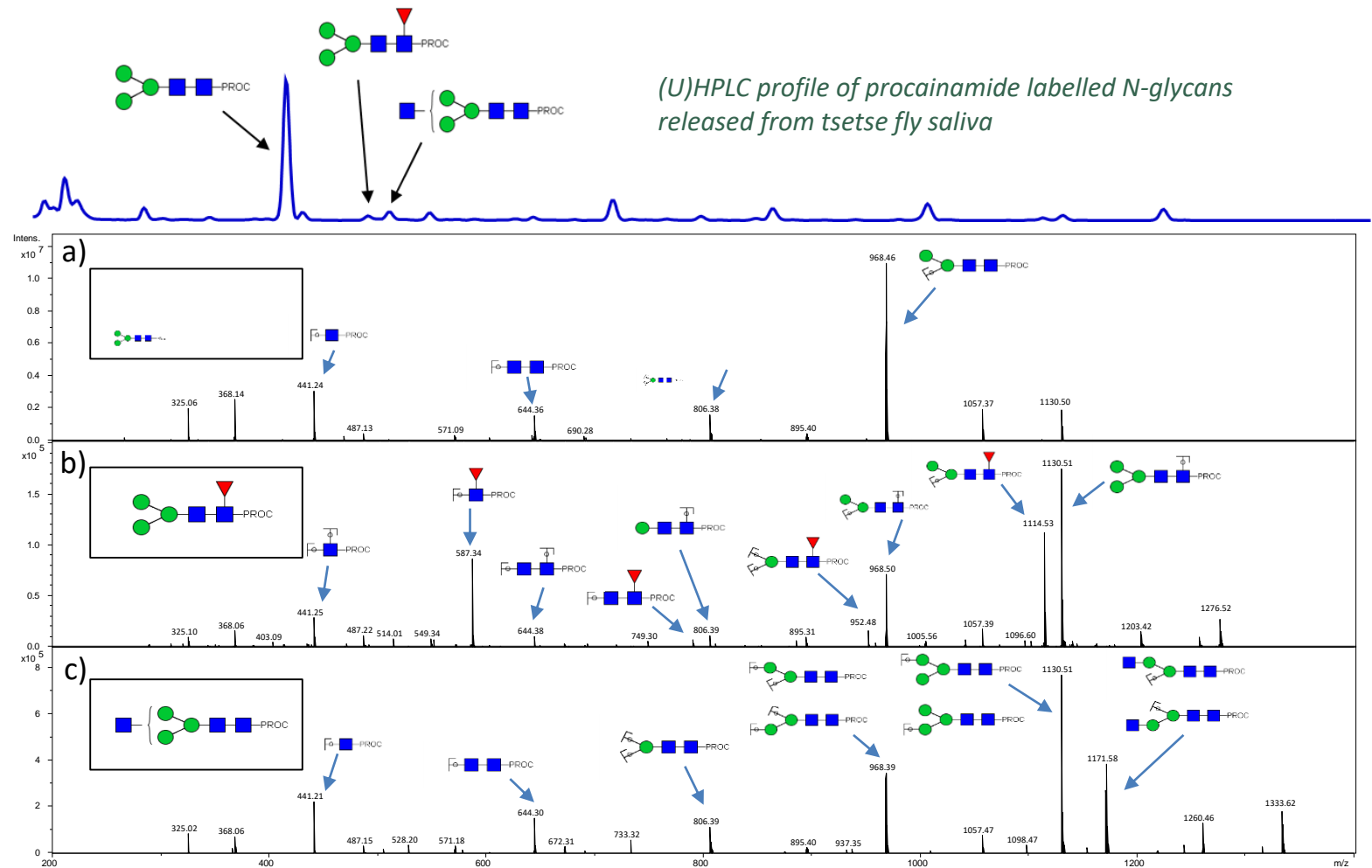
Tsetse fly saliva



Sleeping sickness occurs in 36 sub-Saharan Africa countries where there are **tsetse flies** that transmit the disease. It is transmitted through the saliva of the tsetse fly during feeding

Salivary glycoproteins have been reported to:

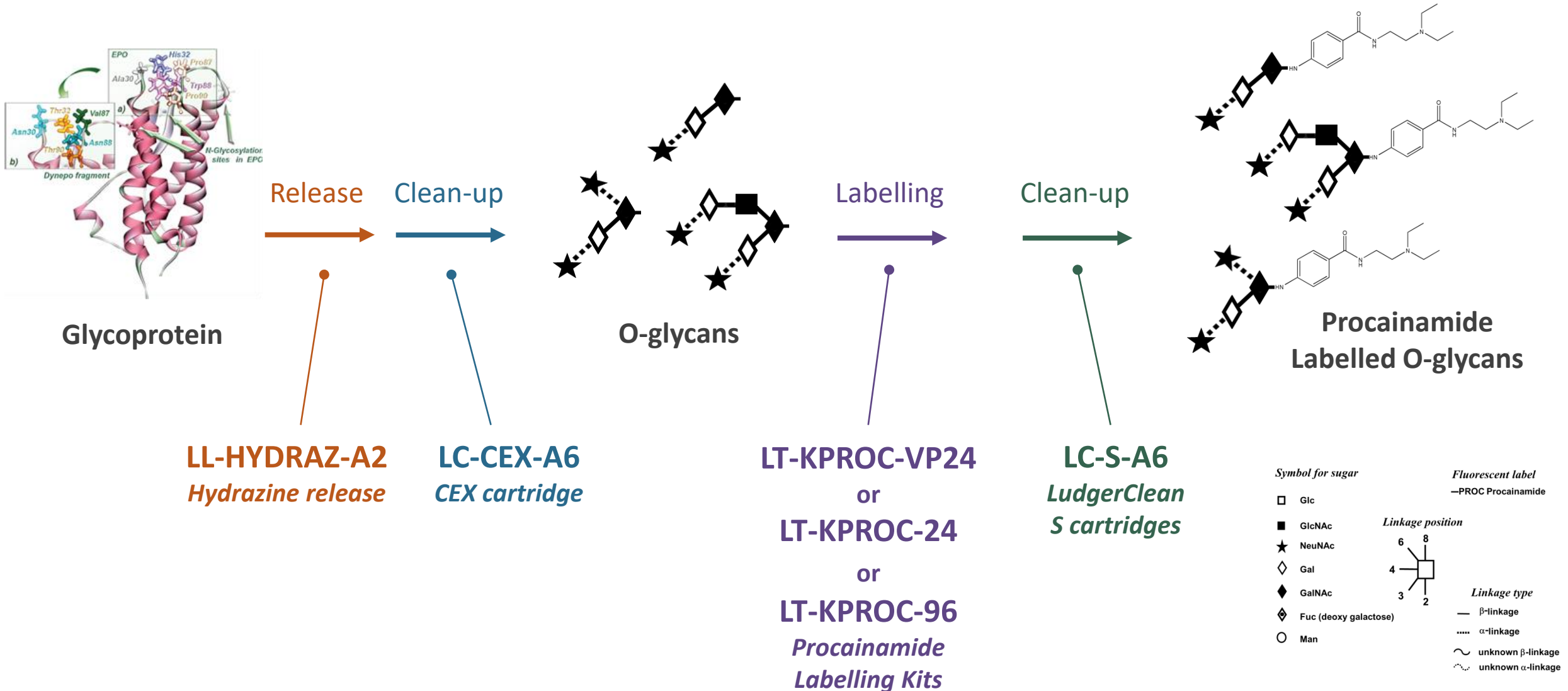
- facilitate host infection through binding and transport of vector-borne diseases to host tissues
- participate in host responses such as inflammation and immune response



How we are using the
LudgerTag Procaïnamide
Labelling System
- for O-glycans

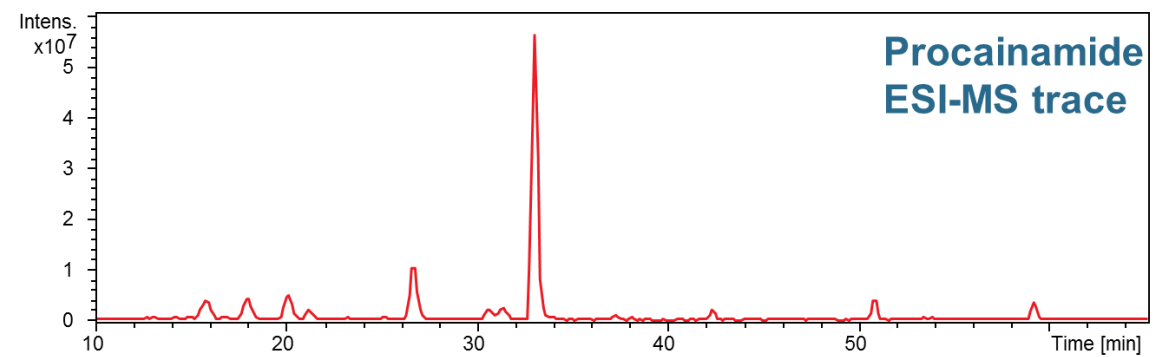
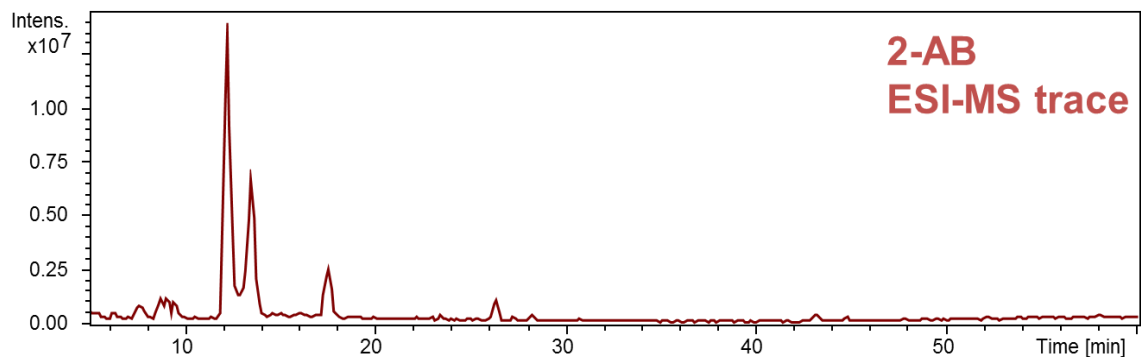
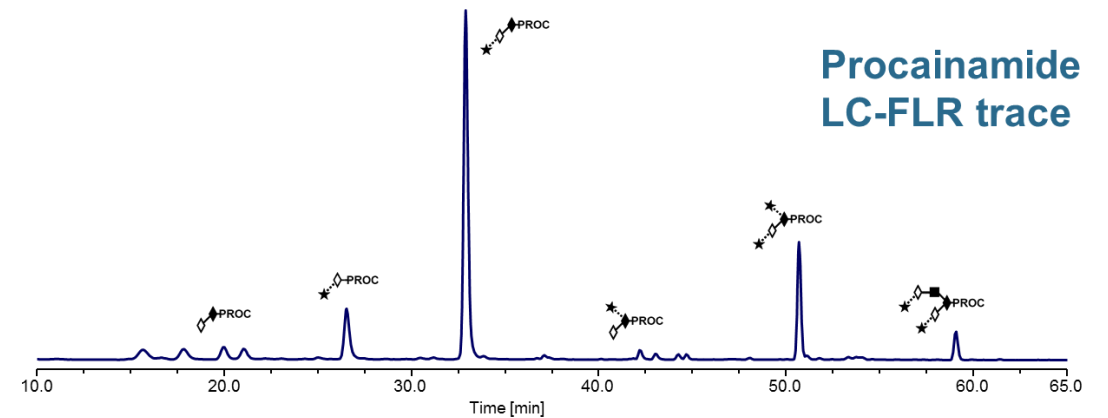
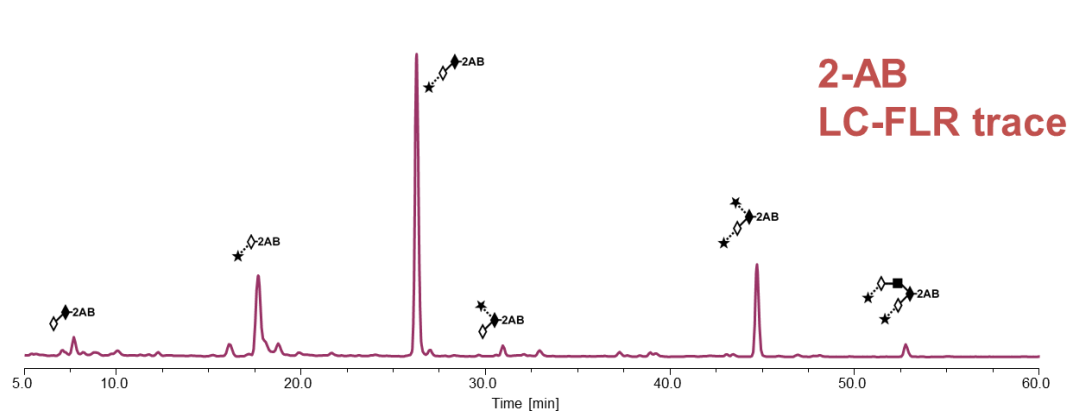


Workflow - LudgerTag Procainamide for O-glycan profiling and identification

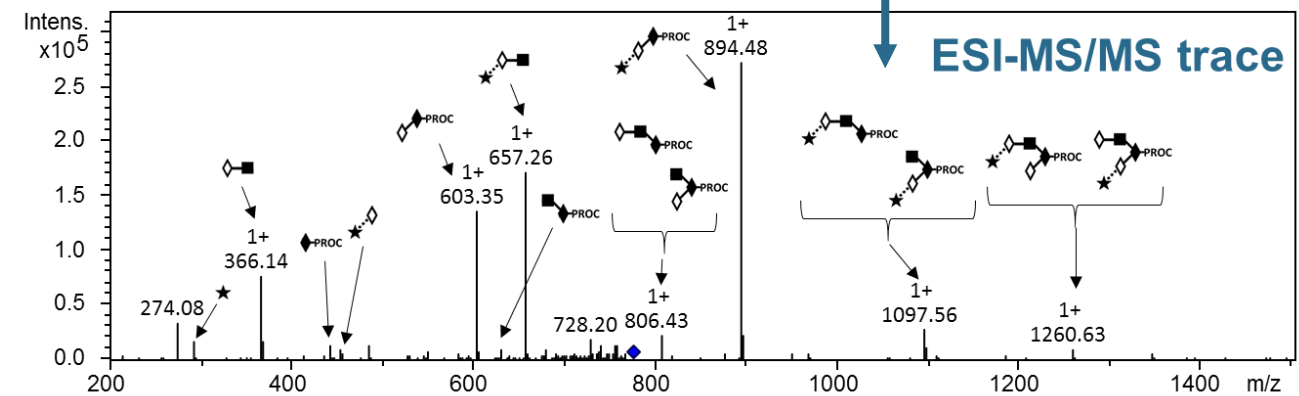
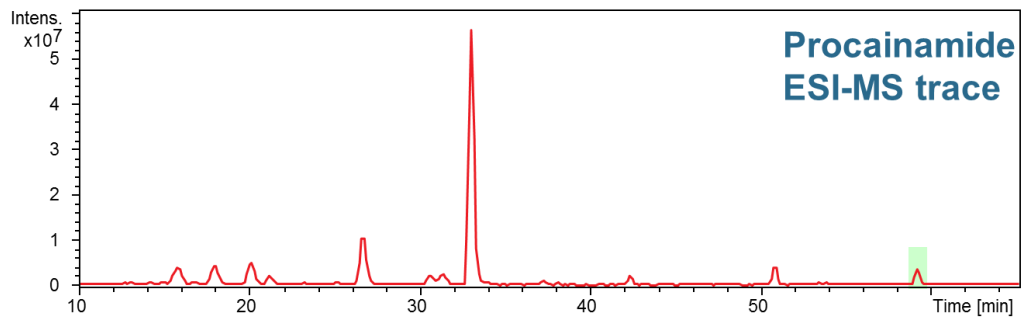
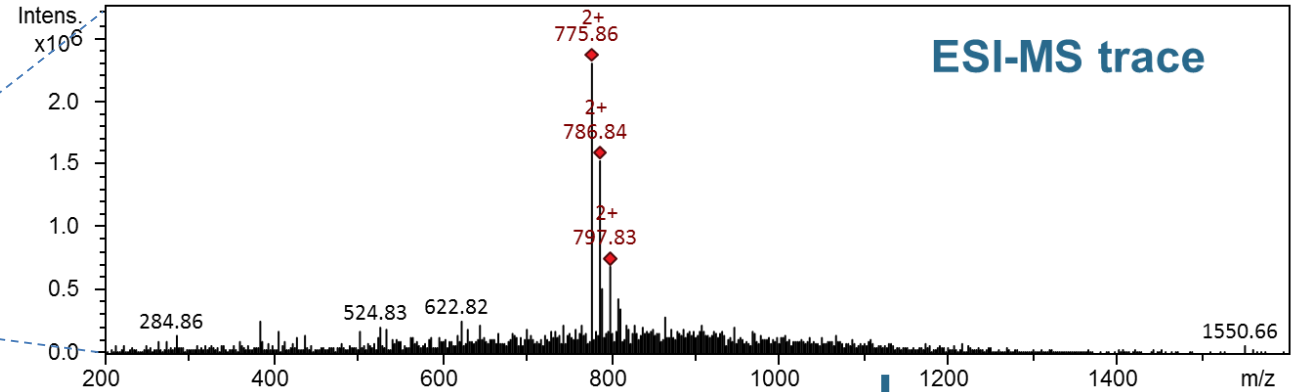
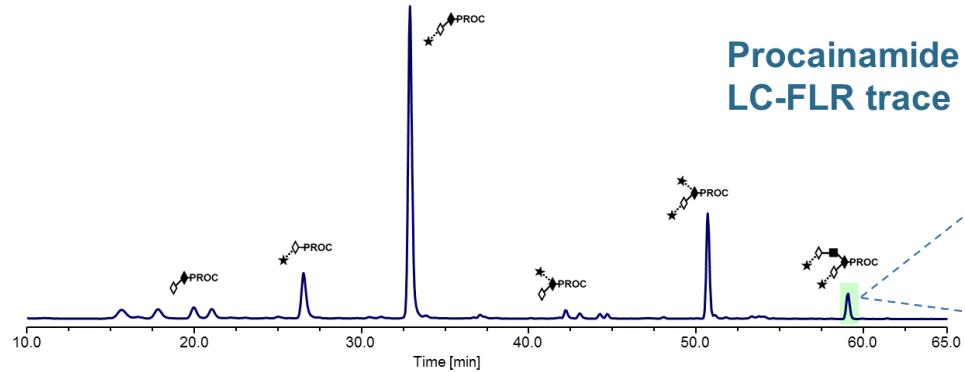


Comparison of 2-AB and Procainamide Labelled O-glycans

Comparable in quantitation and in reliability, using a Waters ACQUITY UPLC Glycan BEH Amide Column (HILIC)



LC-ESI-MS/MS analysis of Procainamide Labelled O-glycans

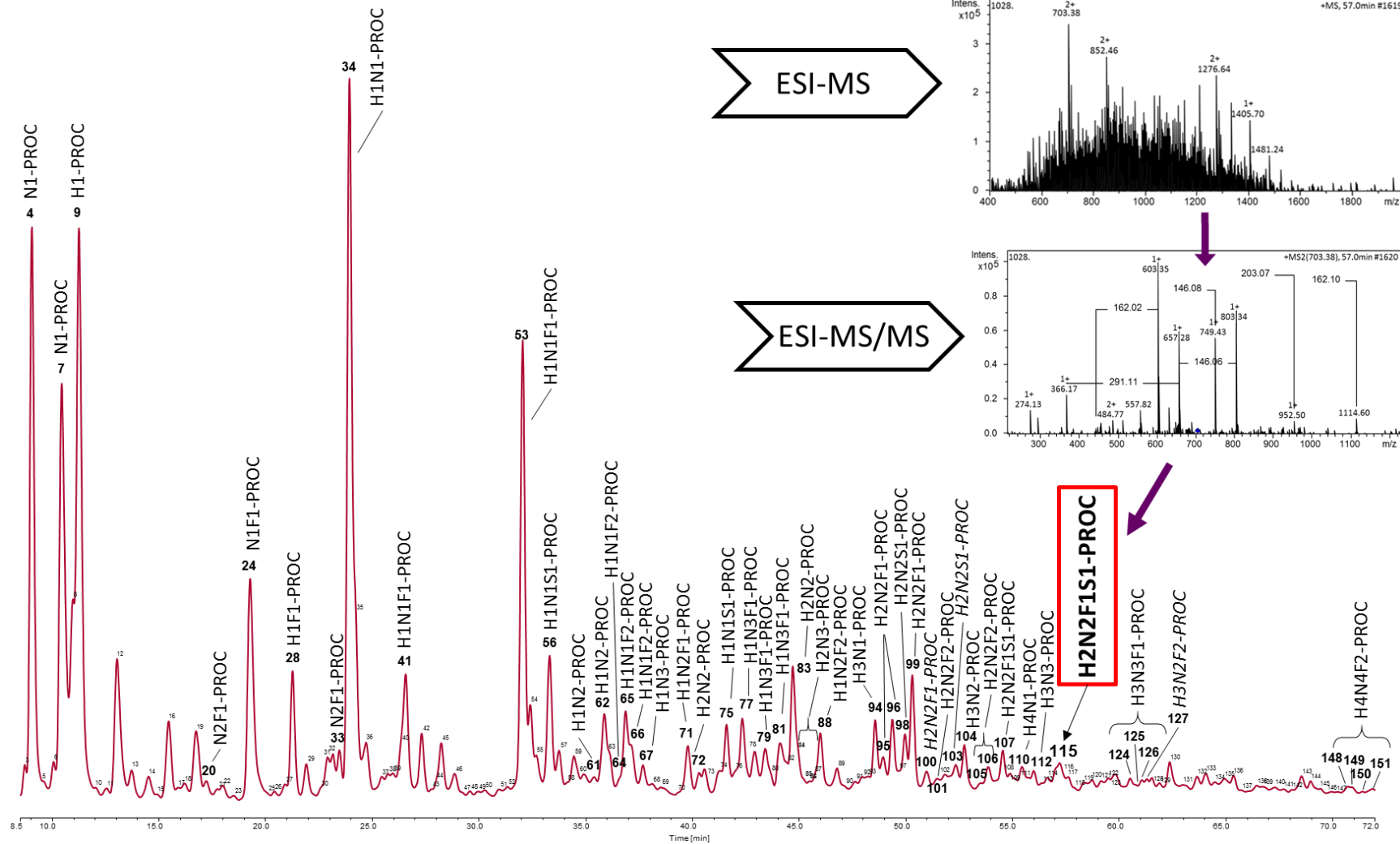


GU 4.91
Rel. % Area = 2.22%

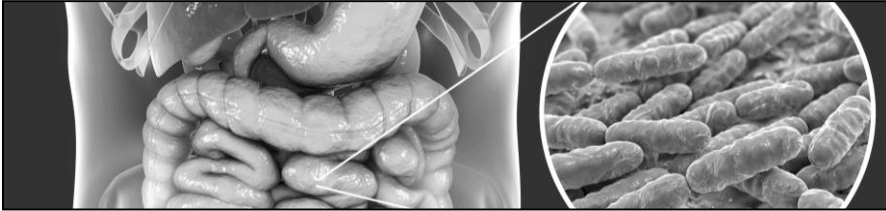
Glycan composition: H₂N₂S₂
Possible structure: di-sialylated core 2 O-glycan

LC-MS analysis of complex biological samples

Human saliva

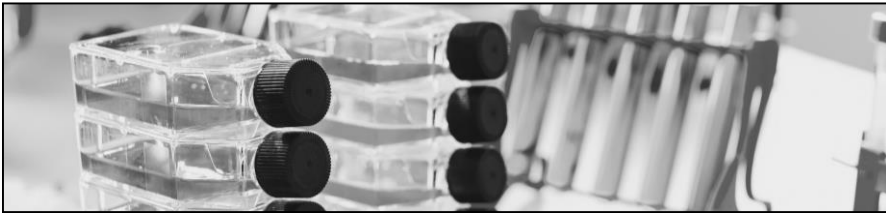


Related publications



Complex N-glycan breakdown by gut Bacteroides involves an extensive enzymatic apparatus encoded by multiple co-regulated genetic loci

Briliūtė J, Urbanowicz PA, Luis AS, Baslé A, Paterson N, Rebello O, Hendel J, Ndeh DA, Lowe EC, Martens EC, Spencer DIR, Bolam DN, Crouch LI. Nat Microbiol. 2019 Jun 3. doi: 10.1038/s41564-019-0466-x. [Epub ahead of print]



Engineering and stable production of recombinant IgE for cancer immunotherapy and AllergoOncology

Crescioli S, Chiaruttini G, Mele S, Ilieva KM, Pellizzari G, Spencer DIR, Gardner RA, Lacy KE, Spicer JF, Tutt ANJ, Wagner GK, Karagiannis SN. Journal of Allergy and Clinical Immunology. 2018 Jan 31. pii: S0091-6749(18)30081-2. doi: 10.1016/j.jaci.2017.12.986. [Epub ahead of print]



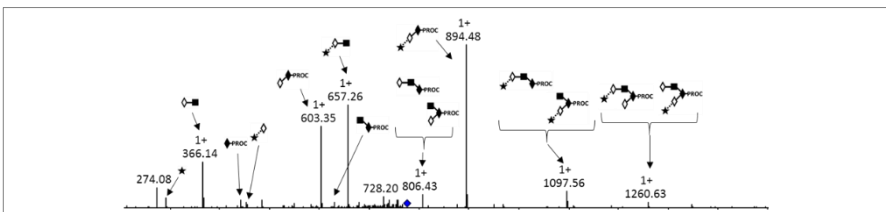
Analysis of Three Epoetin Alpha Products by LC and LC-MS Indicates Differences in Glycosylation Critical Quality Attributes, Including Sialic Acid Content

Thomson RI, Gardner RA, Strohfeltdt K, Fernandes DL, Stafford GP, Spencer DIR, Osborn HMI. Analytical Chemistry. 2017 Jun 20;89(12):6455-6462. doi: 10.1021/acs.analchem.7b00353. Epub 2017 Jun 9.



Variation of Human Salivary O-Glycome

Kozak RP, Urbanowicz PA, Punyadeera C, Reiding KR, Jansen BC, Royle L, Spencer DI, Fernandes DL, Wührer M. PLoS One. 2016 Sep 9;11(9):e0162824. doi: 10.1371/journal.pone.0162824. eCollection 2016.



Comparison of procainamide and 2-aminobenzamide labeling for profiling and identification of glycans by liquid chromatography with fluorescence detection coupled to electrospray ionization-mass spectrometry

Kozak R, Tortosa C, Fernandes D, Spencer D. Analytical Biochemistry. 2014 October;486:38-40. doi:10.1016/j.ab.2015.06.006. Epub 1 October 2015

How to start using the Procainamide Glycan Labelling System

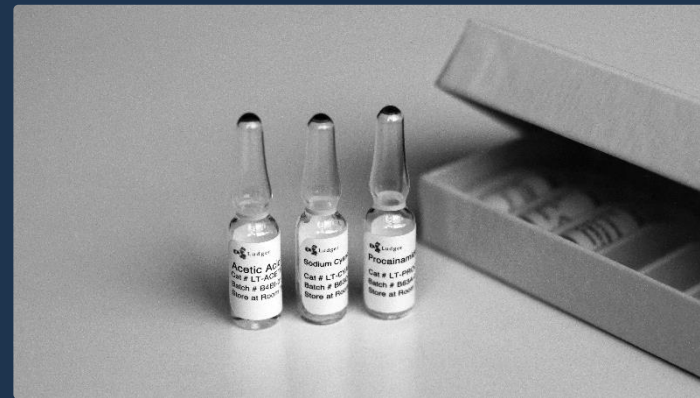
If you have a question



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Request a quotation



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Sales Team
info@ludger.com