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Flash Characterization of mAbs Using a Combination of Reagents via Automated On-Line Microdroplet Reaction

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Introduction

Recently, an unmodified Agilent Jet Stream ESI source (AJS) was used to demonstrate "Flash Characterization" of mAbs by microdroplet facilitated reductions or enzyme digestions with good reproducibility and high yield [1]. These results motivated us to evaluate treatments that combined enzymatic digestion, reduction, and/or deglycosylation of mAb in a single microdroplet experiment. New optimization parameters for the Agilent Injection Program on Agilent 1290 Infinity II LC were presented. Microdroplet experiments, including GlycINATOR ® deglycosylation, the combination of IdeS cleavage and DTT reduction and IdeS cleavage and GlycINATOR ® deglycosylation were examined for reproducibility, intact mAb remaining after treatments and simplified subunit characterization. GlycINATOR is an endoglycosidase that cleaves N-linked glycans and leaves the core GlcNAc residue with or without a fucose on the asparagine. Additional antibodies of Herceptin and sigmamab are also studied with the combination treatment in the microdroplet reaction.

Experimental

The mAbs, enzymes, and DTT were automatically combined and injected in flow injection (FIA) mode using an Agilent Injection Program on Agilent 1290 Infinity II LC. The UHPLC system was coupled with an Agilent 6545XT AdvanceBio LC/Q-TOF system using the AJS in ESI positive mode for ultrafast microdroplet reaction. Spectra of the intact mAbs, digested, reduced or deglycosylated fragments were collected using the Q-TOF and analyzed with Agilent MassHunter Quantitative Analysis (for Q-TOF) and BioConfirm v12 software.

Agilent 1290 Infinity II LC with 6545XT AdvanceBio LC/Q-TOF System

Experimental

Antibody and Reagent Preparation

- All mAbs including NIST IgG1 were diluted to 0.5 mg/mL in 5 mM Ammonium Bicarbonate (ABC)
- IdeS (FabRICATOR ®)and Glycinator ® were diluted to 1 unit/µL in 5mM ABC
- DTT was prepared at 10 mg/mL in 5 mM ABC

Chromatographic Conditions

UHPLC: Agilent 1290 Infinity II
Flow injection analysis (FIA)
Column oven temperature: Ambient
Injection volume: 0.1 to 0.5 µL
Autosampler: 5 ± 2°C

Mobile Phase A: 5 mM Ammonium Bicarbonate (ABC)
Gradient

Time, min	Flow Rate, mL/min	%A
0	0.3	100
0.1	0.3	100
0.2	0.025	100
1.9	0.025	100
2.0	0.3	100
3.5	0.3	100

Injector Program

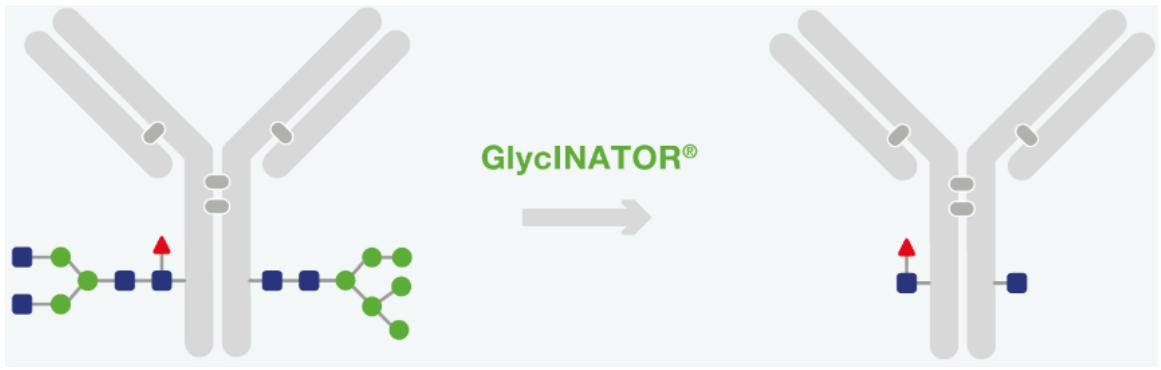
Function	Parameter
Draw	Draw 1.0 or 2.0 µL from reagent
Draw	Draw 0.1 to 0.5 µL from mAb
Mix	Mix air and repeat five times
Wait	Wait 0.5 min
Draw	Draw 1.0 or 2.0 µL from reagent
Mix	Mix air and repeat five times
Remote	Set remote line "Start" for 125 ms
Wait	Wait 0.5 min
Inject	Inject

Optimized MS Conditions-Agilent 6545XT LC/Q-TOF

Parameters	
Drying gas temperature	350 ºC
Drying gas flow	13 L/min
Nebulizer gas	60 psi
Sheath gas temperature	400ºC
Sheath gas flow	12 L/min
Capillary voltage	4500 to 5000 V
Nozzle voltage	2000 V
Ion mode	AJS ESI Positive
Fragmentor	380V
Skimmer	45V
MS range	*m/z* 1350-10000
Acquisition rate/Time	2 spectra/s

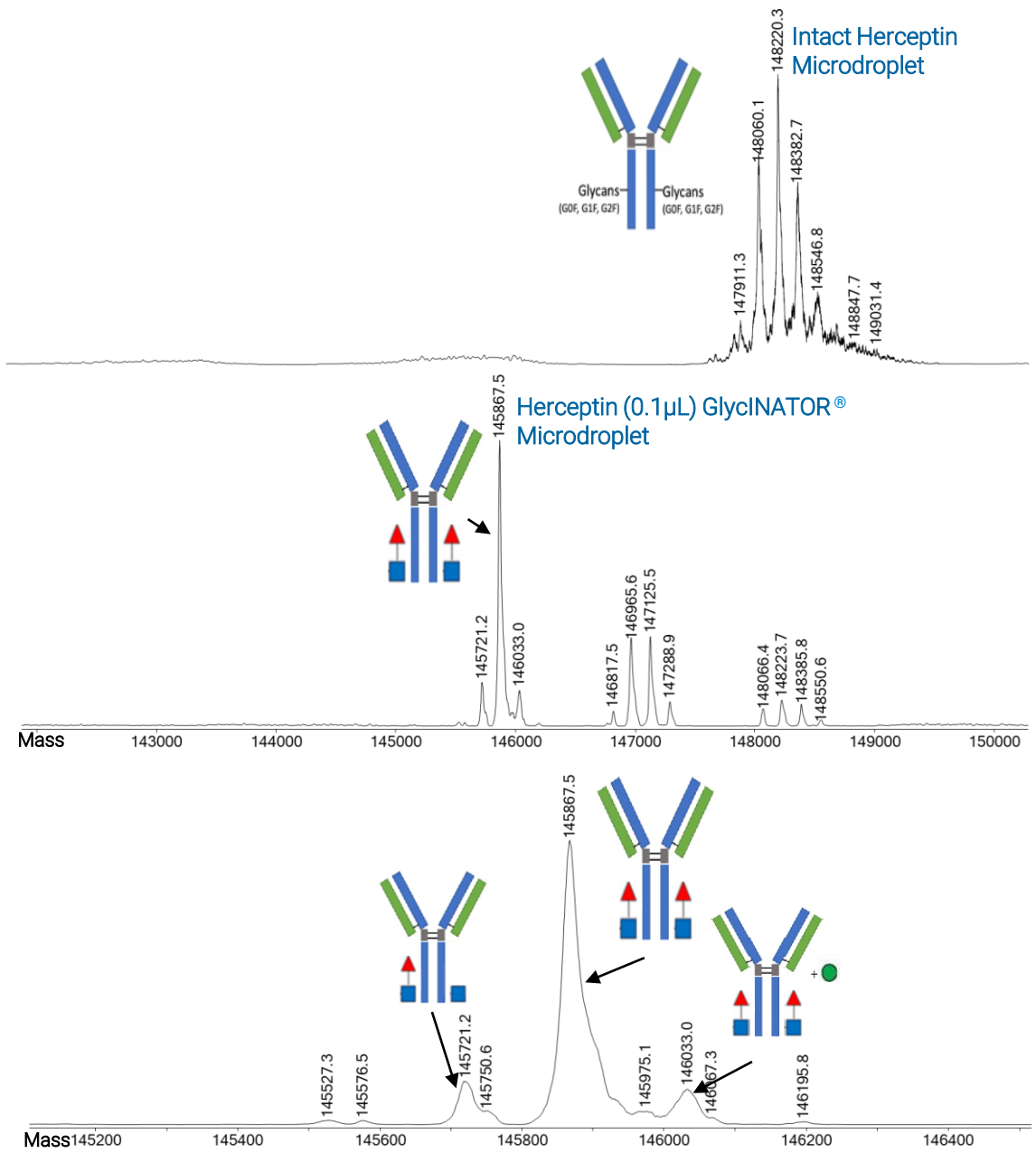
Results and Discussion

Microdroplet GlycINATOR® Deglycosylation[2]

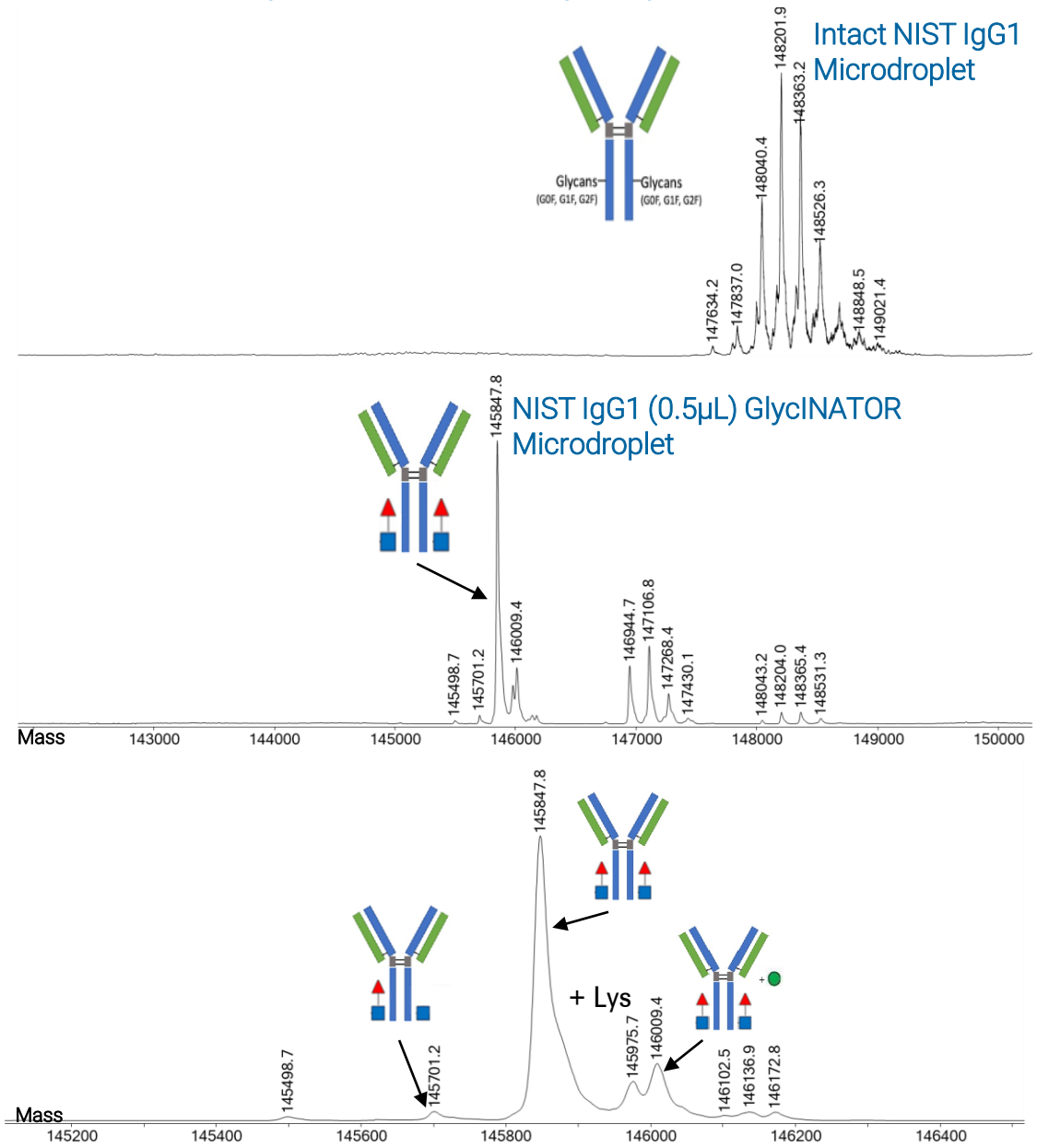


- GlycINATOR ® (EndoS2) is an endoglycosidase from Streptococcus pyogenes that specifically hydrolyzes glycans at the Fc N-glycosylation sites of IgG.

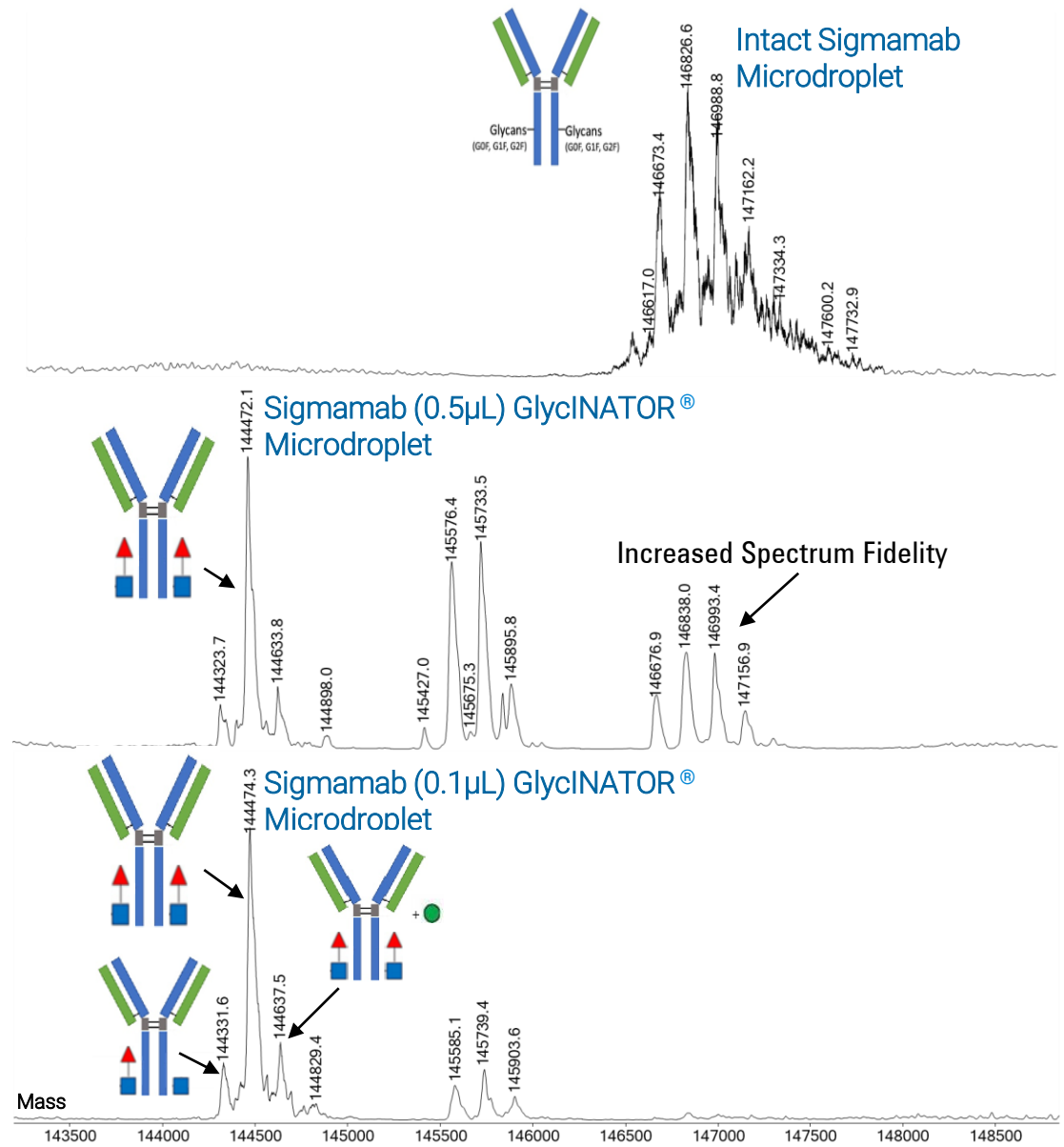
Microdroplet GlycINATOR® Deglycosylation on Herceptin



Microdroplet GlycINATOR® Deglycosylation on NIST IgG1



Microdroplet GlycINATOR® Deglycosylation on Sigmamab



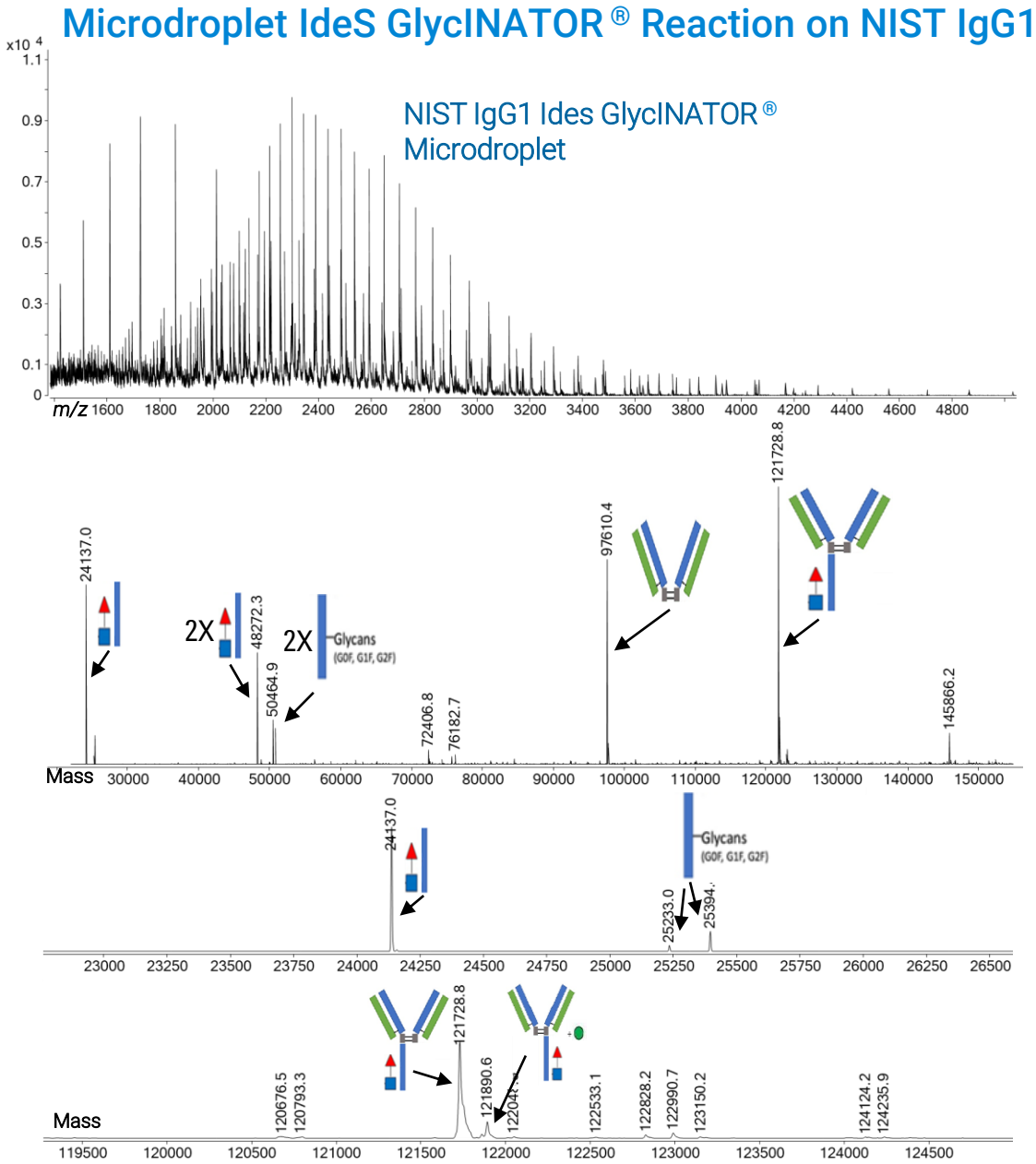
Reference

[1]Lau, J, et al. Flash Characterization of Antibodies via Microdroplet Reactions in an Unmodified Jet Stream Source. Agilent Application Note 5994-6752EN, 2023

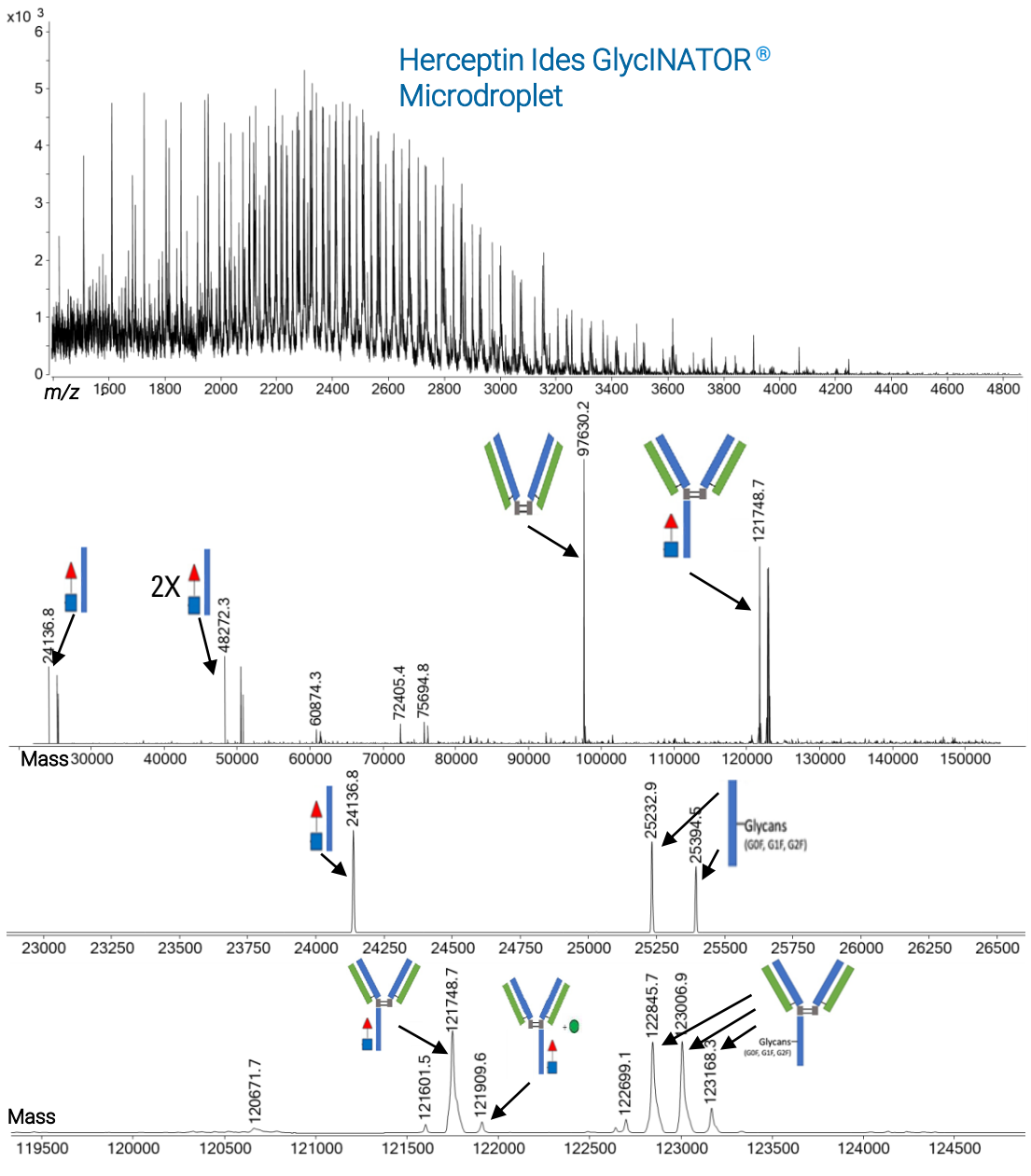
[2]Genovis, Https://www.genovis.com/smartenzymes/antibody-deglycosylation/glycinator/

Results and Discussion

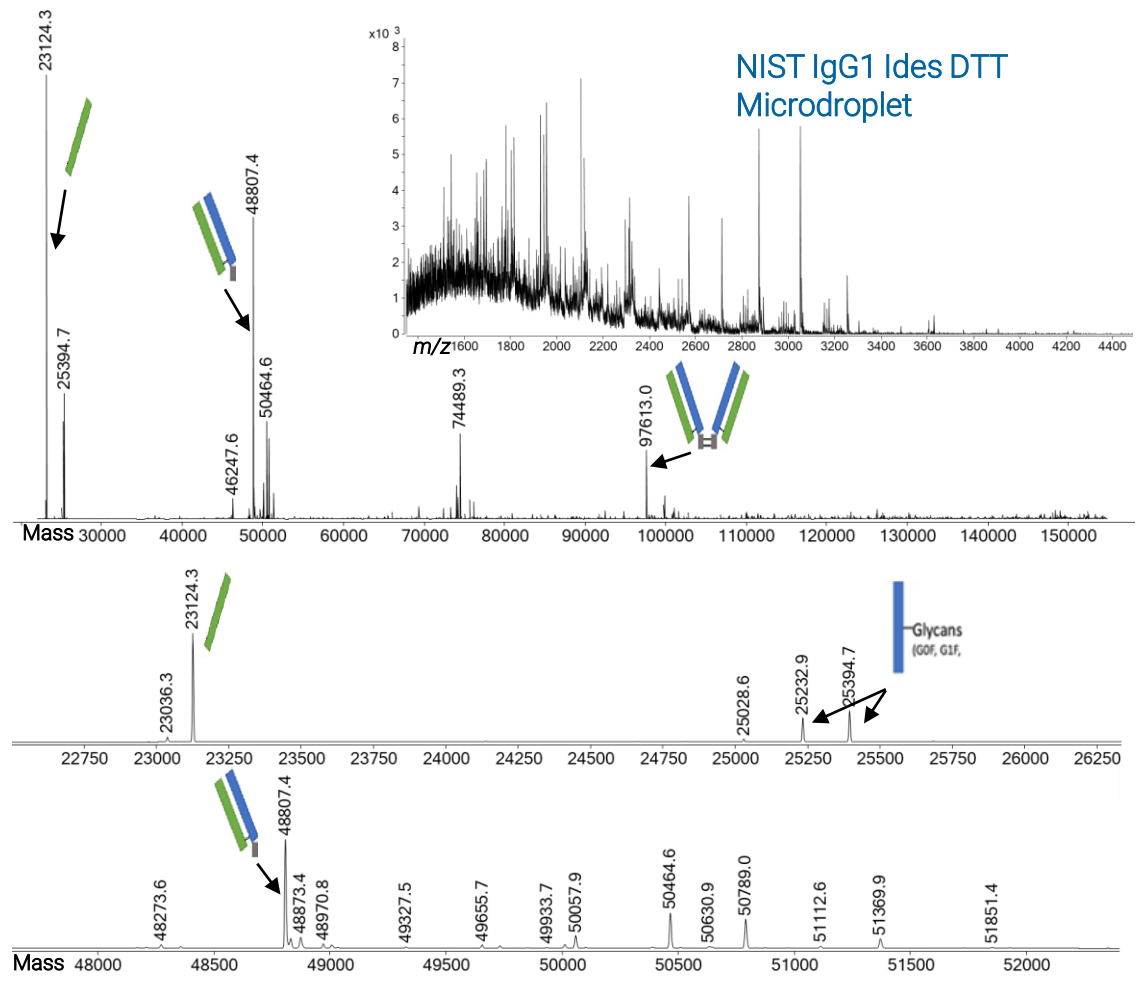
Microdroplet IdeS GlycINATOR® Reaction on NIST IgG1



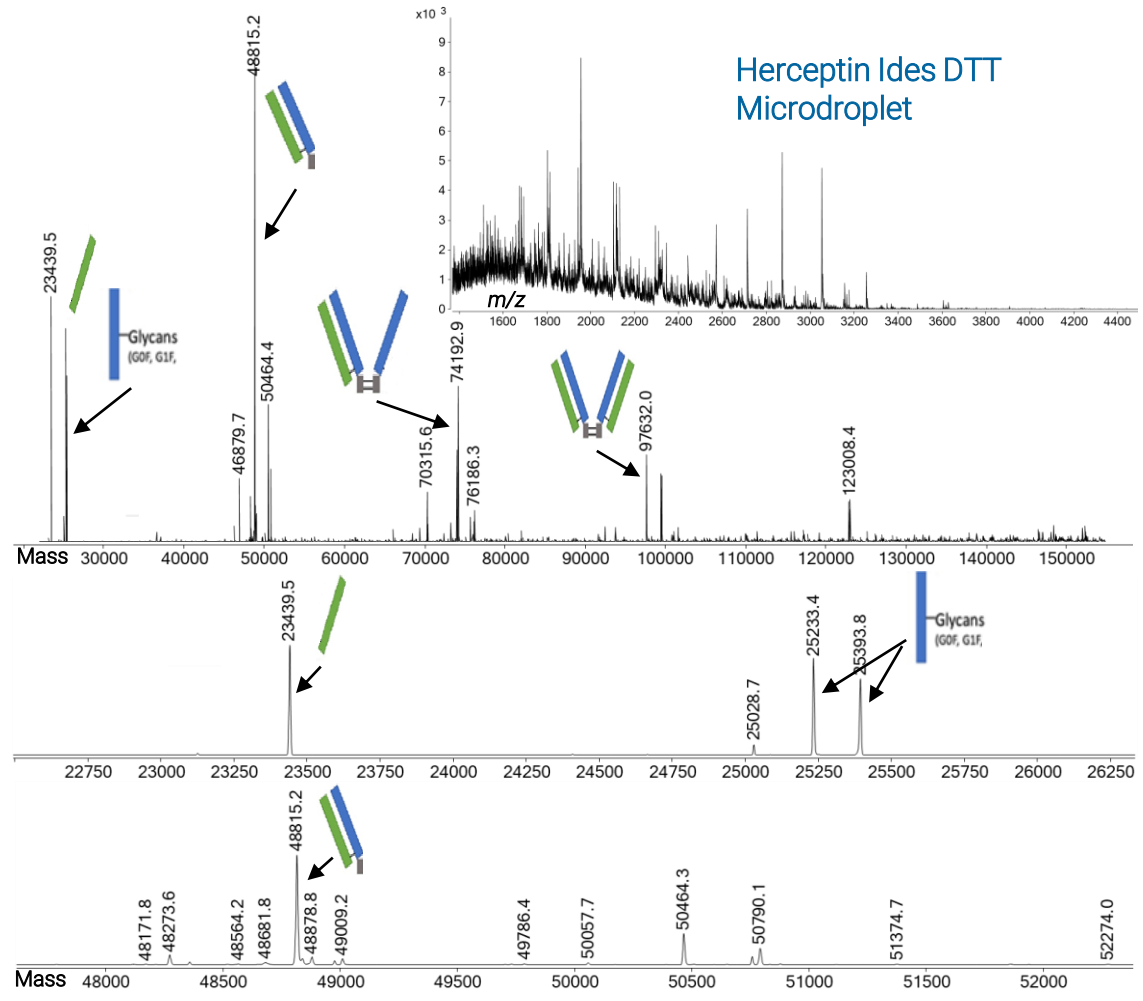
Microdroplet IdeS GlycINATOR® Reaction on Herceptin



Microdroplet FabRICATOR ®(IdeS) / DTT Reaction on NIST Ig



Microdroplet FabRICATOR ®(IdeS) / DTT Reaction on Herceptin



Conclusions

- Flash Characterization of mAbs with an automatic injector, combination treatments, and microdroplet reaction in the AJS source demonstrates simplification, reproducibility, and cost reduction of protein characterization.
- Combination treatments are viable with microdroplet reactions to accelerate the reduction, cleavage, and deglycosylation of native IgG1 significantly.

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