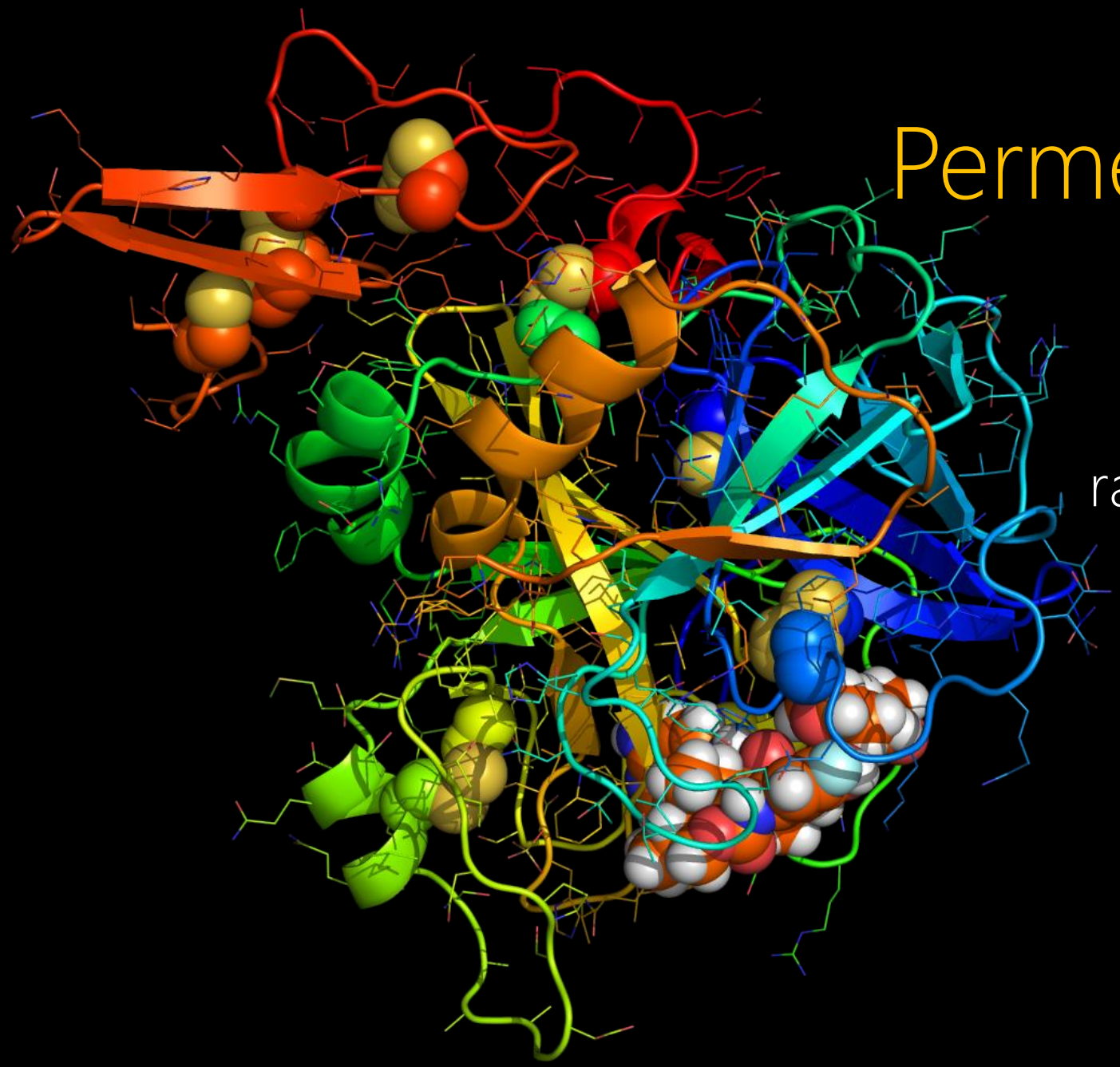
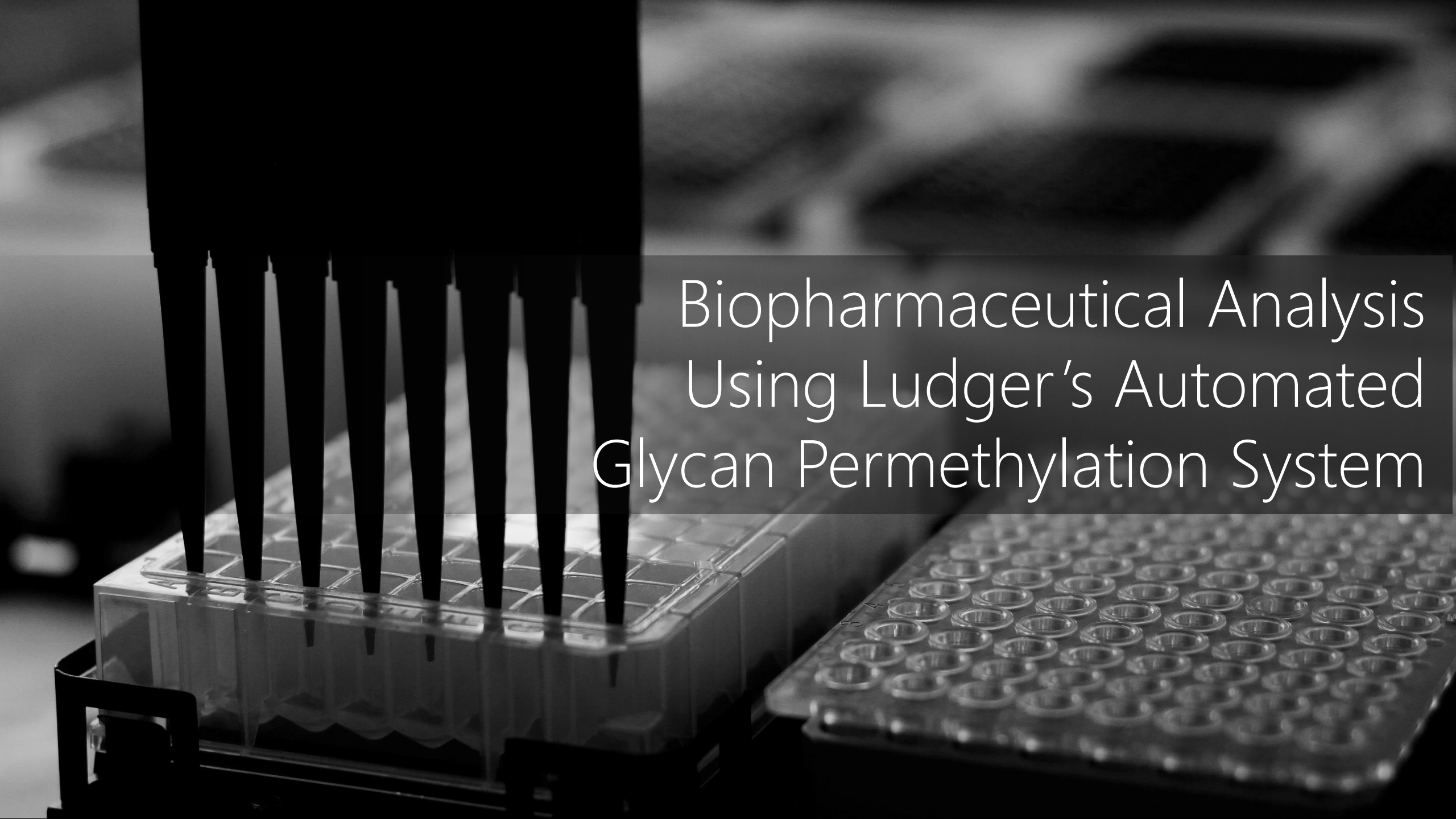


Permethylation of Glycans

Part 2 – Data Presentation

LudgerTag™ technology to enable rapid, reliable, high-throughput (HT) MALDI-TOF-MS analysis



Biopharmaceutical Analysis Using Ludger's Automated Glycan Permethylation System

Achieving Efficiency in Drug Glycosylation Studies Using a Two Stage Strategy

Stage 1:

Detailed Glycan Characterisation

Orthogonal Glycoanalyses

Few representative samples

Stage 2:

High Throughput (HT) Glycomics

Streamlined Glycoanalyses

Many samples

Time

Quality

Money

In Stage 1,
Quality, Time and Money per sample is high.

This approach is used for a few representative samples.

In stage 2,
Streamlining keeps Quality high, while Time and Money per sample are lower.

This approach is used for HT glycoprofiling.

More Detail on the Two Stage Strategy for HT Glycoprofiling Studies

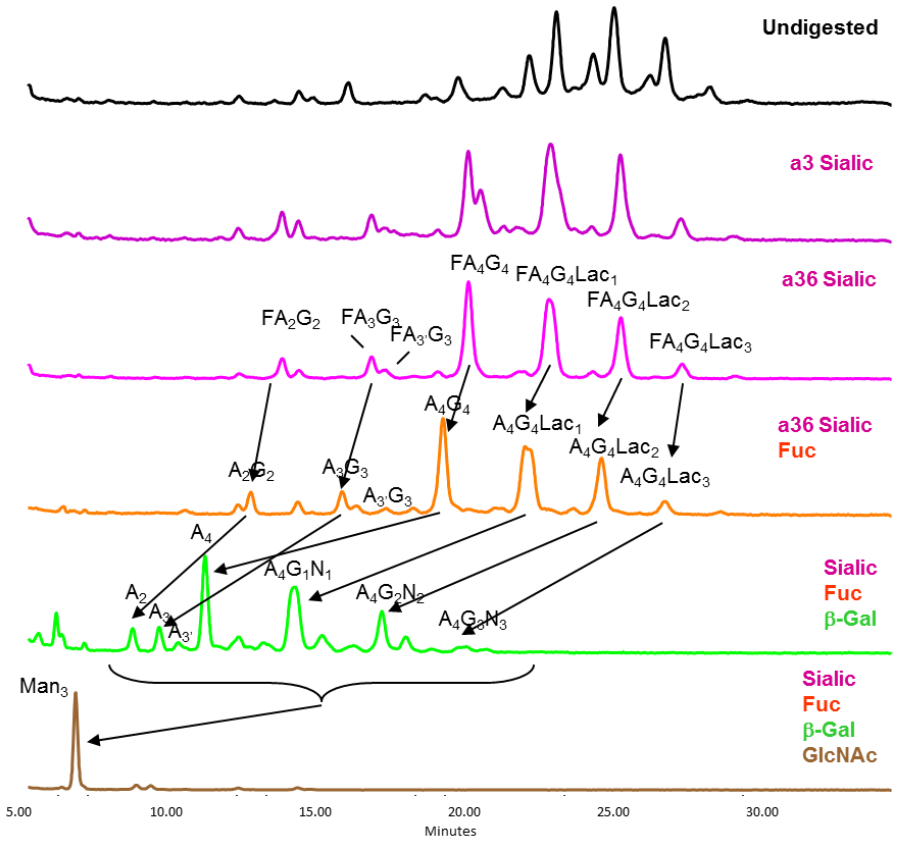
	Stage 1 Detailed Glycan Characterisation	Stage 2: HT Glycomics Studies
Aim 1	Identify and measure all the drug's GCQAs (Glycosylation Critical Quality Attributes)	Quantitative measurement of high priority GCQAs
Aim 2	Prioritise the GCQAs according to impacts on clinical safety and efficacy profiles	Score / categorise / stratify the samples based on GCQA measurements
Glycoprofiling Methods	Use several orthogonal methods	Use MALDI-TOF analysis of permethylated glycans if it fulfils aim
Workflows	Typically complex	Must be streamlined
Number of Samples	Few	Many
Structural detail	High detail of many glycoparameters	Focus on GCQAs
Analysis time per sample	Long	Short
Sample throughput	Low	High
Cost per sample	High	Low

Stage 1: Detailed Glycan Characterisation Using Orthogonal Glycoprofiling Techniques

Example shown, recombinant human erythropoietin (rhEPO)

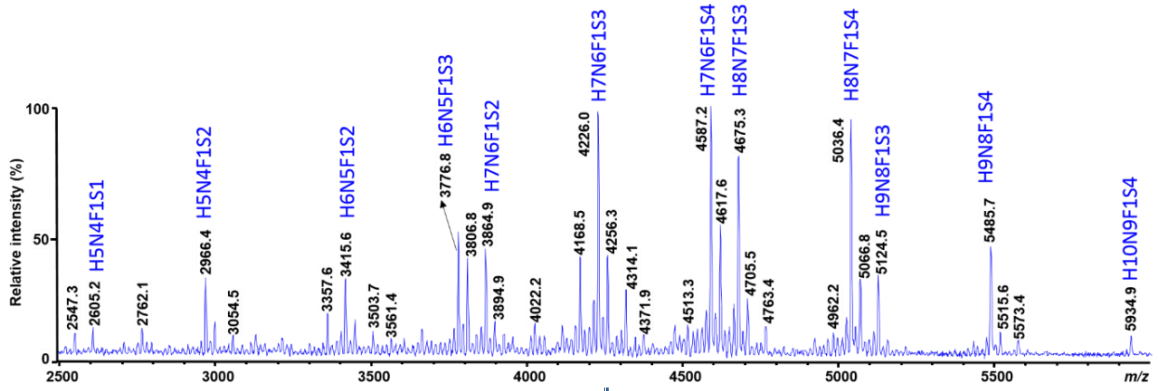
1A: Enzymatic Sequencing

2-AB labelled rhEPO N-glycans analysed using UHPLC and exoglycosidase sequencing performed to allocate or confirm structures.



1B: Permethylation of glycans

Glycan compositions can be assigned after MALDI-TOF-MS analysis of permethylated glycans. (Permethylated rhEPO N-glycans shown in spectra as an example).



1C: Peak Assignment

The data from fluorescent labeling and exoglycosidase digestion was combined along with the glycan composition data obtained from permethylated MALDI-TOF-MS analysis of rhEPO N-glycans to confirm peak assignments.

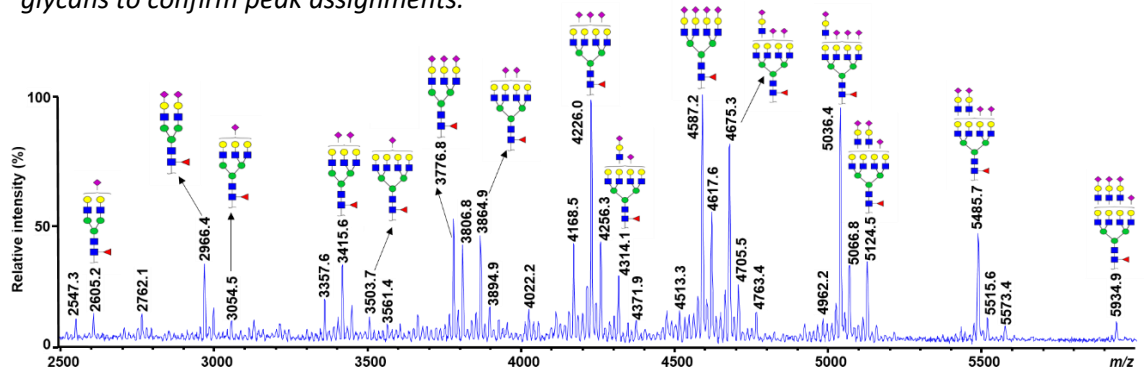


Figure 1. Structural N-Glycan Characterisation of a Highly Sialylated, recombinant human Erythropoietin (rhEPO).

Stage 1: Confirm that Permethylation and MALDI-TOF-MS Analysis can Reliably Measure the GCQAs of your Drug

(e.g. potentially immunogenic non-human glycans, possible CQAs)

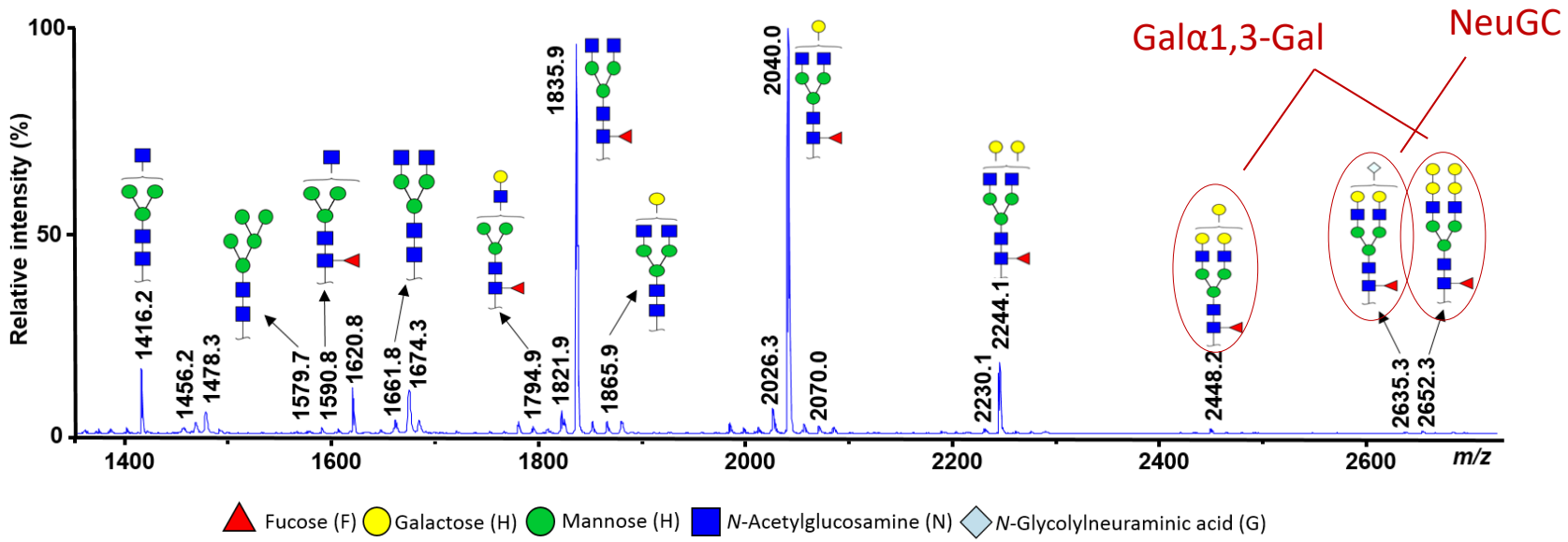


Figure 2: MALDI-TOF-MS spectrum of IgG1 mAb N-glycans permethylated on the liquid handling robot. **The N-glycan structures in the spectrum were established and peak assignments were confirmed through data obtained from procainamide labeling and exoglycosidase digestion of the mAb.**

- Automated N-glycan release, HILIC-SPE enrichment, permethylation and MALDI-TOF-MS was performed on a commercially available IgG1 mAb standard.
- This identified the major N-glycan structures depicted in figure and also the low abundant glycans with *m/z* values of 2635.3 (H5N4F1Sg1-core fucosylated, biantennary, digalactosylated with one *N*-glycolylneuraminic acid); 2448.2 (H6N4F1 - core fucosylated, biantennary, mono-galactosylated, with one alpha-linked galactose (Galα1,3-Gal)); and 2652.3 (H7N4F1 - core fucosylated, biantennary glycan with two Galα1,3-Gal residues).
- Galα1, 3-Gal epitope and *N*-glycolylneuraminic acid (NeuGC) are non-human glycosylation features, reflecting possible critical quality attributes (CQAs) due to the potential immunogenic characteristics of the mAb. We identified these glycosylation features after permethylation of the IgG1 mAb standard.

Stage 2: Use the Streamlined Permethylation and MALDI-TOF-MS System for the HT Studies

Time taken:

Permethylation and liquid-liquid extraction of 96 samples
can be performed in under 5 hours using the liquid handling robot.

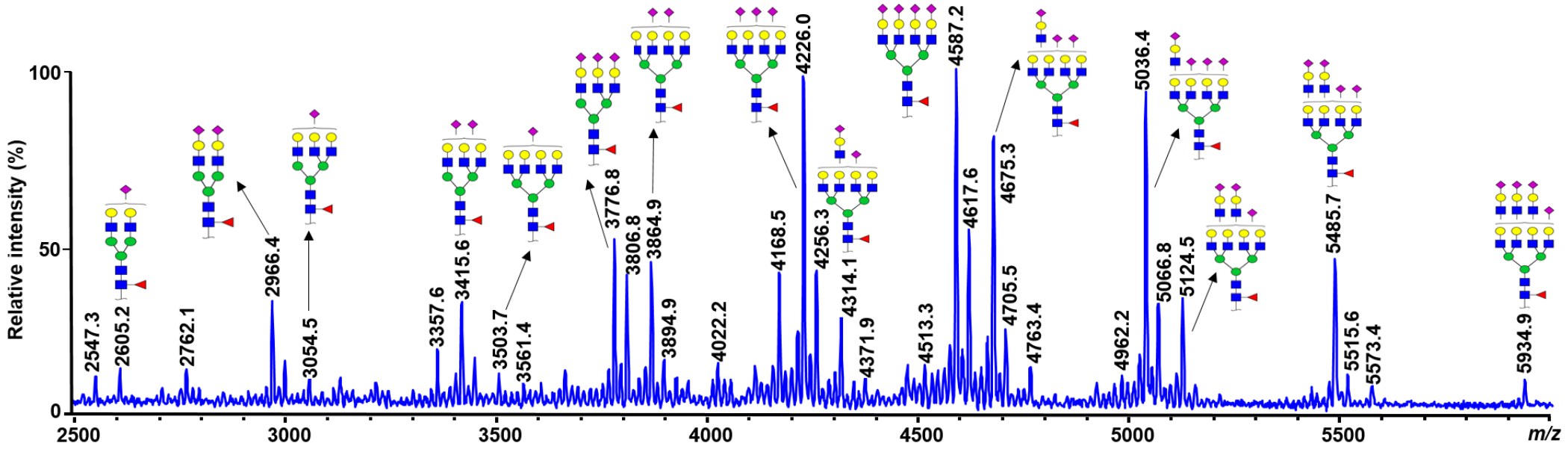


Figure 3. MALDI-TOF-MS spectrum of 25 µg rhEPO N-glycans released, enriched and permethylated using the liquid handling robot.

Permethylation is also Suitable for O-glycans

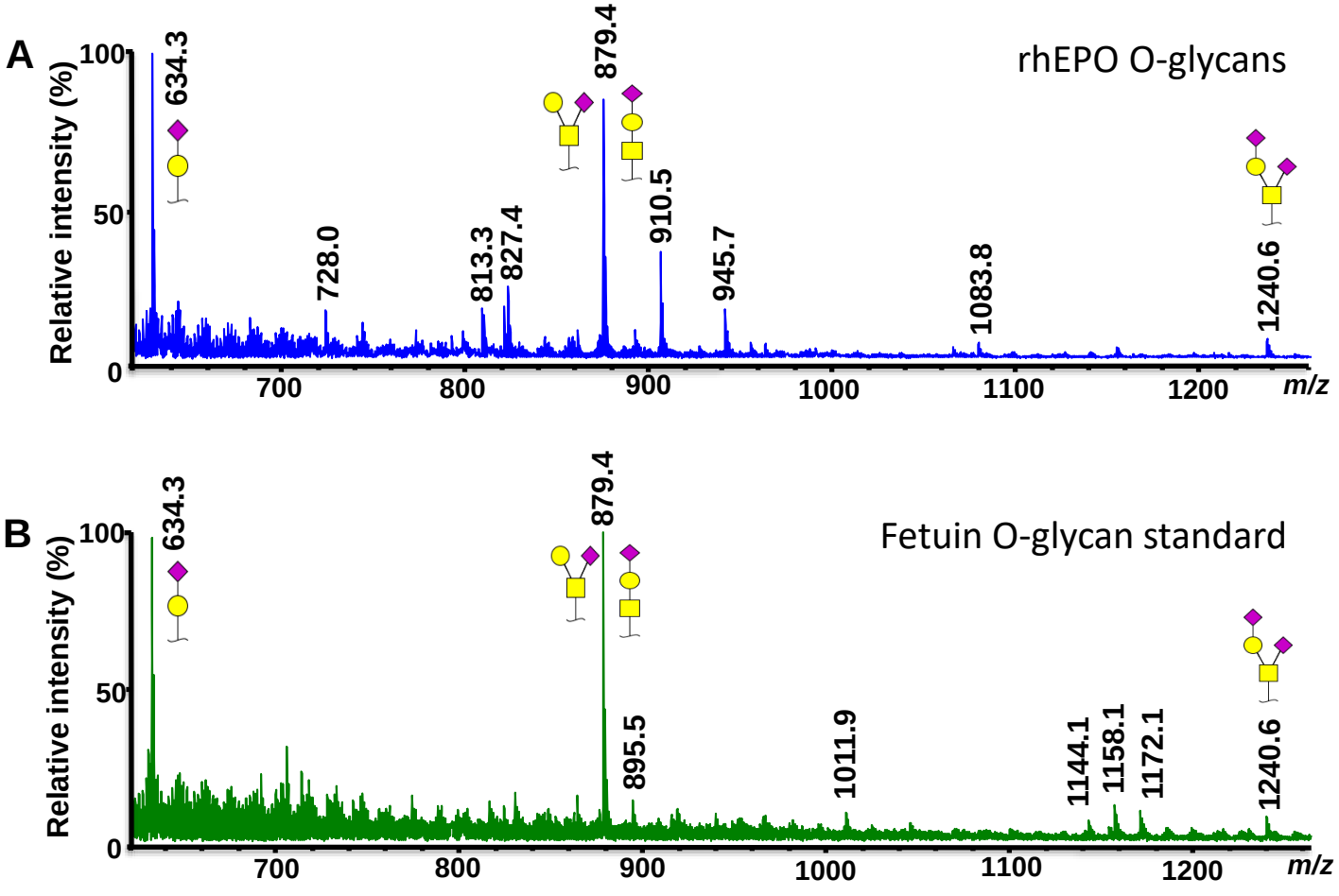
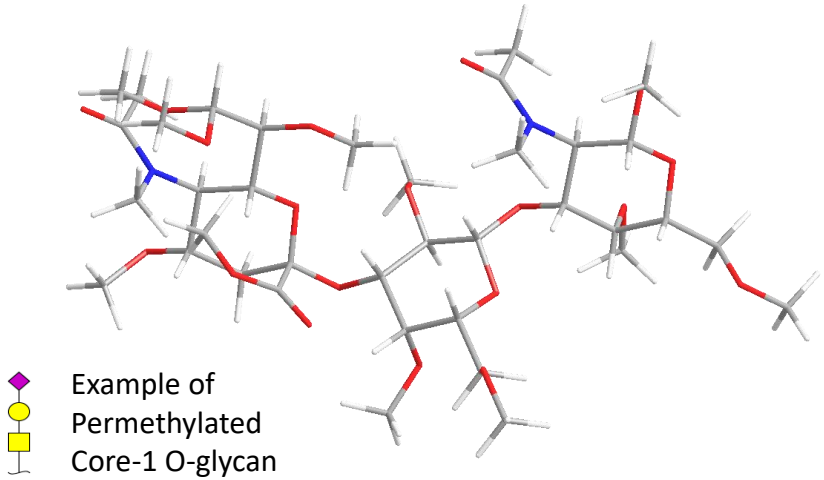


Figure 4. MALDI-TOF-MS spectra of O-glycans permethylated by the liquid handling robot after manual hydrazinolysis. Spectra of (A) rhEPO O-glycans and (B) Fetuin O- glycan standard.

Note: Unassigned peaks are possible artefacts resulting from hydrazinolysis release.



- Here we demonstrate the practical applicability of the automated HT permethylation and MALDI-TOF-MS of O-glycans released from rhEPO and bovine fetuin samples.
- O-glycans were manually released by hydrazinolysis and cleaned-up using cation exchange LudgerClean CEX cartridges. Aliquots of the released and enriched O-glycans were later permethylated on the robot.
- The rhEPO O-glycans contain mono-sialylated core 1 (*m/z* 879.4) and disialylated core 1(*m/z* 1240.6) and a peeled product resulting from hydrazinolysis release (*m/z* 634.3) and the obtained results were comparable to HILIC UHPLC data.

Using Permethylation Technology in a QbD Study: Monitoring the Impact of Cell Culture Conditions on IgG4 Glycoform Patterns

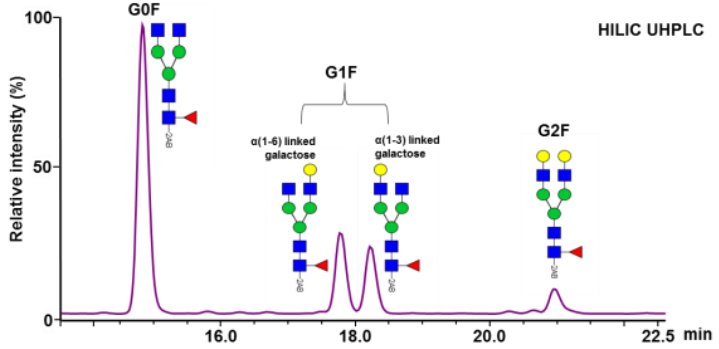
Study focused on monitoring the alterations in the levels of Galactosylation

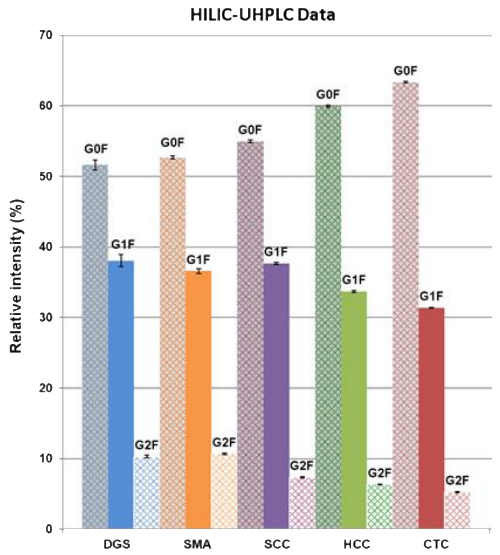
Stage 1

HILIC-UHPLC

Typical chromatogram of IgG4 mAb after automated 2-AB labeling

Minimum of 48 hours required for data acquisition of 96 samples



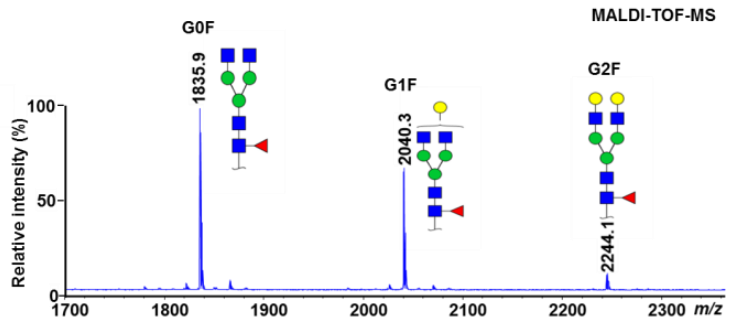


Stage 2

MALDI-TOF-MS

Typical MALDI spectra after automated permethylation

Less than 1.5 hours required for data acquisition of 96 samples



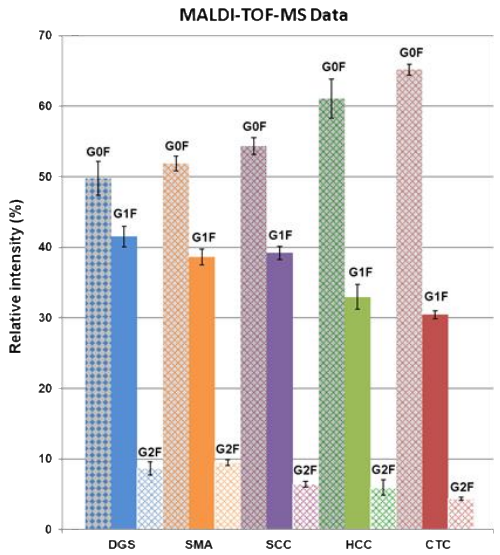


Figure 5. Relative quantitation of glycan species (G0F), (G1F), and (G2F) structures from different bioreactor conditions. Error bars depict standard deviation (SD) with acceptable error range.

Key to bioreactor conditions: Direct Gas Sparging - DGS; Silicone Membrane Aeration - SMA; Standard Culture Condition - SCC; Hypothermic Culture Condition - HCC; Control Temperature Condition - CTC

Result: The relative quantitation and SD data for the both methods HILIC UHPLC and MALDI-TOF-MS which show high comparability between the two data sets. From Figure 5 we can conclude that the histogram shows similar trends and conclusions.

How to Start Using the Ludger Permethylation Kit

1. Submit Samples for Automated High-throughput Permethylation

We can perform sample preparation and analysis for you in our laboratories and send you a data analysis report

2. Method Transfer

We can transfer the methods to your lab and provide technical support

3. Try Permethylation kit in-house

Contact us for a quotation and place your order Catalogue # LT-PERMET-96

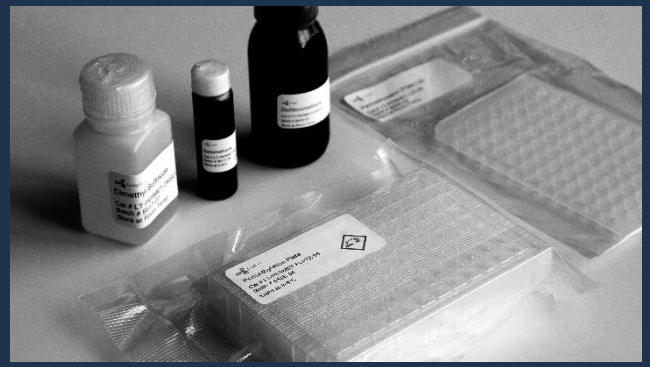
Contact Us

If you have technical questions

CLICK to contact Archana

Archana Shubhakar
Senior Scientist
archana.shubhakar@ludger.com

To request a quotation:
For services, method transfer or LT-PERMET-96 kit



CLICK to contact Sales

Sales Team
Quotations: info@ludger.com
Orders: sales@ludger.com