

Poster Reprint

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“Flash Characterization” of Antibodies via Microdroplet Reactions in an Unmodified Jet Stream Source

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Introduction

Antibody characterization with IdeS enzyme digestion, reduction with tris(2-carboxyethyl)phosphine (TCEP), triethylphosphine (TEP) or dithiothreitol (DTT), and enzymatic deglycosylation requires incubation (minimum 30 minutes) in bulk solution to observe significant reaction products. Recently, attention has been drawn to the use of microdroplet reactions for antibody analysis (Gunawardena, et al., *Anal. Chem.* 2023, 95, 3340-3348). The microdroplet reactions are attractive due to the rapid reaction rate (microseconds) and high reaction yield achieved in the ESI spray chamber. The NIST IgG1 mAb was used to optimize microdroplet reaction conditions on two reactions, IdeS cleavage and disulfide bond reduction, using the Agilent JetStream ESI source. Optimized conditions were applied to several commercial antibodies to test robustness and broad applicability of the conditions. The experiments demonstrated exceptional results and in addition to the time savings, the cost of analysis is dramatically lowered due to the reduction in enzyme and antibody consumption for characterization. The optimized workflow we call "Flash Characterization".

Experimental

The antibodies and reagents were 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 6546 Q-TOF system using the Agilent Jet Stream ESI source in positive mode for ultrafast microdroplet reactions. Spectra resulting from intact antibodies and digested or reduced antibody fragments were analyzed with Agilent MassHunter Quantitative Analysis and BioConfirm v10 software.



Agilent 1290
Infinity II LC
with 6546
QTOF
LC/MS 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 was diluted to 1 unit/ μ L in 5mM ABC
- TCEP, TEP and DTT were 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: 1 μ L

Autosampler: 5 \pm 2 $^{\circ}$ 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

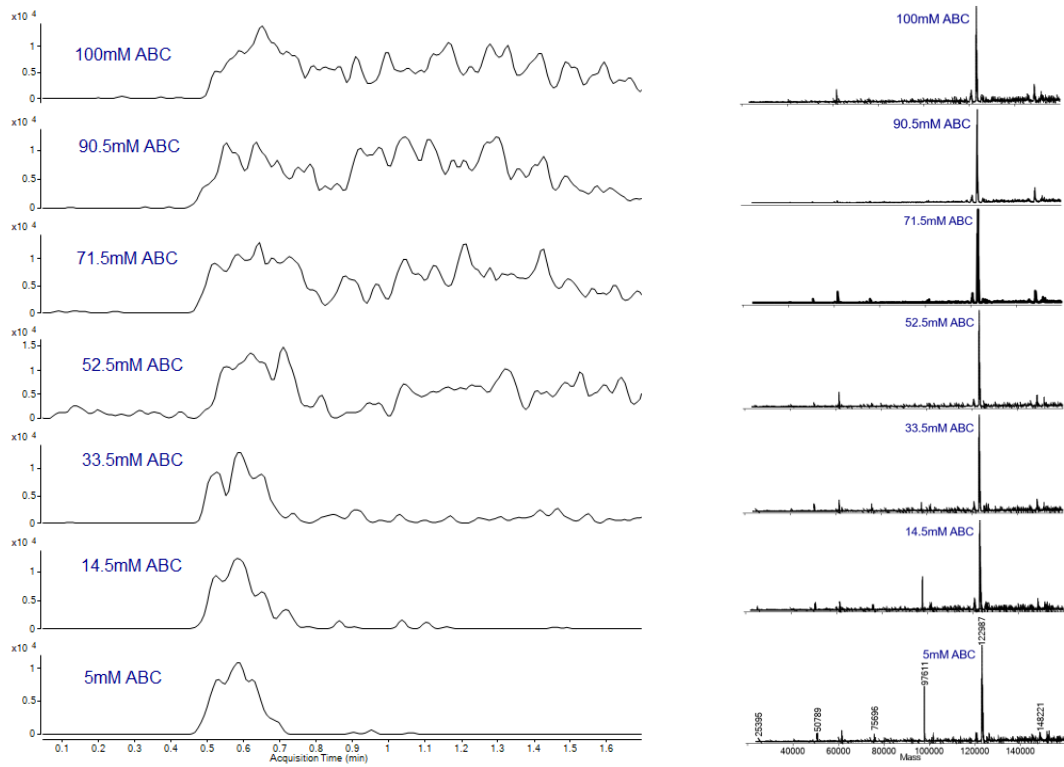
Injector Program

Function	Parameter
Draw	Draw 1.0 μ L from reagent
Draw	Draw 1.0 μ L from mAb
Mix	Mix 2 μ L from air and repeat two times
Remote	Set remote line "Start" for 125 ms
Wait	Wait 0.1 min
Inject	Inject

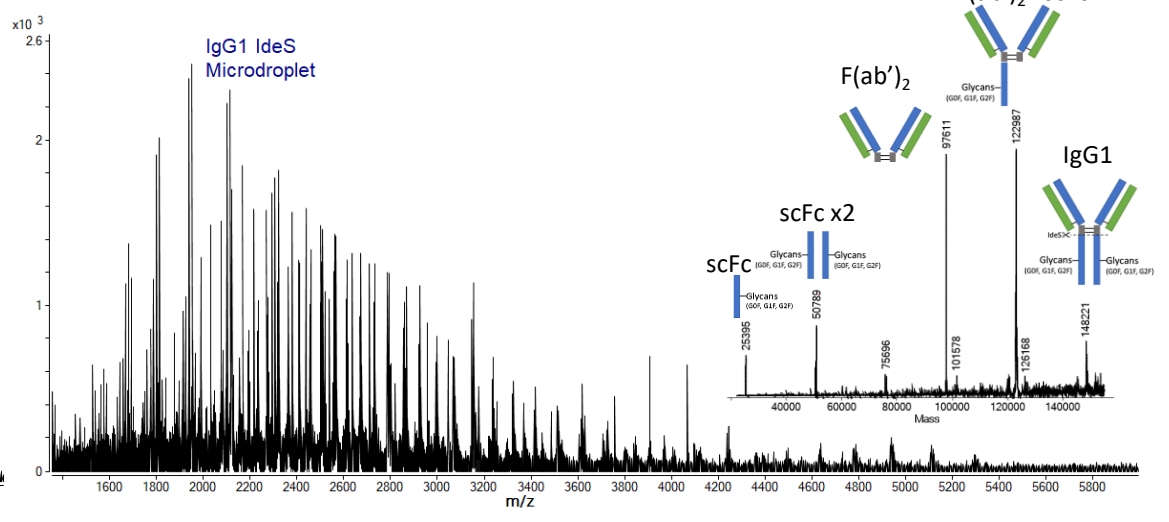
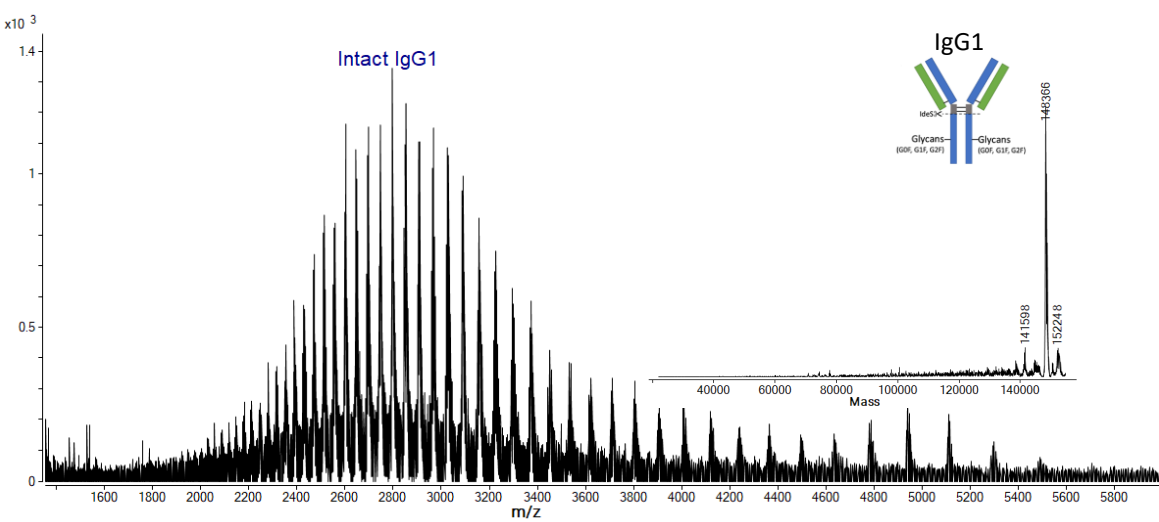
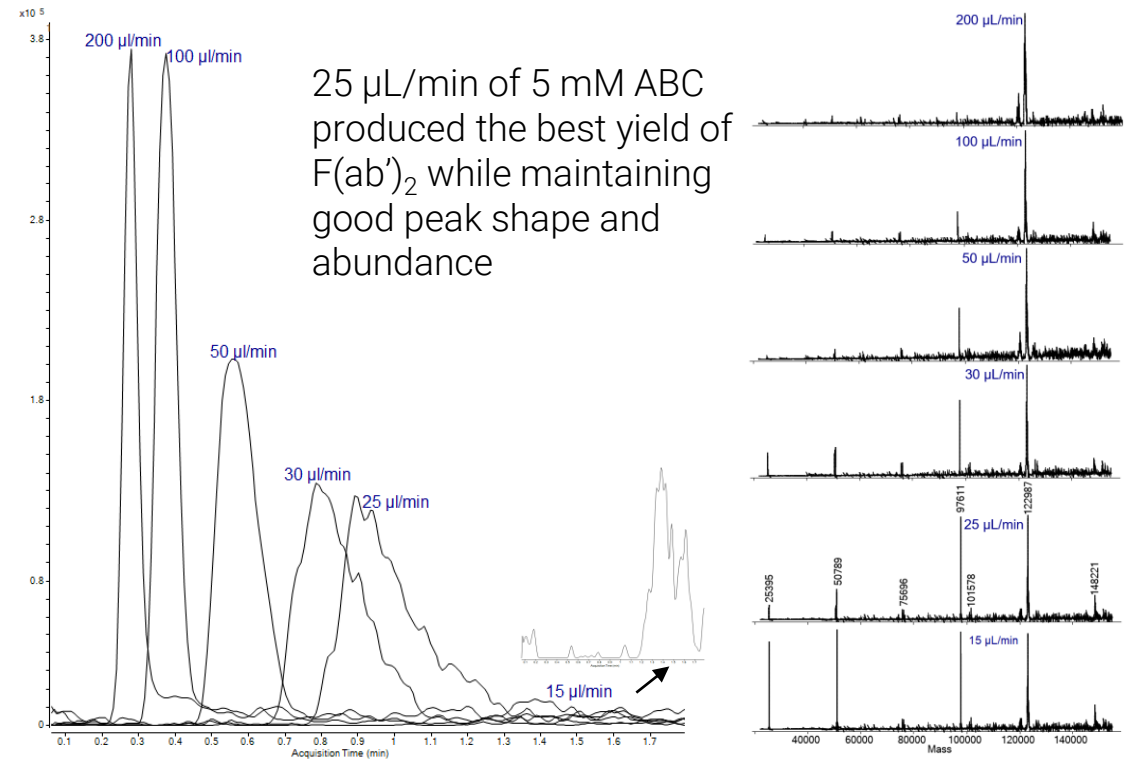
Optimized MS Conditions-Agilent 6546 LC/Q-TOF

Parameters	
Drying gas temperature	365 $^{\circ}$ C
Drying gas flow	13 L/min
Nebulizer gas	60 psi
Sheath gas temperature	400 $^{\circ}$ C
Sheath gas flow	12 L/min
Capillary voltage	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

Effect of ABC Concentration on NIST IgG1/IdeS F(ab')₂ Yield



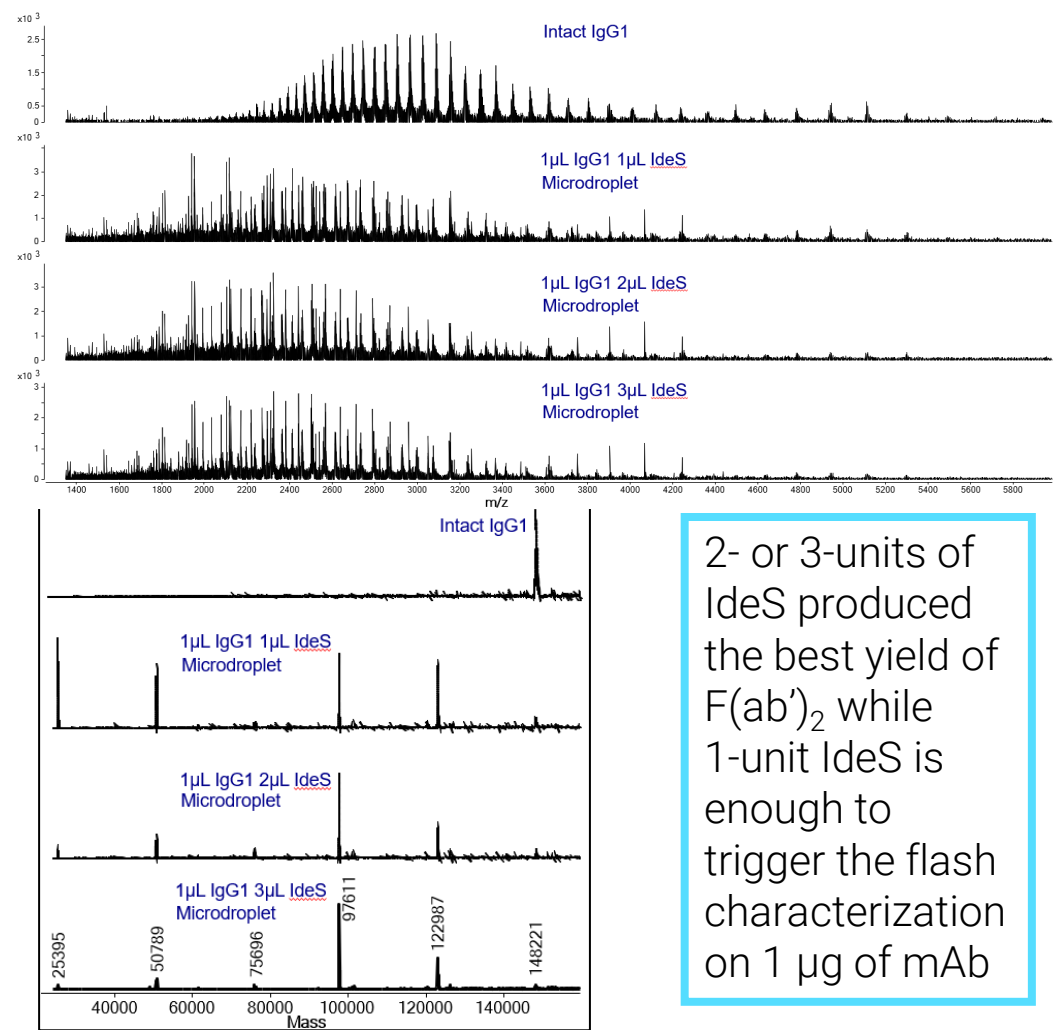
Flow Rate Effect on NIST IgG1/IdeS F(ab')₂ Yield



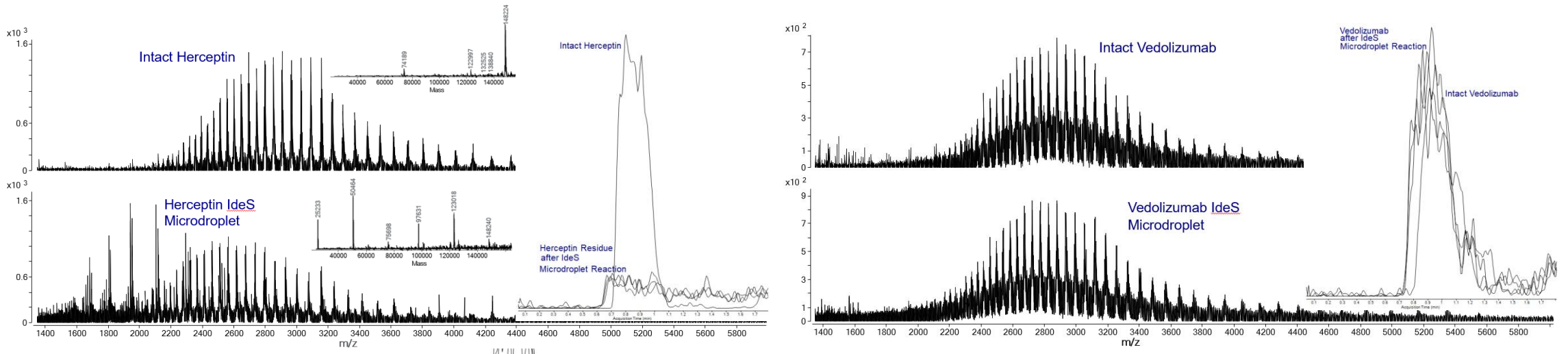
Nist mAb IgG1/ IdeS Microdroplet Reproducibility @ 1U IdeS

Run	Remaining Intact IgG1 after IdeS Microdroplet Reaction
1	403094
2	417371
3	380727
4	382958
5	437117
6	404127
7	395262
8	418881
9	403459
10	390050
Average	403305
Precision %	4.3
IgG1 – no IdeS	2598747 (Intact IgG1)
Average Conversion, %	85

Effect of IdeS Amount on NIST IgG1/IdeS F(ab')₂ Yield



Microdroplet IdeS reaction on Herceptin and Vedolizumab

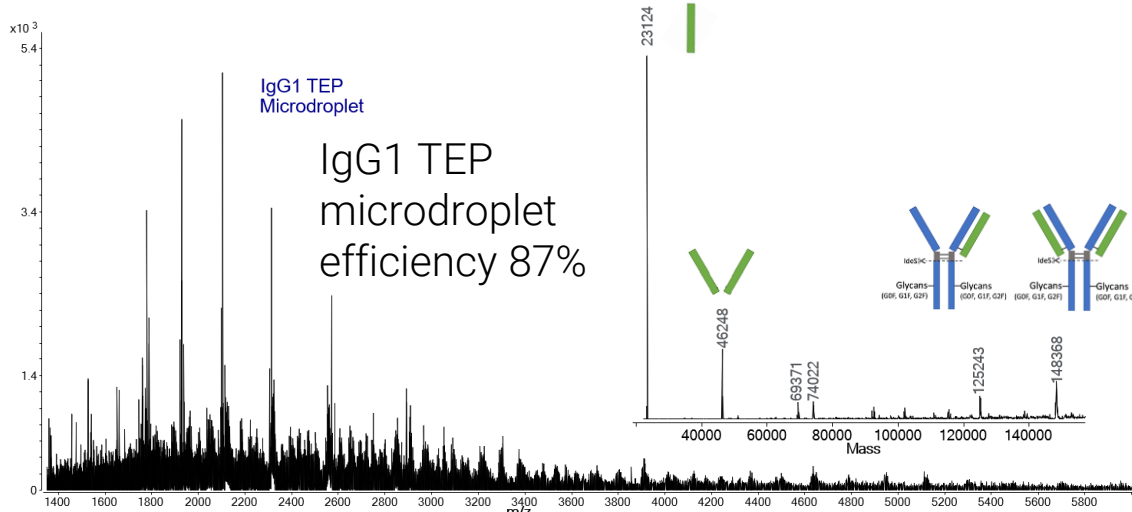
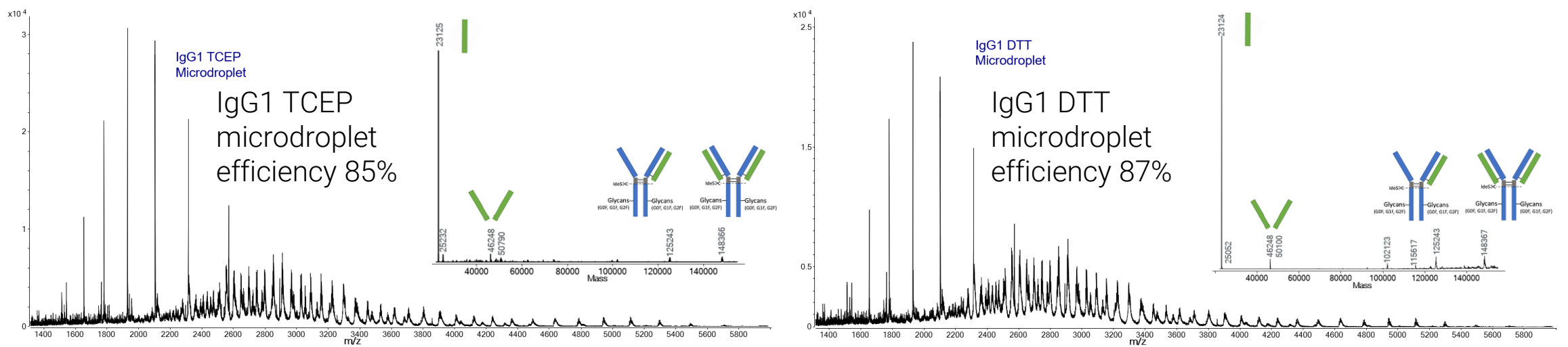


mAb	Mass of F(ab') ₂ Da	Response of Intact mAb	MS response of mAb F(ab') ₂ after IdeS Microdroplet Reaction			Average Conversion % (n=3)	Precision % (n=3)
			1	2	3		
Adalimumab	97719	4826294	854558	880255	784104	83	5.9
Bevacizumab	98770**	593679	59623	64895	55712	89	7.7
Herceptin	97631	2603028	333711	351471	347472	87	2.7
Nivolumab	95824**	578475	75817	81608	71283	87	6.8
Rituximab	96714	1154326	140117	147817	144427	88	2.7
Sigmamab	96300**	499829	124836	128147	114772	77	5.7
Trastuzumab	97629	1207714	123903	121589	115809	90	3.5
Vedolizumab*	-	801472	857924	890857	878026	0	1.9

*Vedolizumab (LAGAPS) sequence doesn't contain LGGP motif for IdeS cleavage as in Rituximab (LLGGPS) and Nivolumab (FLGGPS)

**with 2- or 3-units IdeS

Microdroplet Reduction of NIST IgG1 mAb Disulfides with TCEP, TEP, and DTT



Conclusions

- Ultrafast Microdroplet reaction with 85% efficiency is achieved with an unmodified JetStream electrospray spray source
- Ultrafast microdroplet enables "Flash Characterization" of mAbs
- Microdroplet reaction leads to dramatic time savings and cost reduction on enzyme and reagents usage

<https://www.agilent.com/en/promotions/asms>

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