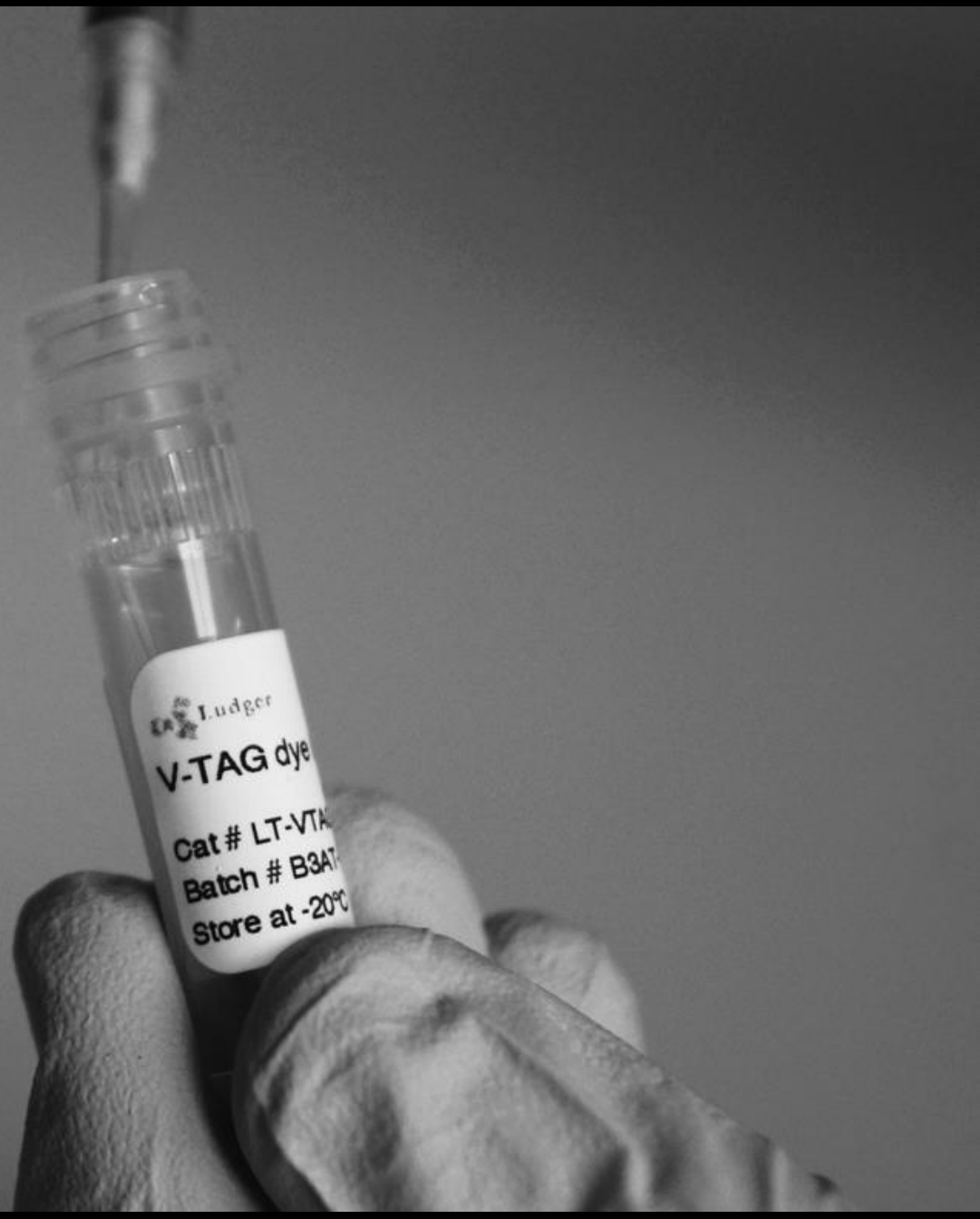
mAb Glycopeptide Profiling with V-Tag

Adding reliable glycoprofiling to your peptide mapping workflow with ease and simplicity

Ludger



Who is the V-Tag Glycoprofiling Technology for?

V = Velocity

The Ludger V-Tag Glycoprofiling Technology is for mAb developers, both innovator and biosimilar companies, who need to glycoprofile* mAb samples reliably, at an affordable cost and with a fast turnaround time.

Our typical V-Tag clients are those who want to:

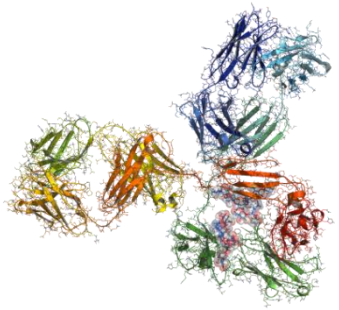
Monitor their mAb drug's glycosylation during the product lifecycle. This includes showing the comparability of glycosylation throughout the drug lifecycle as well as biosimilarity to an innovator's drug

Integrate glycoprofiling into the peptide mapping workflow. The V-Tag labeled glycopeptides are analysed using orthogonal platforms, MALDI-MS and UHPLC

*Glycoprofile = a map of the drug's glycosylation containing structural ID and relative abundance of each glycan species

Options for Analysing mAb Glycosylation

Three types of mAb-derived molecules can be analysed to gain information about mAb glycosylation

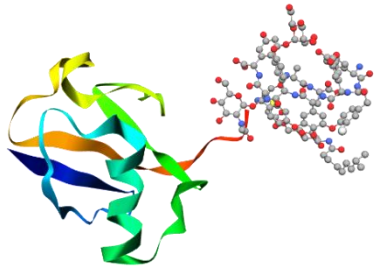


1. Intact Glycoprotein

Using lectin affinity, CE or MS

✓ *Provides information on molecular weight and* ***glycoform distribution***

X *Poor structural information for the glycans. Insufficient for regulatory work or lot release*

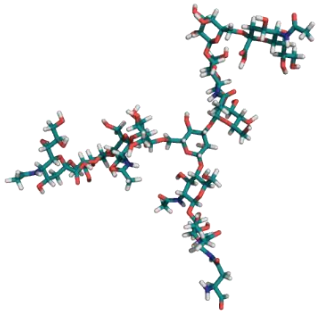


2. Glycopeptides

Using MS and UHPLC

✓ *Provides both* ***glycan identity*** *and* ***quantitation*** *as well as* ***glycan attachment site*** *and* ***site occupancy***

V-Tag is designed for glycopeptide mapping



3. Glycans

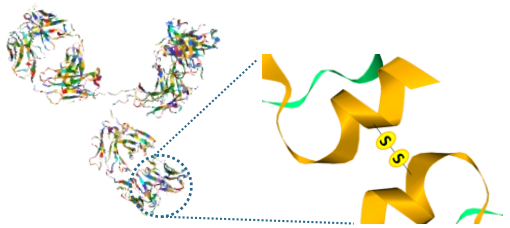
Using MS, UHPLC, CE

✓ *Provides* ***glycan identity*** *and* ***quantitation***

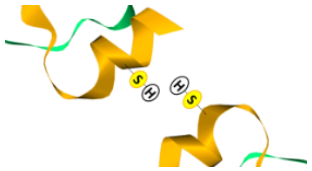
X *Need to release glycans – extra work and expense. No glycosylation site information (for mAbs with Fab and Fc glycosylation)*

Adding Glycoprofiling to the Traditional Peptide Mapping Workflow

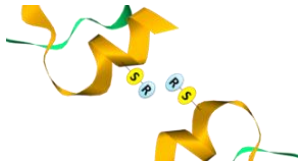
Incorporating glycopeptide mapping can reduce the time and costs of glycoprofiling work in drug production monitoring



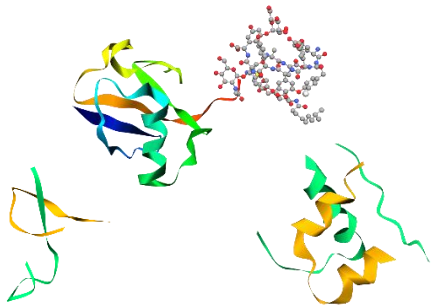
Intact mAb



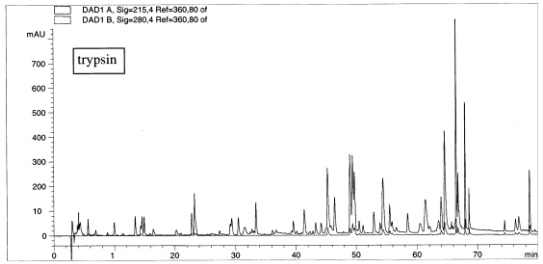
Reduced mAb



Reduced, Alkylated mAb



Peptides + Glycopeptides



*RP-HPLC peptide map of anti-CD4 IgG-1 **

Glycopeptide signals attenuated in a complex mixture

Peptide Mapping

Glycopeptide Mapping

The V-Tag system labels and enriches mAb glycopeptides efficiently, allowing determination of their structures and relative quantities

* J. Bongers et al. J. Pharm. Biomed. Anal. **2000**, 21, 1099-1128.

Highlights of the V-Tag System

Reliable mAb Glycoprofiling

Allows glycan identification and quantitation using the orthogonal analyses of MALDI-MS and UHPLC. Provides data comparable to gold-standard glycoprofiling methods based on 2-AB or 2-AA

Minimal Sample Needed

Excellent glycopeptide mapping using as little as 10 µg of mAb glycoprotein

Quick and Easy

Labeling and enrichment is completed within 2 hours

Validated for GMP Labs

Validated to ICH Q2(R1) standards and tested in glycoprofiling labs

Integrates Easily with Peptide Mapping Workflow

Adds onto your existing peptide mapping workflow, without requiring extra steps for glycan release

Automatable for High-Throughput Studies

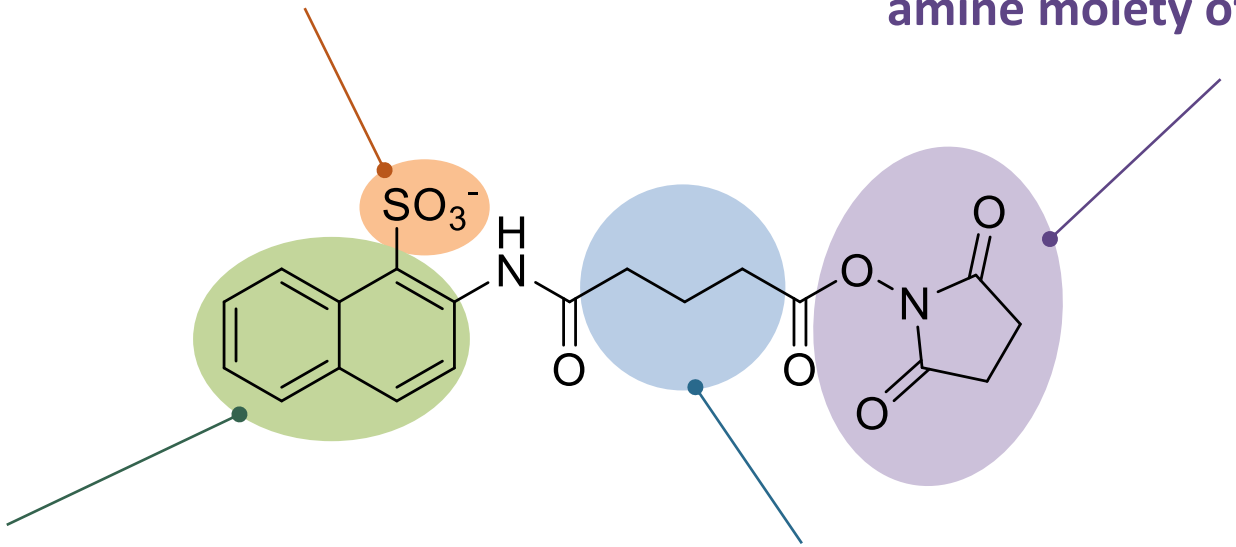
The procedure is compatible with 96-well plate based assays, enabling high-throughput studies using a liquid handling robot

Anatomy of V-Tag

The molecular aspects that make V-Tag work

Sulphate anion to improve analysis in negative ion mode on MALDI

Amine reactive succinimidyl ester to react with the N-terminus amine moiety of the peptide



Fluorescent group for detection in UHPLC

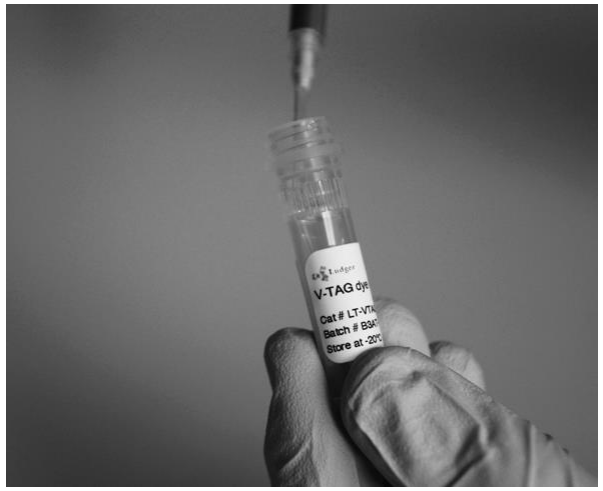
λ_{ex} = 250nm, λ_{em} = 360nm

Simple, **non-reactive alkyl chain** to link the fluorescent moiety to the reactive succinimidyl ester

Components of the LT-VTAG-24 Kit

1. Labeling

2. Enrichment



Reaction buffer and solvent

PBS Buffer Tablet
LT-PBS-TAB-0.01M

Amine reactive fluorescent labeling reagent

V-Tag Labeling Dye
LT-VTAG-01

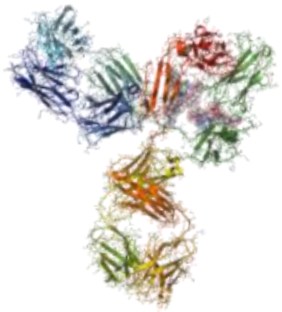
HILIC resin cartridge

LudgerClean A Cartridges (LC-A)
LC-A-24

Solvent involved in HILIC clean-up and enrichment

TFA 10% (aq.)
LC-TFA10PC-01

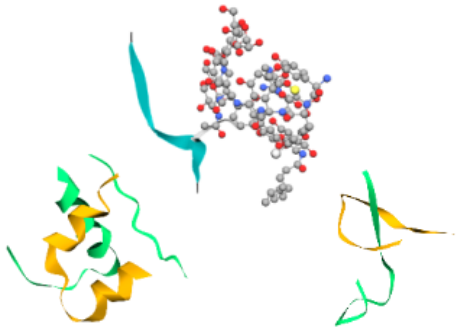
Workflow for the V-Tag System: Stage 1 - Labeling



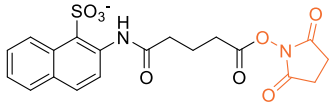
mAb

Protease digestion

Use your enzyme of choice
e.g. sequencing grade trypsin



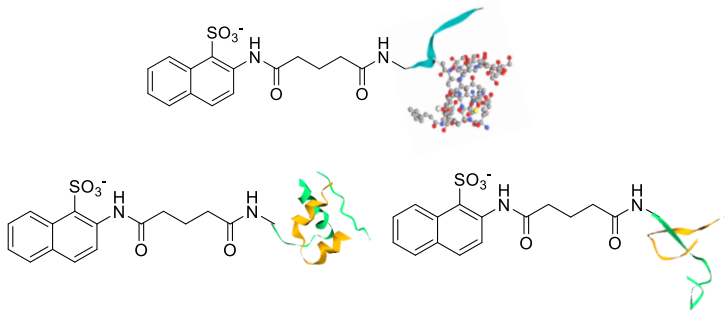
Glycopeptides + peptides



Labeling

V-Tag Dye
+ PBS Buffer

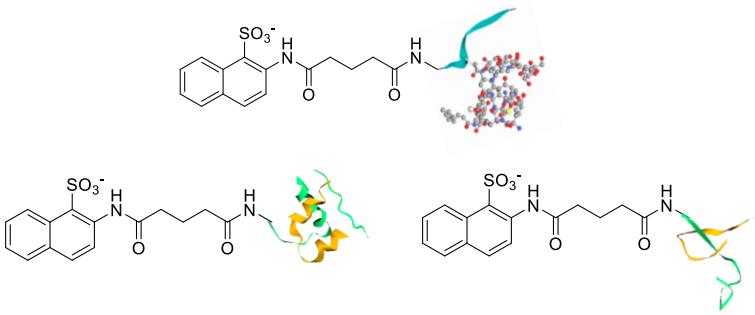
Labels the amine on the
N-terminus of the peptide



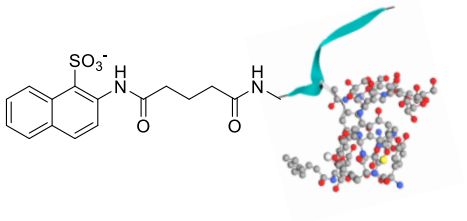
V-Tag labeled peptides and glycopeptides

Workflow for the V-Tag System: Stage 2 – Enrichment

Analysis by MALDI-MS and UHPLC



V-Tag labeled peptides and glycopeptides



V-Tag labeled glycopeptides

Enrichment

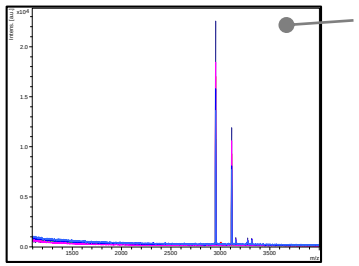
LT-VTAG-24 Kit

LC-A cartridges with TFA solutions

Separation of mixture using HILIC cartridge. Conditions optimised for recovery of glycopeptides with glycosylation patterns preserved

Orthogonal Analyses

MALDI-MS and UHPLC

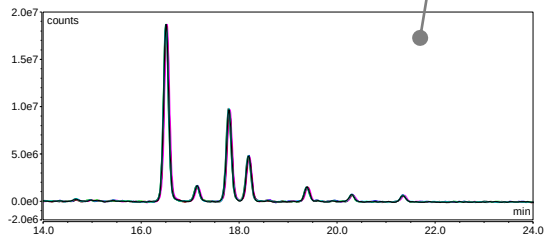


MALDI-MS

For glycan Identification

UHPLC

Quantitation



IgG-1 V-Tag labeled glycopeptides

V-Tag Workflow: Simple and Easy

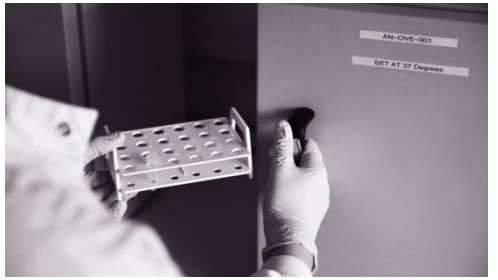
Labeling

Buffering the mAb digest



Label the digest

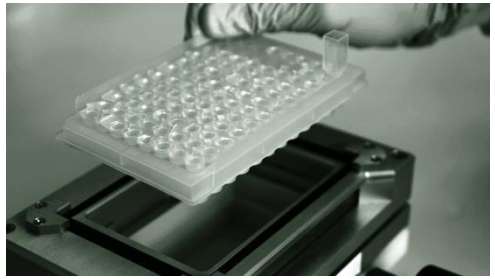


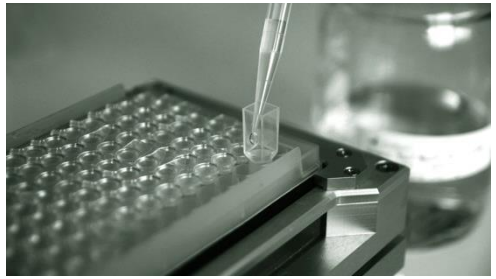
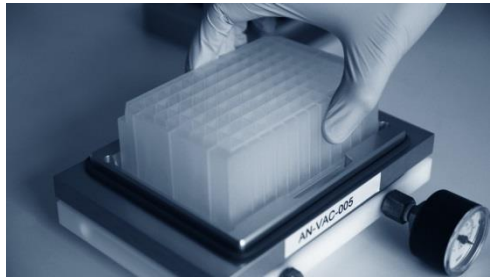
Enrichment

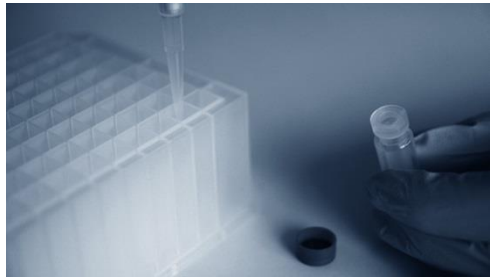
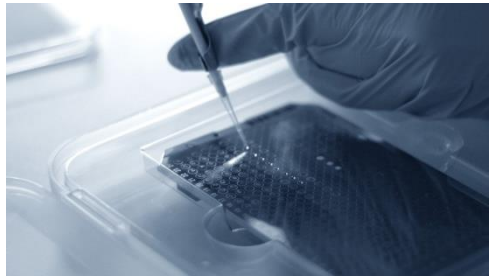
Remove peptides





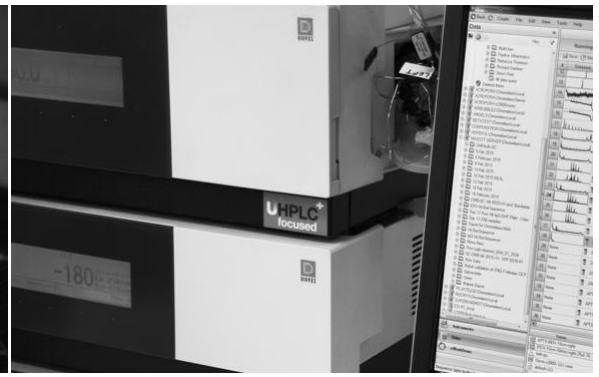

Elute glycopeptides

Set-up of Orthogonal Glycoanalytical Platforms

Typical setup for analysis of V-Tag labeled glycopeptides by UHPLC and MALDI-MS



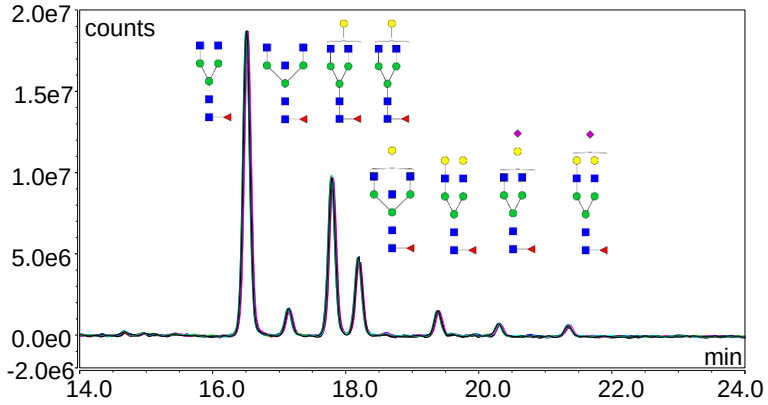
Thermo Scientific Dionex U3000
30 minute gradient
25 µl injection

→

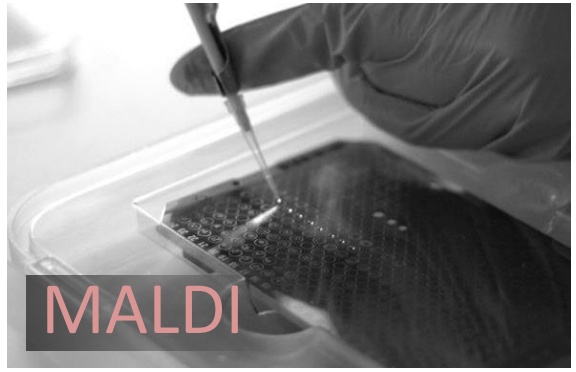
Waters UHPLC Glycan BEH Amide Column (HILIC)
(150mm x 2.1mm)
Temperature: 60 °C

→

FLD Fluorescence Detector
λ_{ex} = 250 nm
λ_{em} = 360 nm



Typical data
V-Tag labeled glycopeptide map of IgG 1



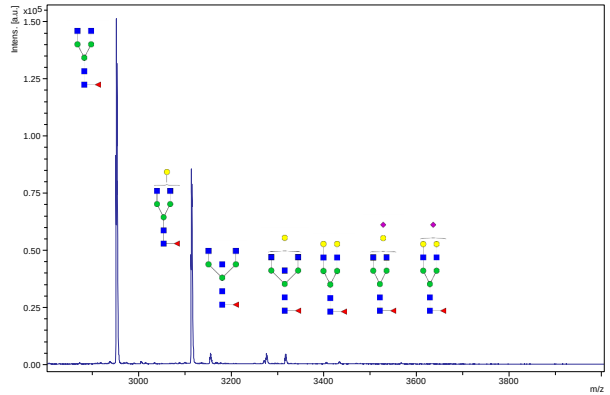
Spot Sample
Matrix: 2,5-dihydroxybenzoic acid (DHB)

→

Load Plate
Bruker Autoflex MALDI-MS instrument

→

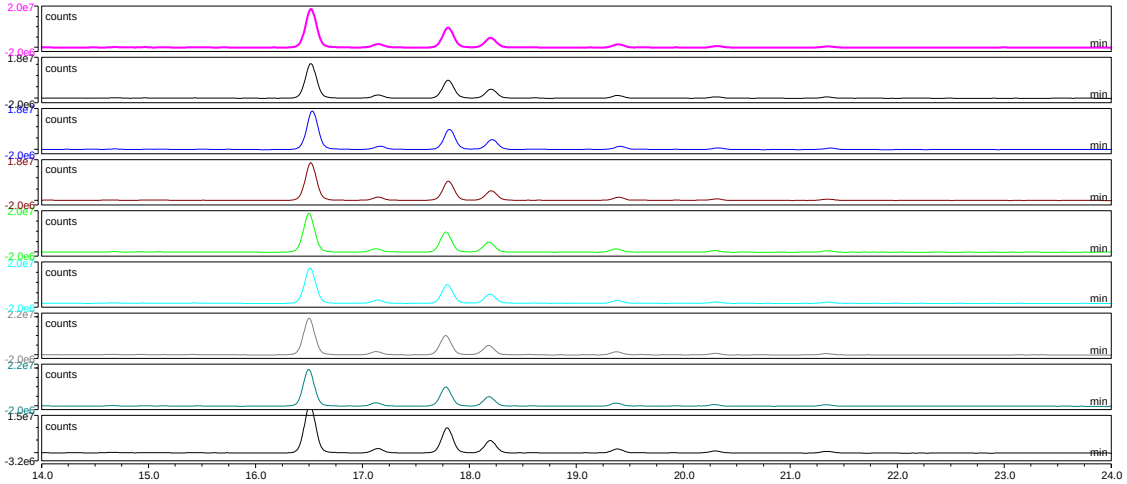
Collect Data
Mode: reflectron negative ion



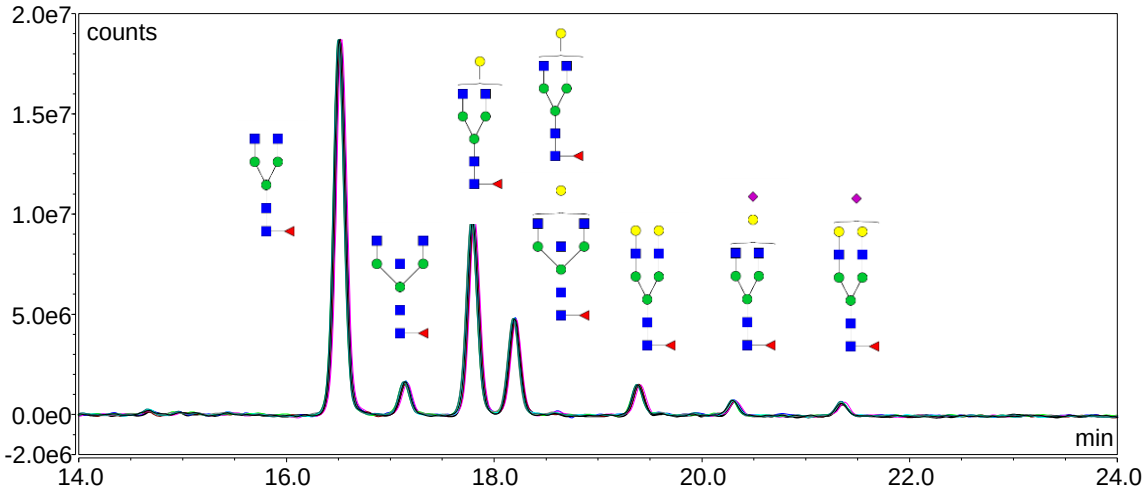
Typical Data
V-Tag labeled glycopeptide map of IgG 1

V-Tag System Has Been Validated to ICH Q2(R1) Level

V-Tag is reliable and robust and can be used for GMP level glycoprofiling of monoclonal antibodies



Stacked IgG 1 V-Tag labeled glycopeptide UHPLC profiles (9 replicates)



Overlaid IgG 1 V-Tag labeled glycopeptide UHPLC profiles (9 replicates)

Validation studies typically show repeatability with CVs for relative abundances < 4%

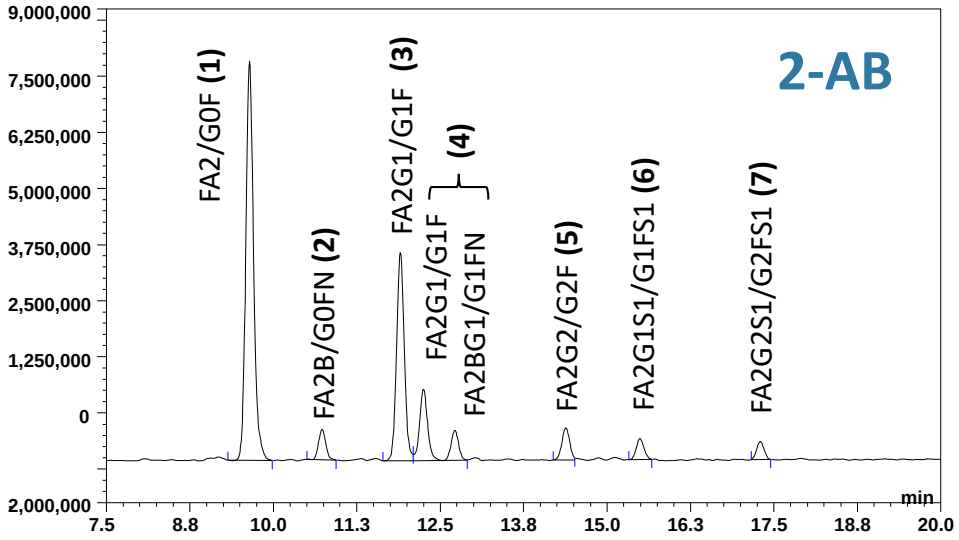
Peak Number		1	2	3	4	5	6	7
Glycopeptide		G0F (FA2)	G0FB (FA2B)	G1F (FA2G1)	G1F + G1FB (FA2G1 + FA2BG1)	G2F (FA2G2)	G1FS1 (FA2G1S1)	A1F (FA2G2S1)
Relative % Area	Av.	50.2	4.2	25.8	12.6	3.8	1.8	1.7
	Std. Dev.	0.35	0.14	0.18	0.26	0.10	0.02	0.04
	CV	0.70	3.20	0.69	2.05	2.54	0.92	2.40

Average relative % area, SD and CVs for V-Tag labeled IgG 1 glycopeptides

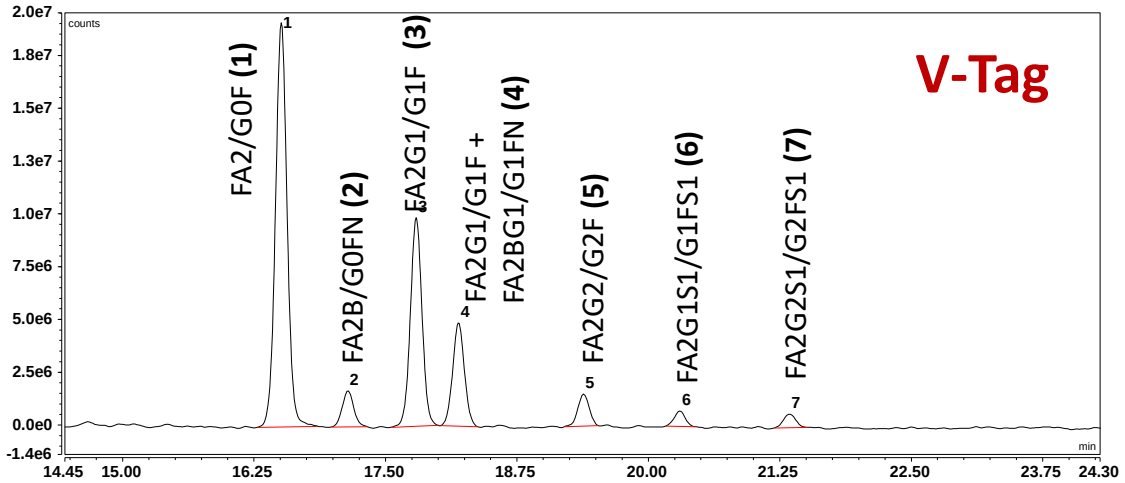
Studies using V-Tag at Ludger

Comparability of 2-AB and V-Tag

V-Tag labeling of glycopeptides gives comparable results to the gold standard 2-AB labeling of glycans

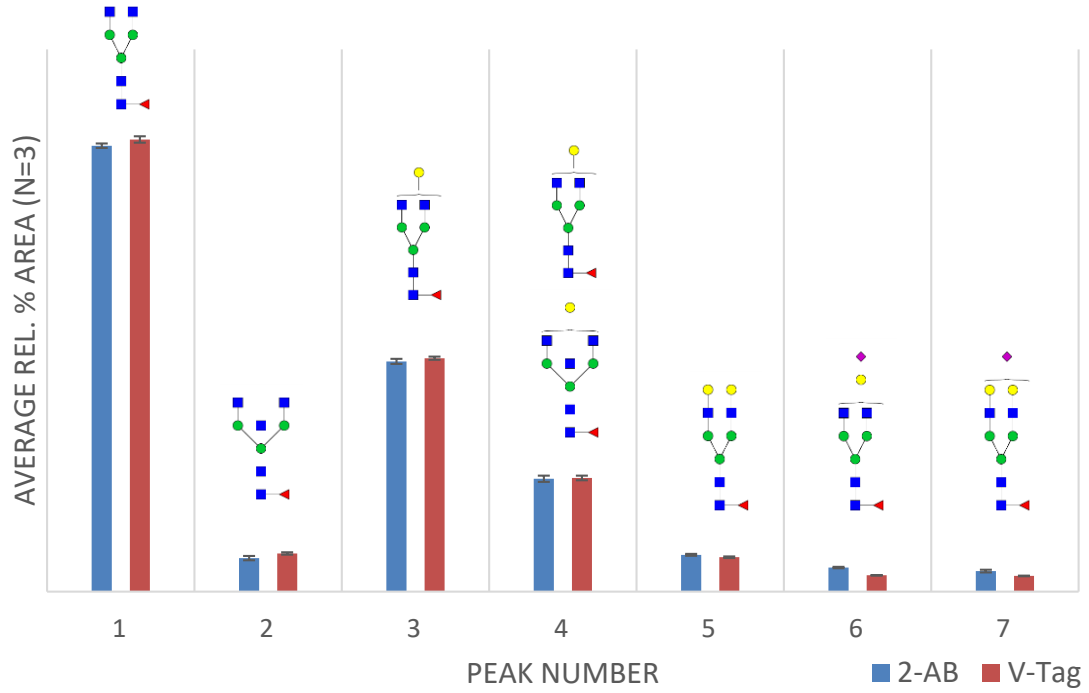


*UHPLC Chromatogram of **2-AB** labeled glycans from IgG-1 mAb (PNGase F release)*



*UHPLC chromatogram of **V-Tag** labeled glycopeptides from IgG-1 mAb (tryptic digestion)*

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



The V-Tag protocol is much shorter than 2-AB

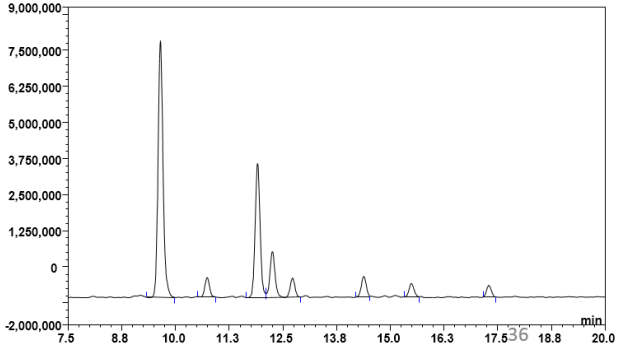
2-AB labeling of N-Glycans

Total: 8 -21 hrs

3-16 hr — *PNGase F release*

4 hr — *2-AB Labeling + Reduction*

1 hr — *Clean-up*



IgG 1 2-AB labeled glycans

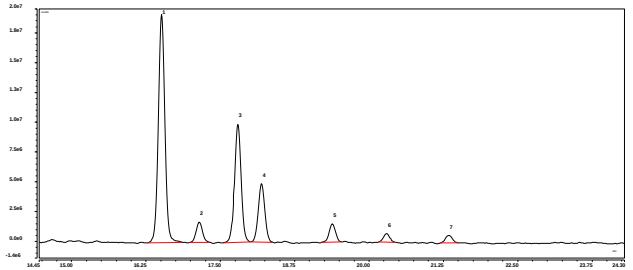
V-Tag labeling of glycopeptides

Total: 3 hrs

1hr — *Trypsin Digest*

1 hr — *V-tag Labeling*

1 hr — *Clean-up and Enrichment*



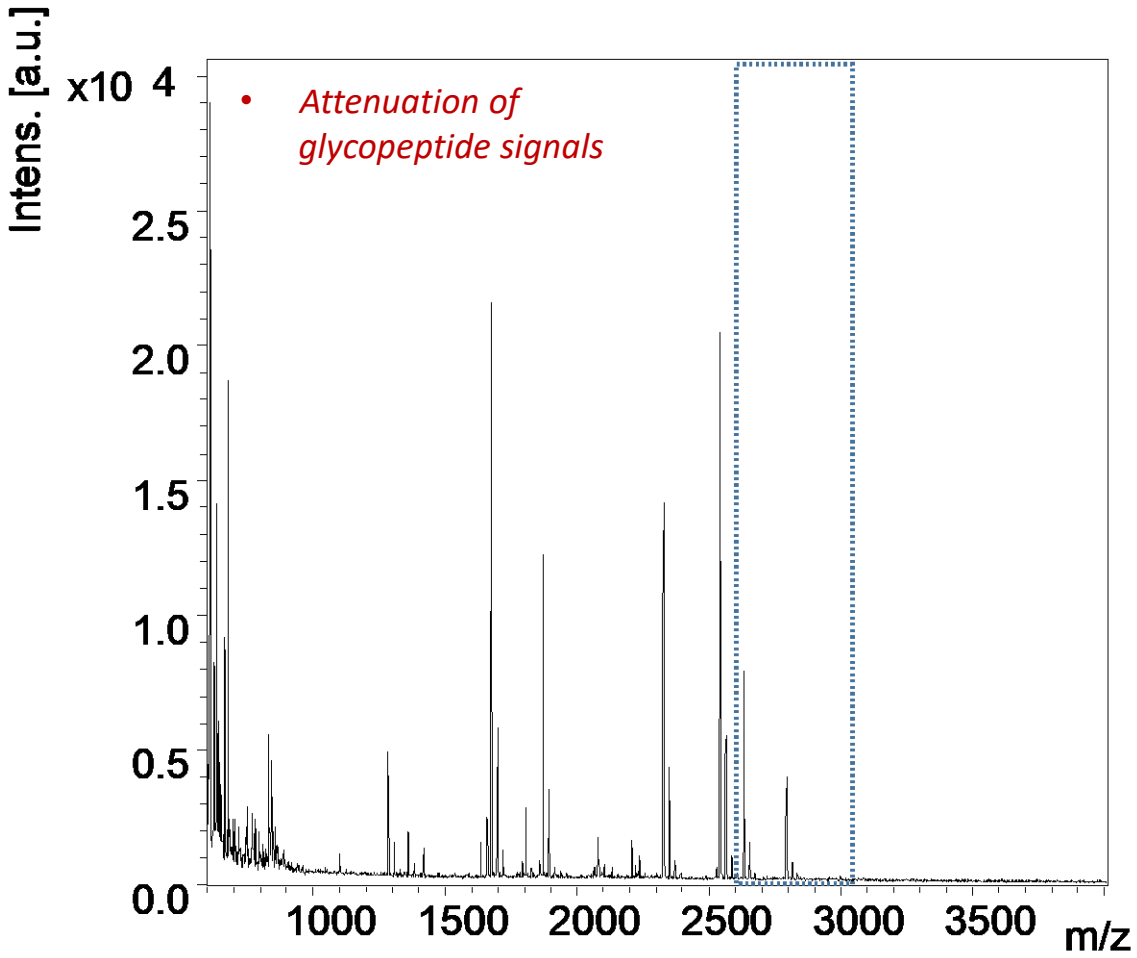
IgG 1 V-tag labeled glycopeptides

V-Tag Greatly Enhances MALDI-MS Analysis of Glycopeptides

The signal for underivatised glycopeptides is suppressed in MALDI-MS but is enhanced after V-tag labeling and enrichment

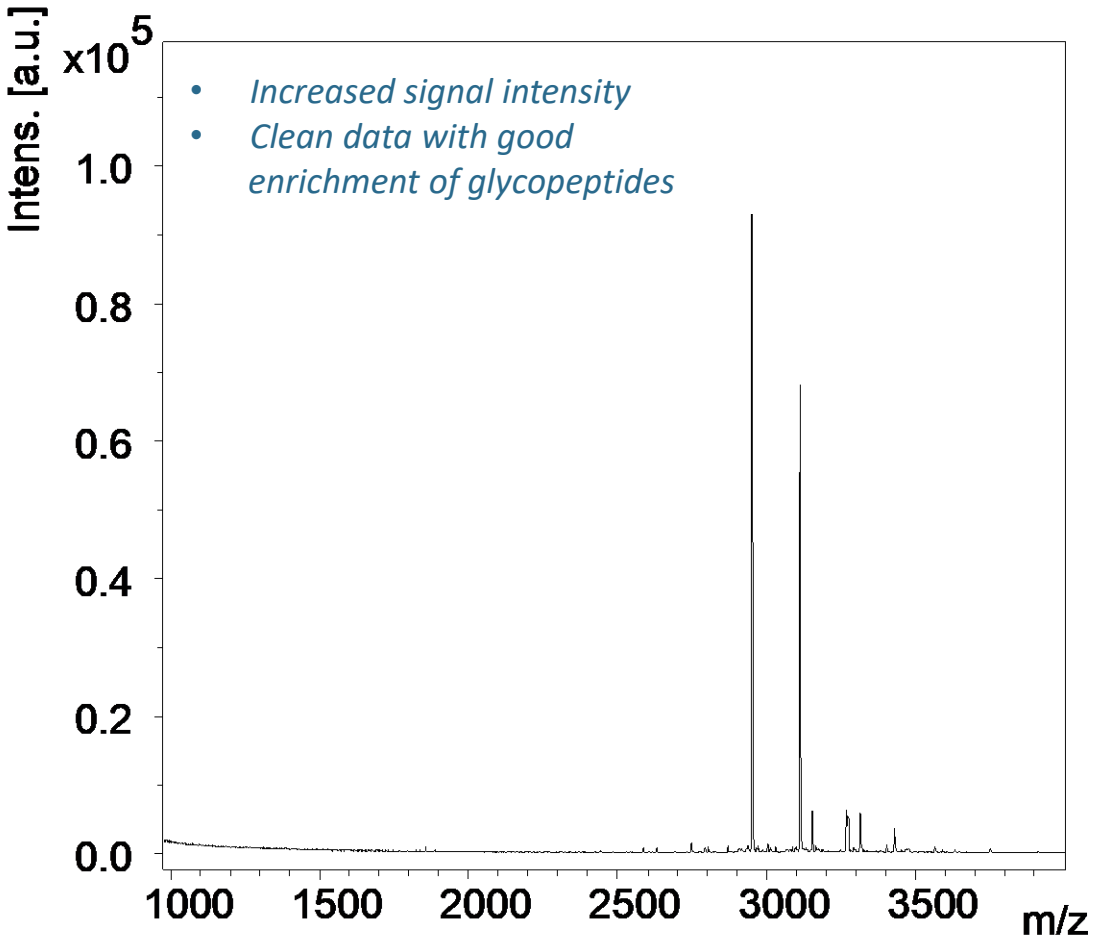
Before V-Tag labeling and enrichment

IgG 1 tryptic peptides and glycopeptides



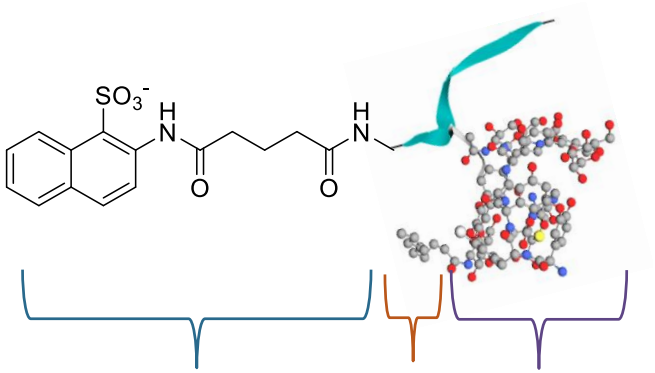
After V-Tag labeling and enrichment

IgG 1 tryptic glycopeptides



V-Tag Allows Identification of Glycopeptides using MALDI-MS

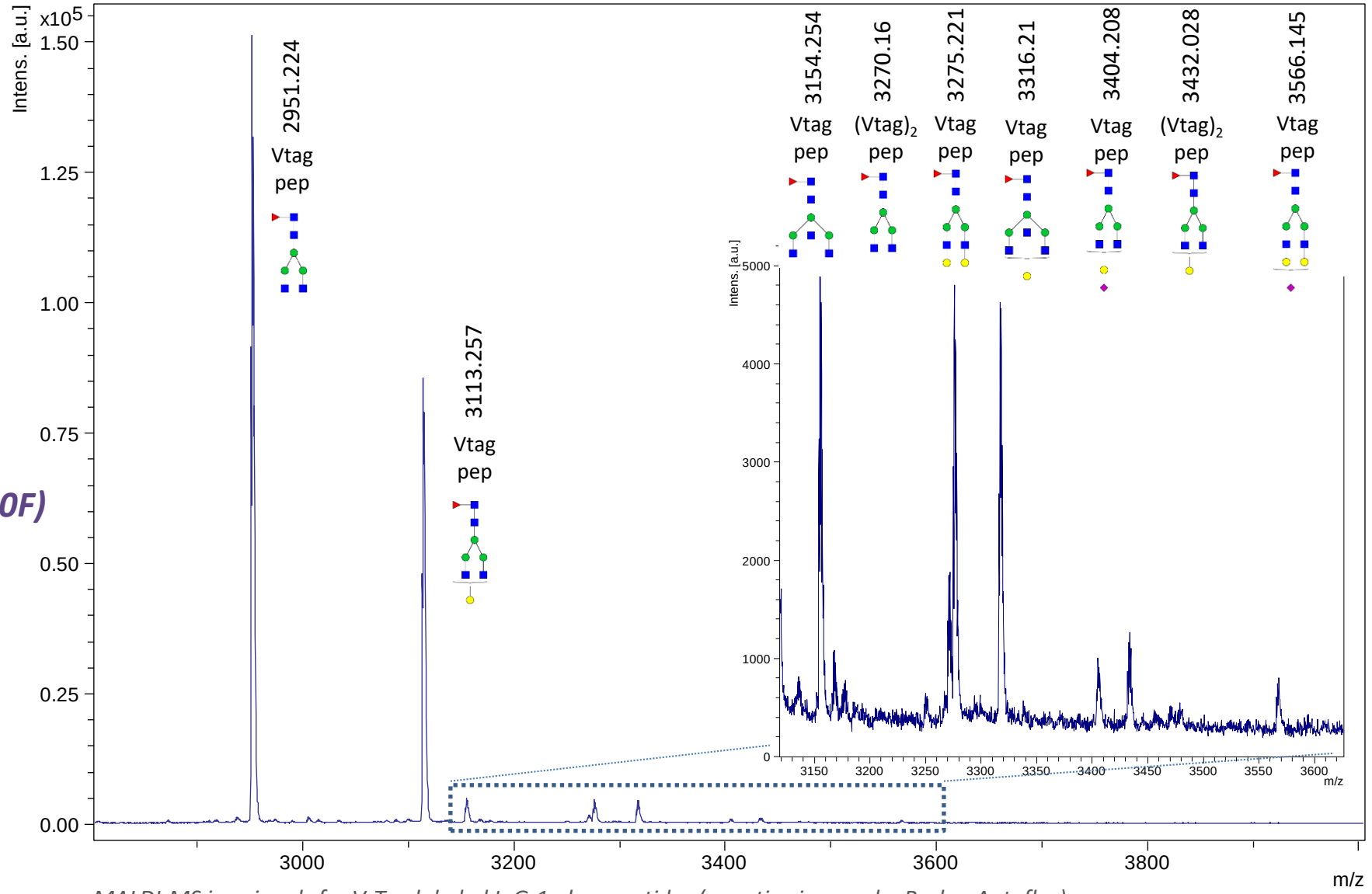
Example of V-Tag labeled IgG-1 mAb glycopeptide analysis by negative mode MALDI-MS



M= V-Tag + EEQYNSTYR + Glycan

M = 319.33 + 1189.52 + 1444.53 (G0F)

M = 2952.11

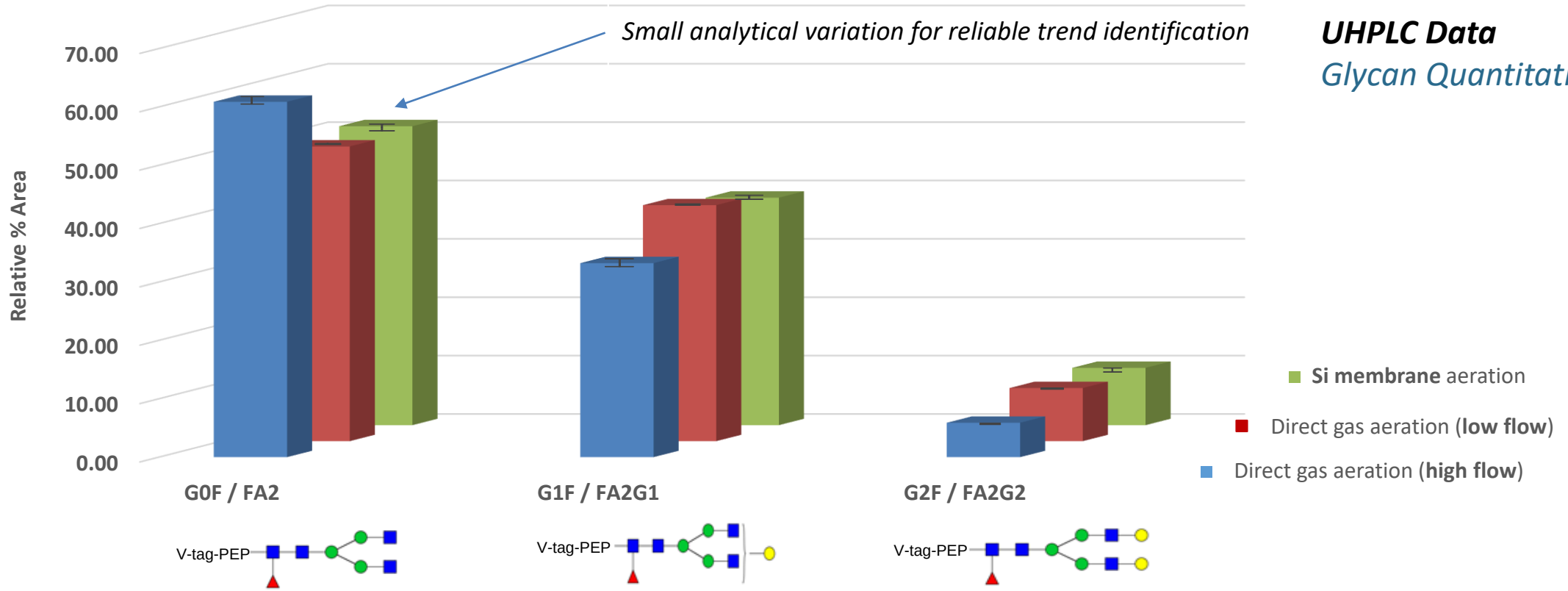


MALDI-MS ion signals for V-Tag labeled IgG-1 glycopeptides (negative ion mode; Bruker Autoflex)

Incorporating V-Tag into your drug programme

QbD Study: The impact of cell culture conditions on glycoform patterns

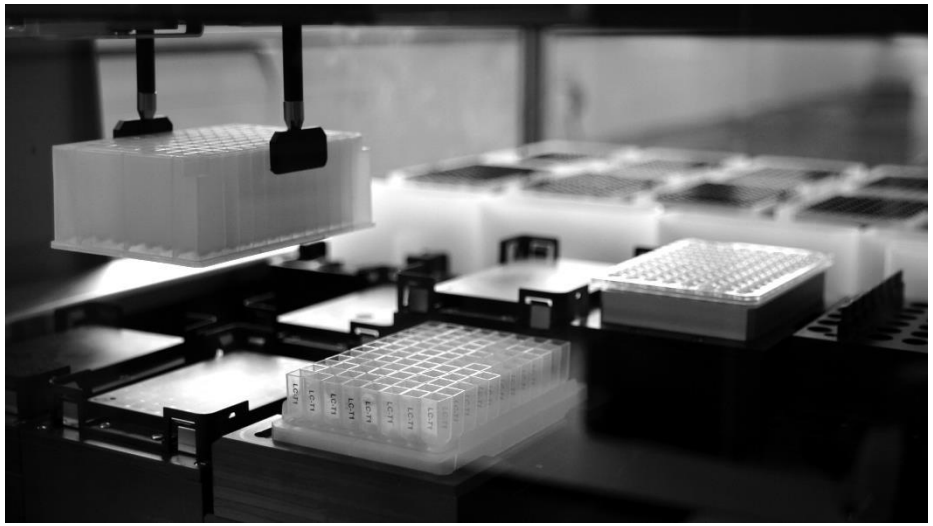
Variations are reliably detected with V-tag system



- *Chinese hamster ovary (CHO) cell line GS-CY01 expressing a mAb was grown in bio-reactors using different aeration conditions*
- *Fc galactosylation patterns analysed (i.e. the ratios of the G0F, G1F and G2F glycans) for different aeration conditions.*
- *Increasing the levels of terminal galactose are known to positively correlate with complement dependent cytotoxicity (CDC) activity*
  - *The cells grown under silicon membrane aeration showed the highest degree of Fc galactosylation (higher abundance of G2F)*

Automated High Throughput Studies using V-Tag

Adapted to 96-well plate system to use with a liquid handling robot



Morning — **Afternoon or Overnight**

Protease Digestion → **V-Tag Labeling** → **Clean-up and Enrichment** → **Sample Preparation for MALDI-MS and UHPLC** → **Data Acquisition** → **Data Analysis**

*The workflow can be completed in **1 day** making this technology a good candidate for high throughput analysis of mAbs*

Next Steps…

If you have a question

CLICK to contact Jenifer

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Senior Scientist
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Request a quotation

CLICK to contact Sales

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