

Rapid Determination of Drug-to-Antibody Ratios in Antibody–Drug Conjugates Using Ultrafast Microdroplet Digestion Technology

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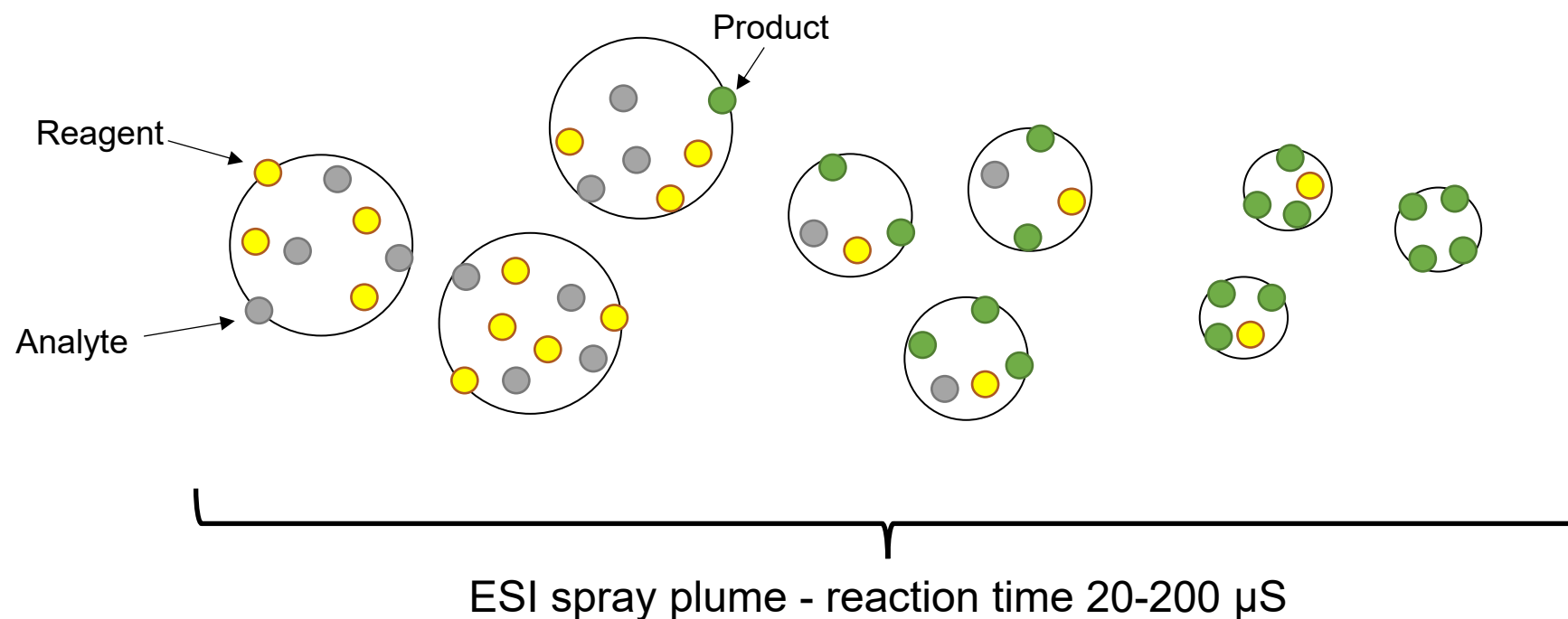
³Johnson & Johnson innovative Medicine, Johnson & Johnson, McKean Road, Spring House, PA, 19477, USA

CASSS, Sep. 16, 2026



What are “Microdroplet Reactions”?

- Fast reactions occurring on 10 μm or smaller droplets

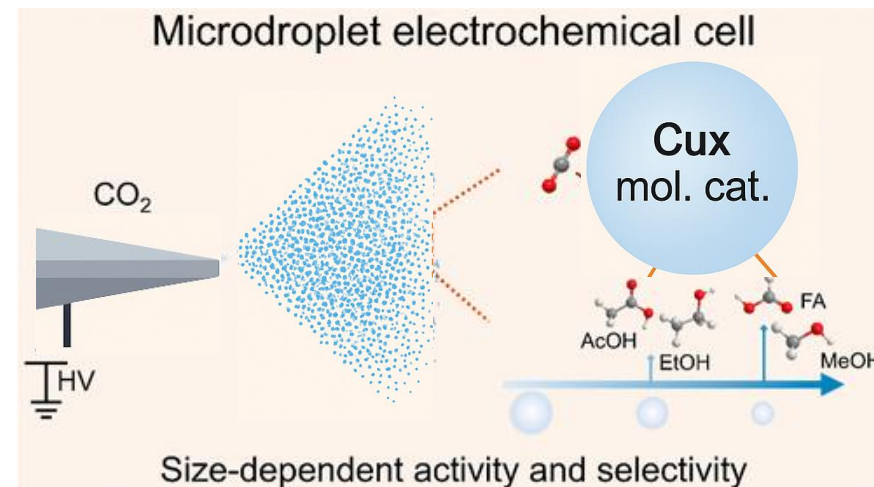
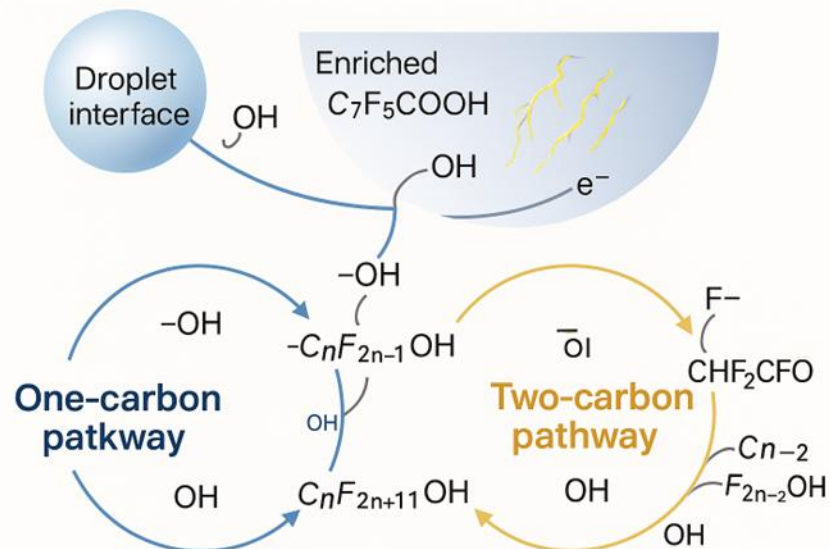
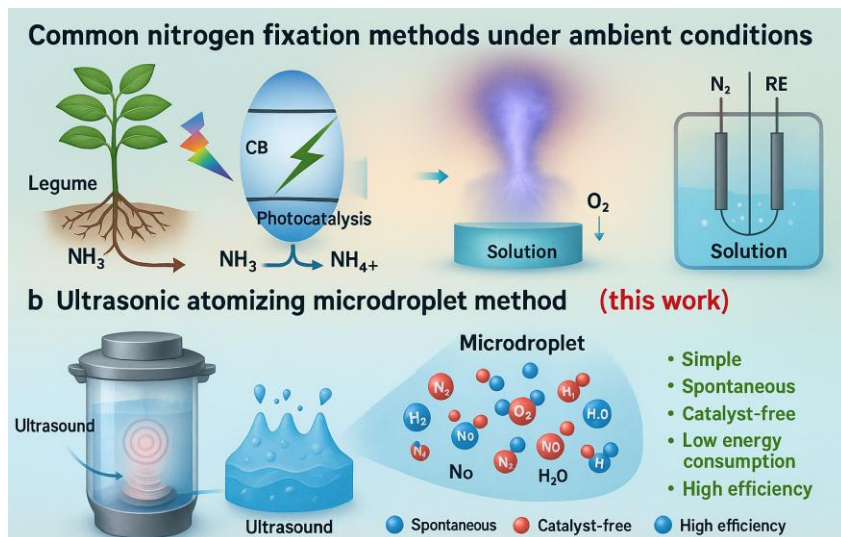


Girod, M.; Moyano, E.; Campbell, D. I.; Cooks, R. G. *Chem. Sci.*, 2011, 2, 501–510

C. Liu, J. Li, H. Chen, R. N. Zare, *Chem. Sci.*, 2019, 10, 9367-9373

Yan, X.; Bain, R.M.; Cooks, R.G. *Angew. Chem. Int. Ed.* 2016, 55 (42), 12960-12972.

Importance of Microdroplet Reactions



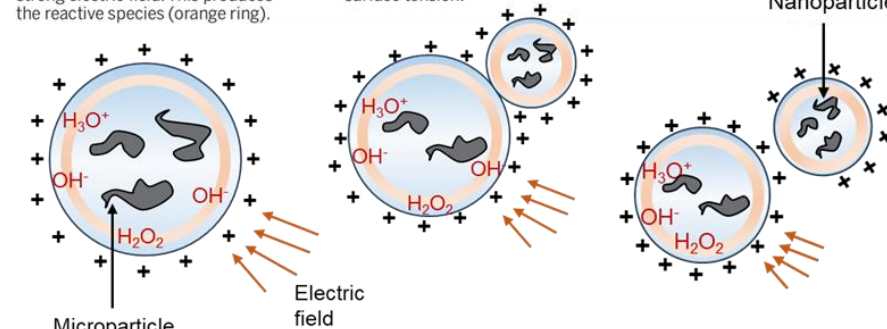
Micro-to-nano transitions in minerals at the air-water interface

Reactions that promote mineral disintegration are accelerated at the air-water interface of microdroplets. Key reactive species are the result of the effects of a high electric field at the surface of the water droplets.

Parent droplet
A charged droplet generates an electric double layer, which gives a strong electric field. This produces the reactive species (orange ring).

Coulomb repulsion
Droplet fission begins when coulombic energy exceeds surface tension.

Nanoparticle formation
Nanoparticles are released in smaller microdroplets.

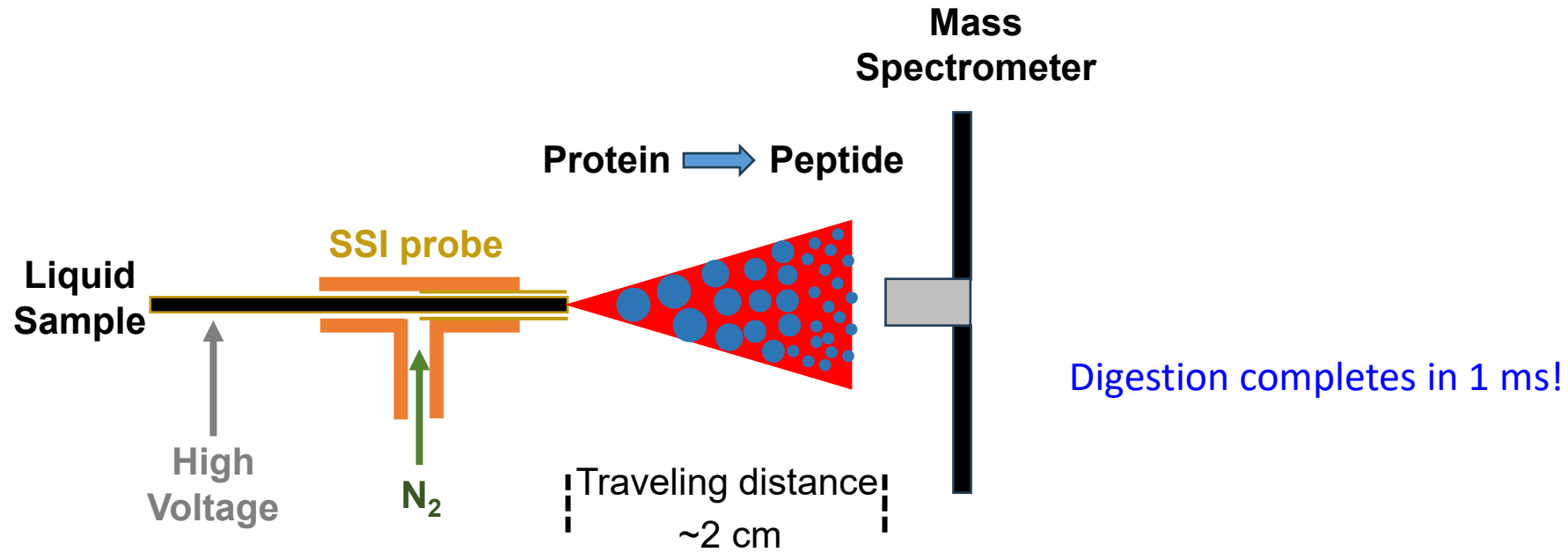


Mineral Disintegration

Dong, J.; et al., *J Am Chem Soc* 2024, 146 (3), 2227-2236; DOI:10.1021/jacs.3c12586
Wang, Y., et al. *J Am Chem Soc* 2025, 147 (3), 2756-2765; DOI: 10.1021/jacs.4c15514

Cooks, R. G.; Holden, D. T., *Science* 2024, 384 (6699), 958-959; DOI: 10.1126/science.adp7627
Xia, D.; et al, *J Am Chem Soc* 2024, 146, 11266-11271; DOI: 10.1021/jacs.4c00435

Microdroplet reactions for protein/DNA digestion

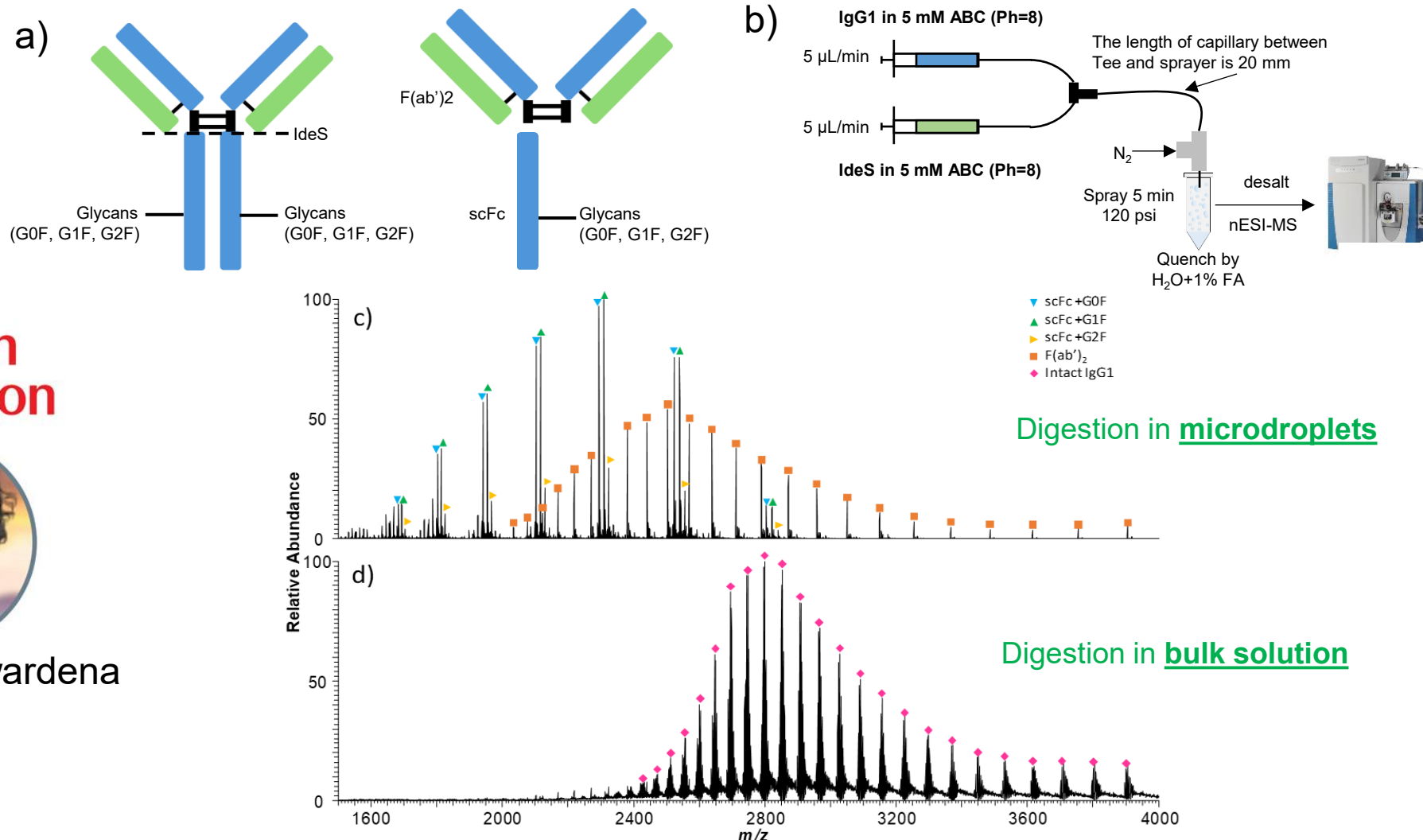


Schematic drawing of the experimental apparatus for the online proteolysis by microdroplet chemistry coupled with mass spectrometry (ESSI-MS).

Zhong, X.; Chen, H.; Zare, RN. *Nat. Comm.* **2020**, 11, 1049.

Xiaoqin Zhong, Hao Chen, Richard N. Zare, *QRB Discovery* 2021, 2:e4, 1–8

Intact Antibody Microdroplet Digestion



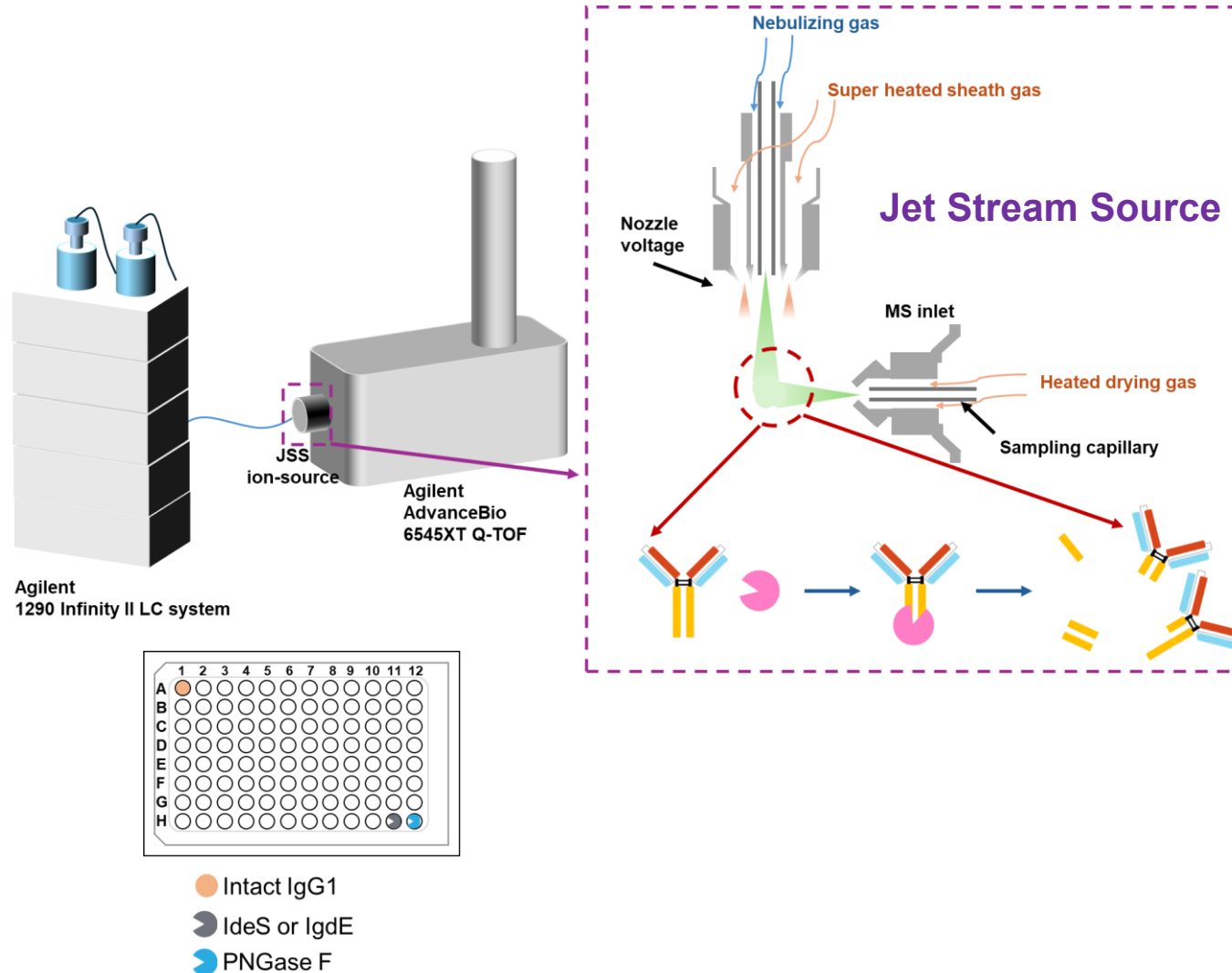
Johnson
& Johnson



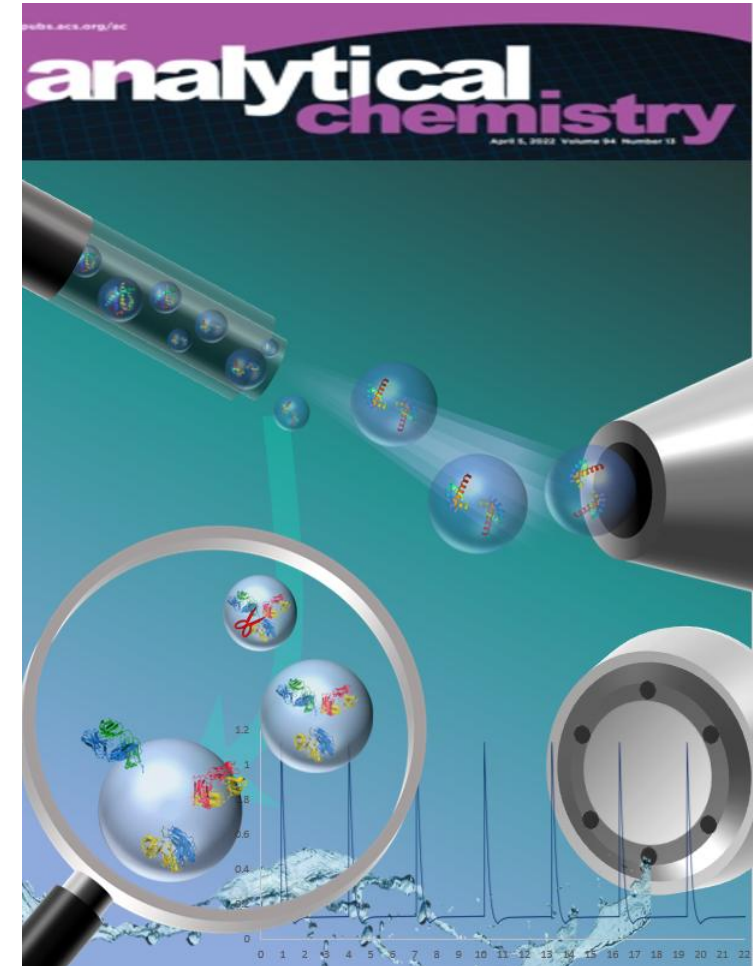
Harsha Gunawardena

Zhao, P., Gunawardena, H., Zhong, X., Zare, RN., Chen, H. *Anal Chem.*, **2021**, 93, 8, 3997–4005.

Rapid Characterization of Antibodies via Automated Flow Injection Coupled with Microdroplet Reactions and Native-pH Mass Spectrometry



2 min per sample analysis

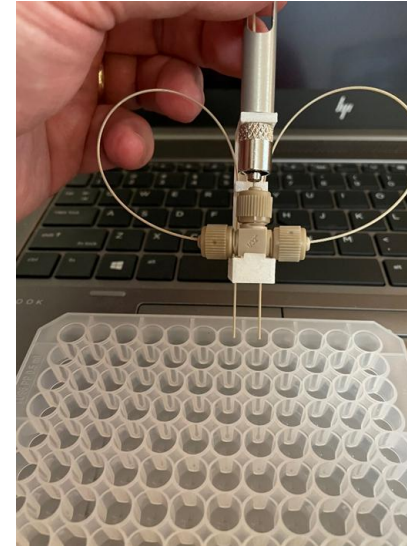


High-Throughput Microdroplet Digestion using RapidFire

RapidFire Blaze mode with dual sipper

**Flash
Characterization
with Dual Sipper
on RapidFire in
Blaze Mode**

dual sipper



**Johnson
& Johnson**

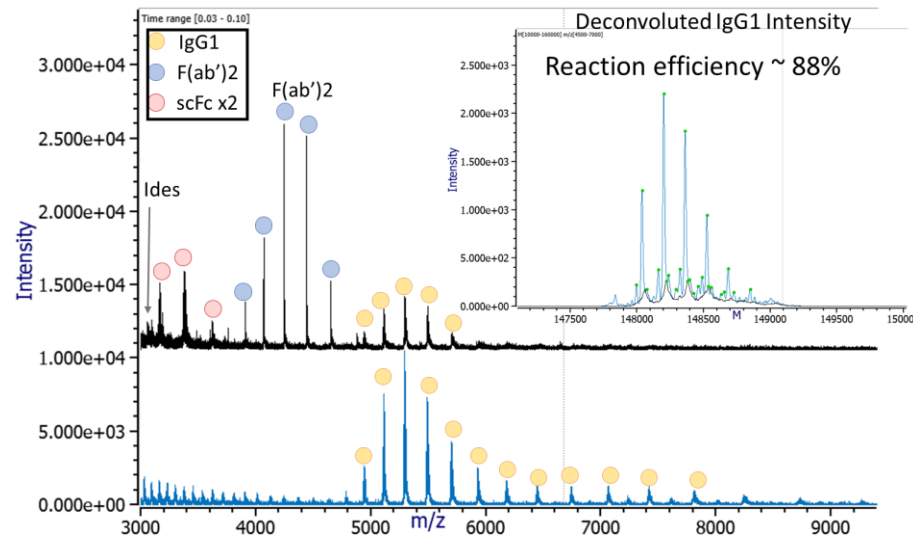


Harsha Gunawardena

 **Agilent**

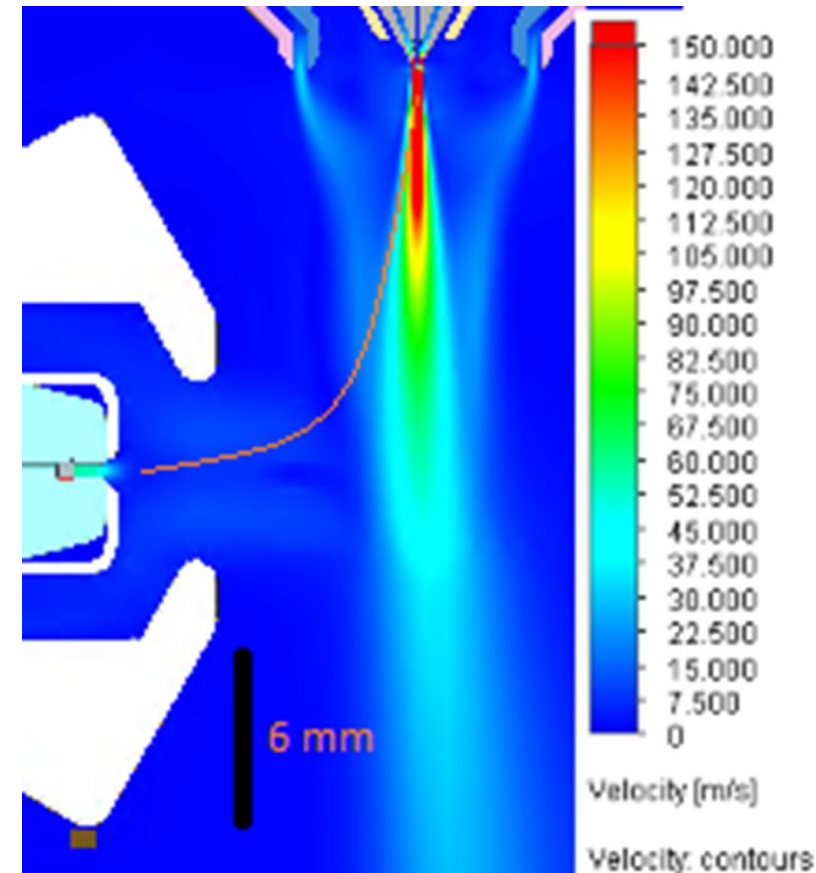
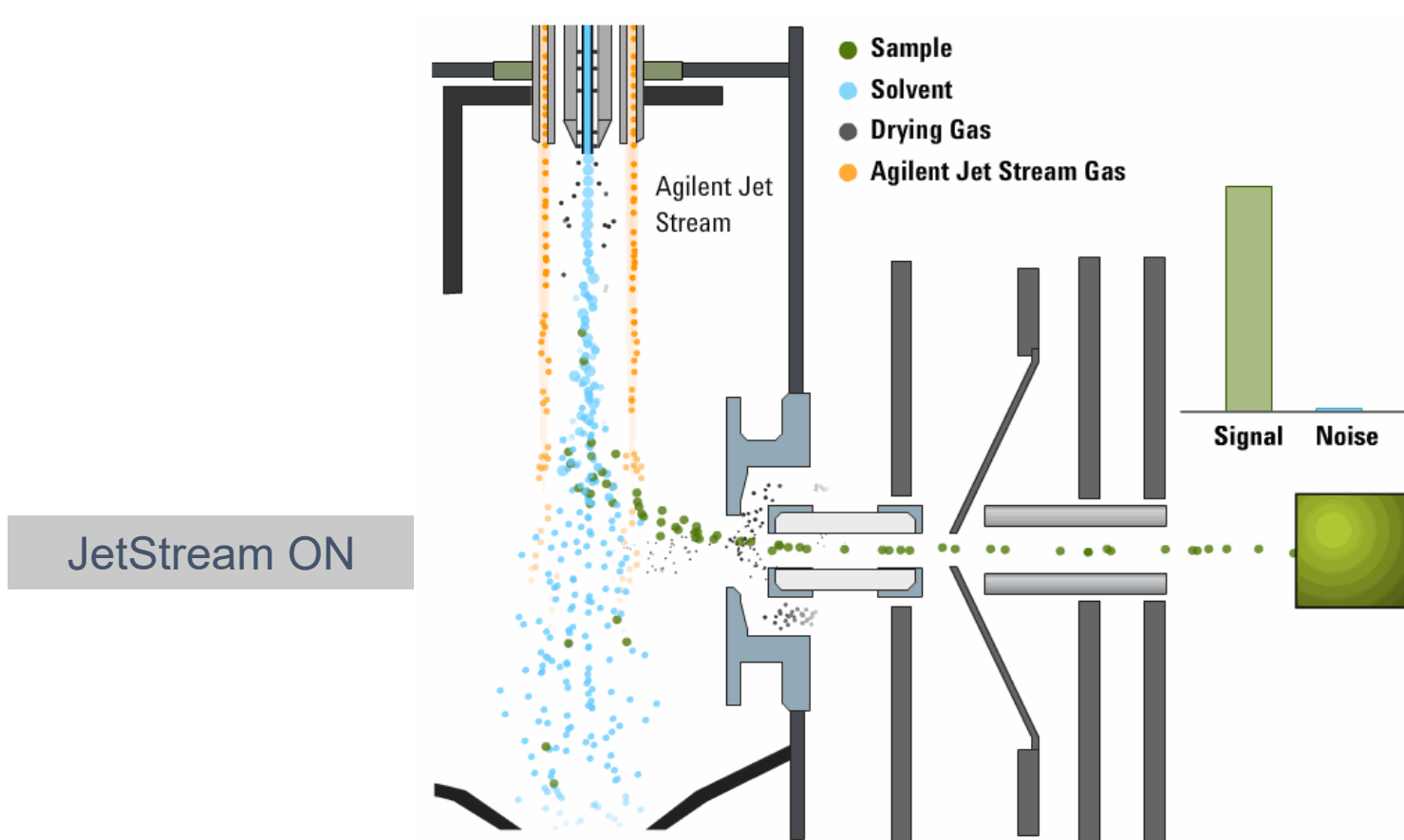


Mike Knierman



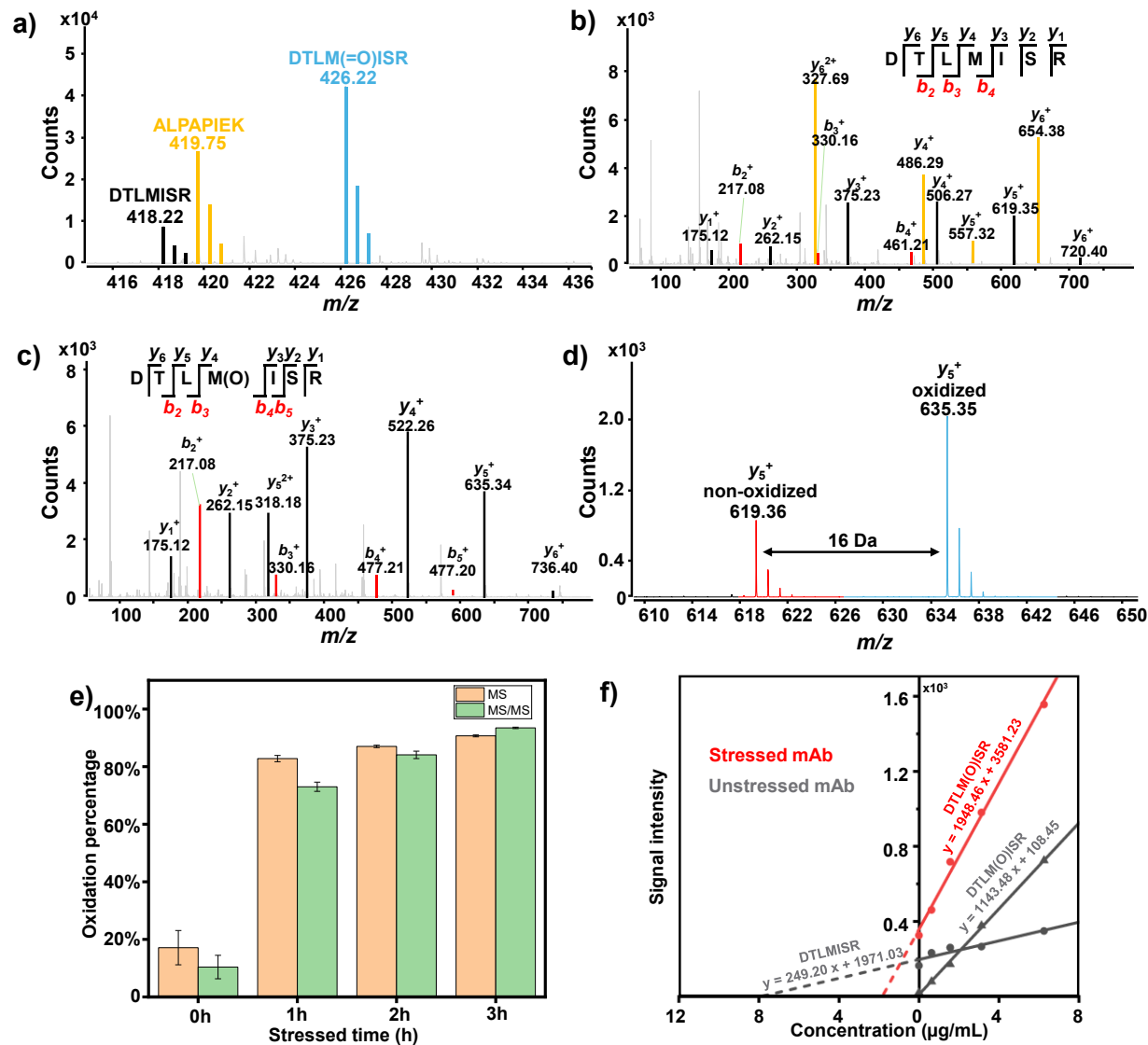
*Automation allows for versatile
sample introduction every 6 sec*

Jet Stream Source Performing Droplet Reactions



- Sub -10 mm droplets are formed within 3 mm from jet at velocity 142 m/s, travel time < 1 ms

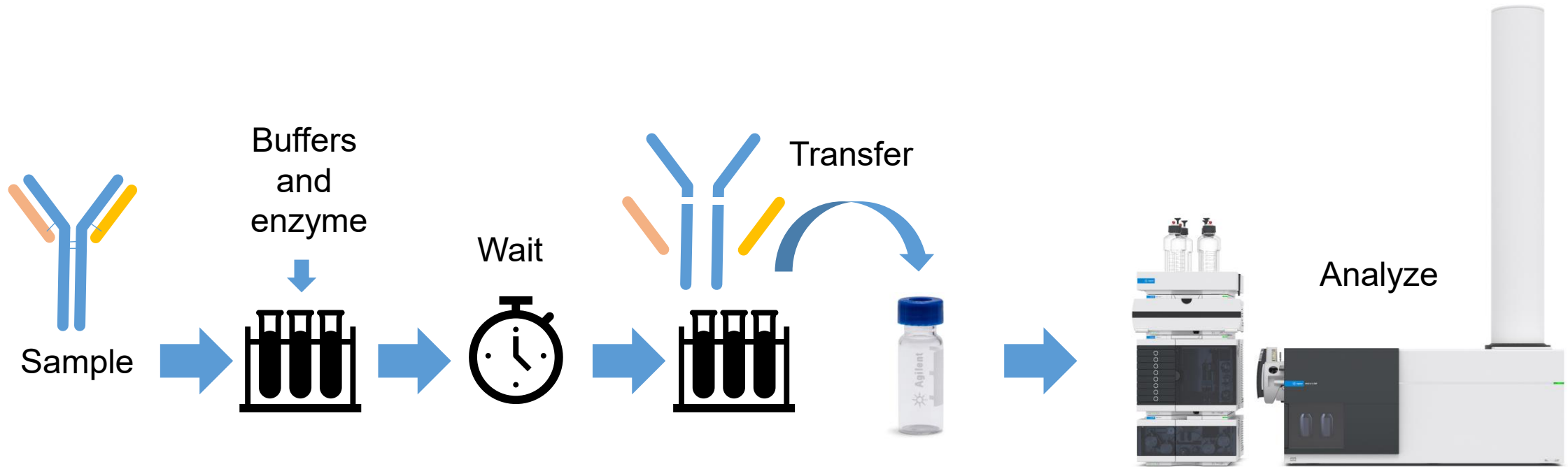
Identification and Quantification mAb Oxidation



Absolute quantification of the intact peptide DTLMISR and the oxidized DTLM(O)ISR peptide with standard-addition method!

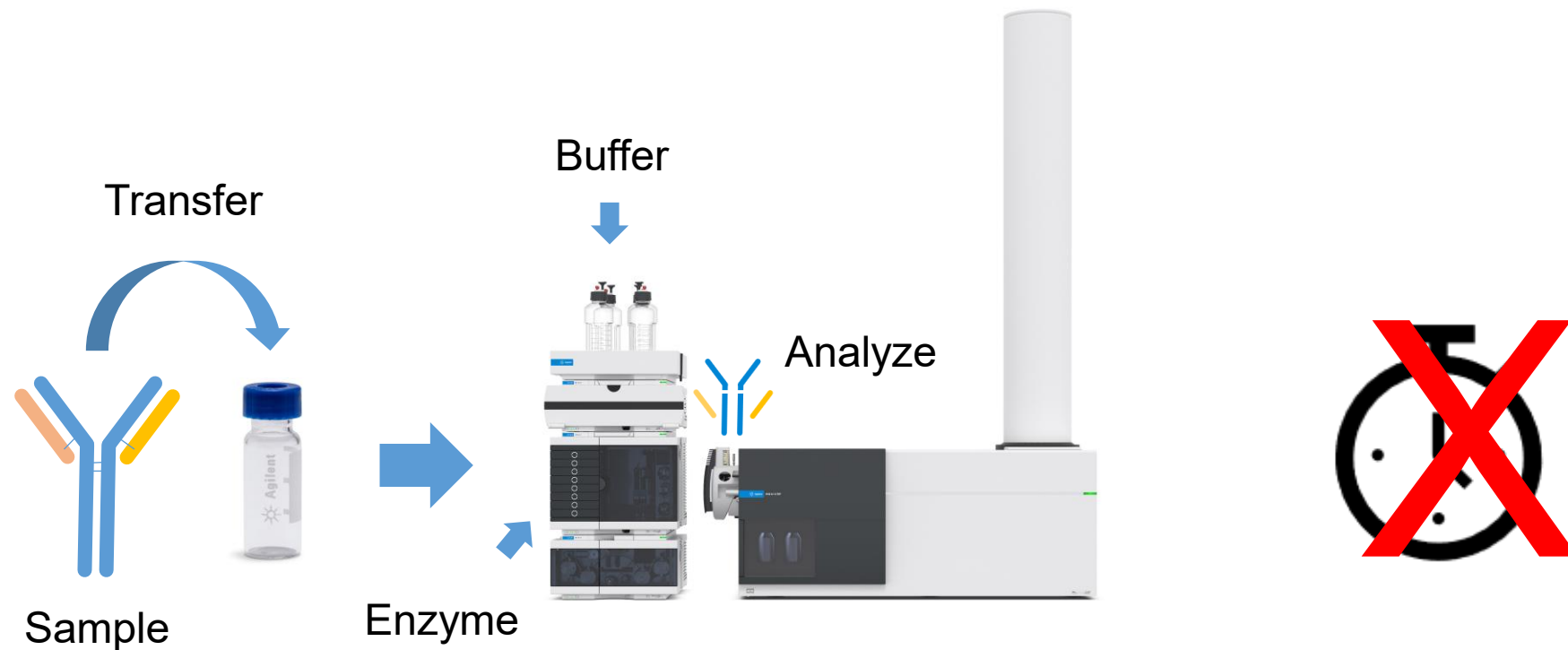
J. Am. Soc. Mass Spectrom., 2026, in press

Current Characterization Workflows



- Reactions happens in the vial in ~1 hour or more

Flash Characterization Workflows

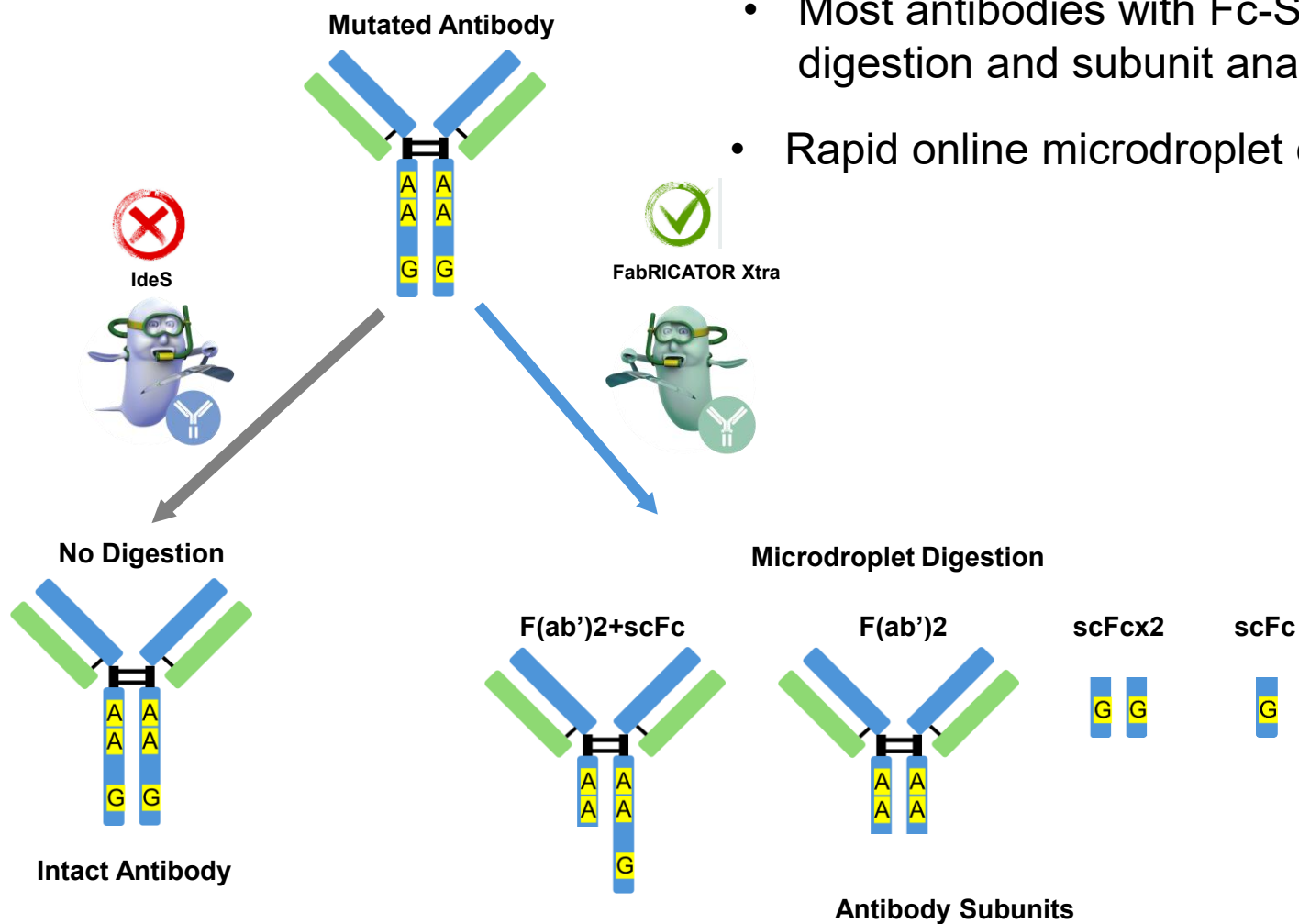


- Reaction happens in the ESI source in **microseconds**; **No HPLC separation**

Enabled by the JetStream source, extended m/z range, autosampler programming, and microdroplet reaction acceleration

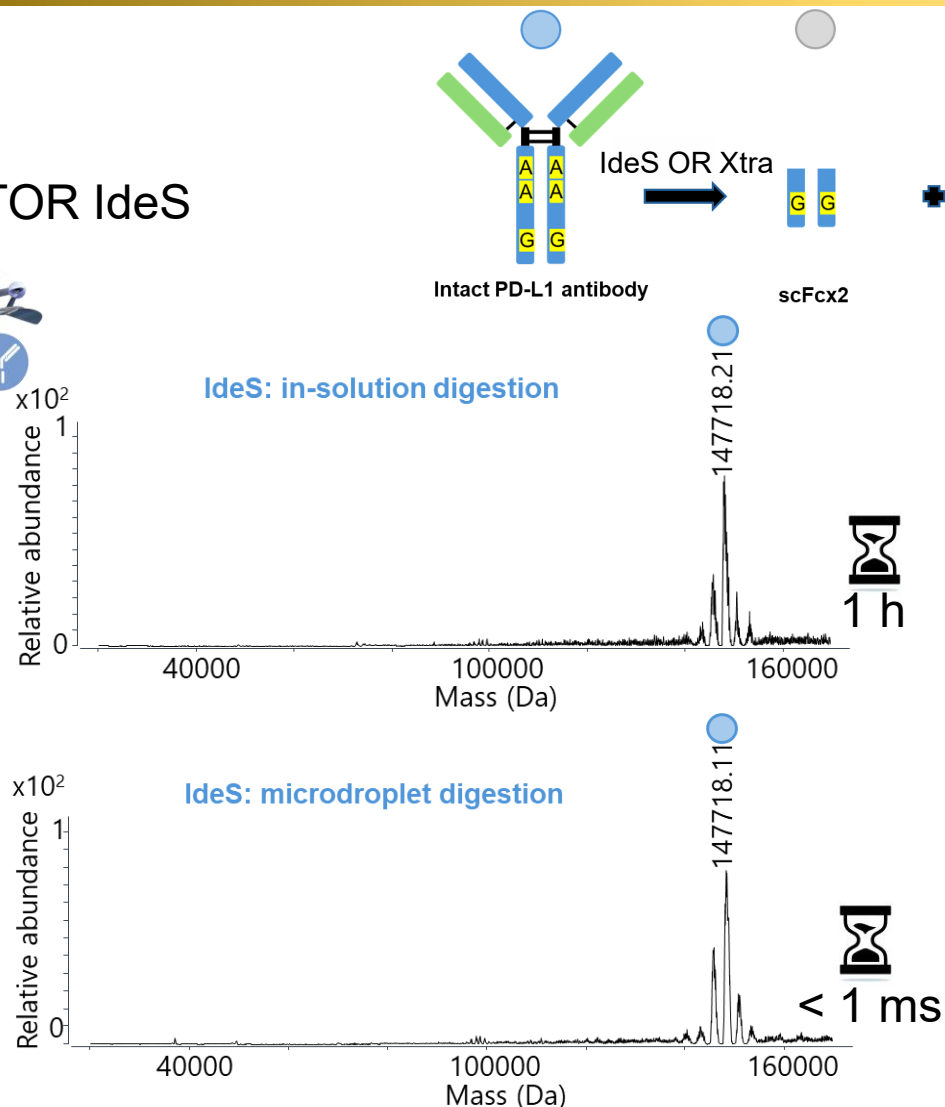
FabRICATOR Xtra Digests Mutated Antibodies in Sub-Millisecond's

- Most antibodies with Fc-Silencing mutations are **resistant** to IdeS digestion and subunit analysis
- Rapid online microdroplet digestion of Fc-Silenced mAb with Xtra enzyme



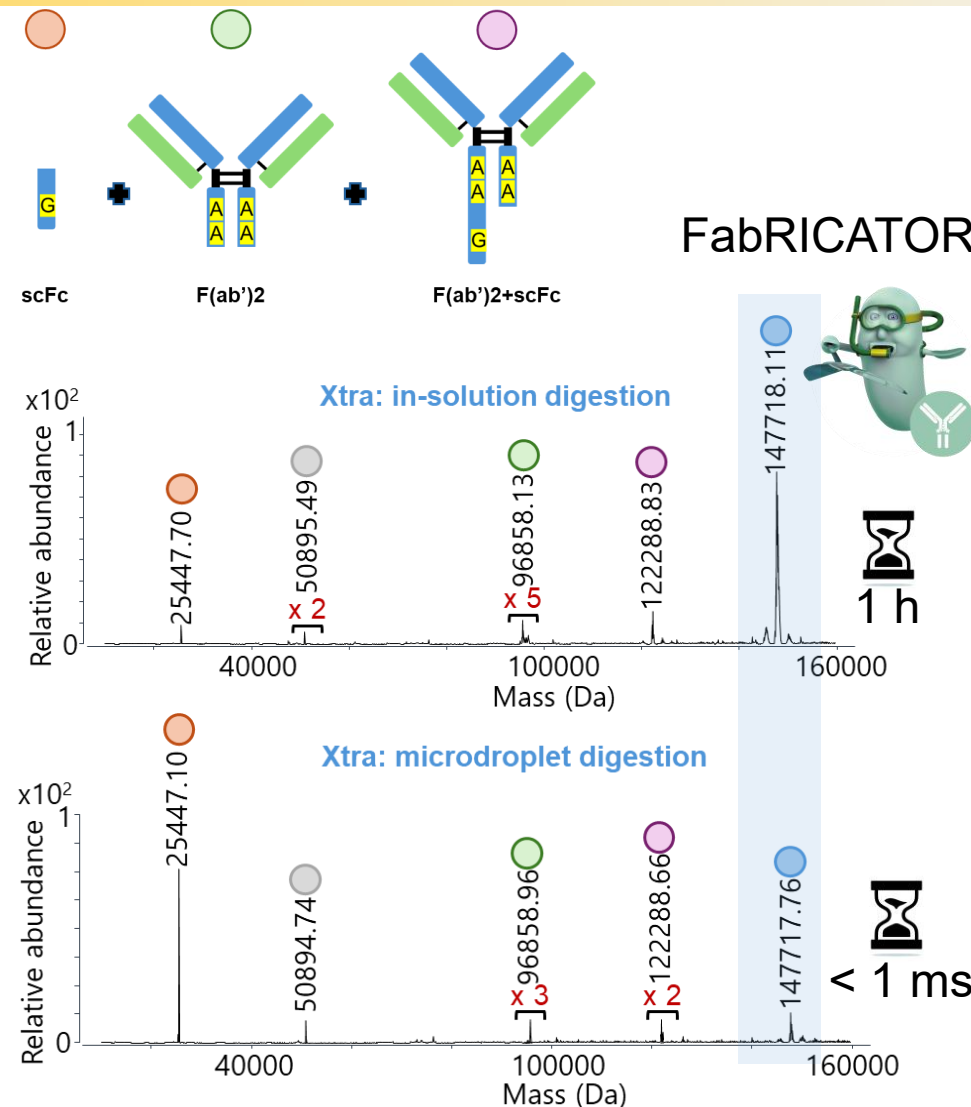
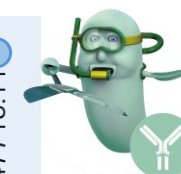
Comparing IdeS or Xtra Digestion of LALA-PG Mutated Anti-PD-L1

FabRICATOR IdeS



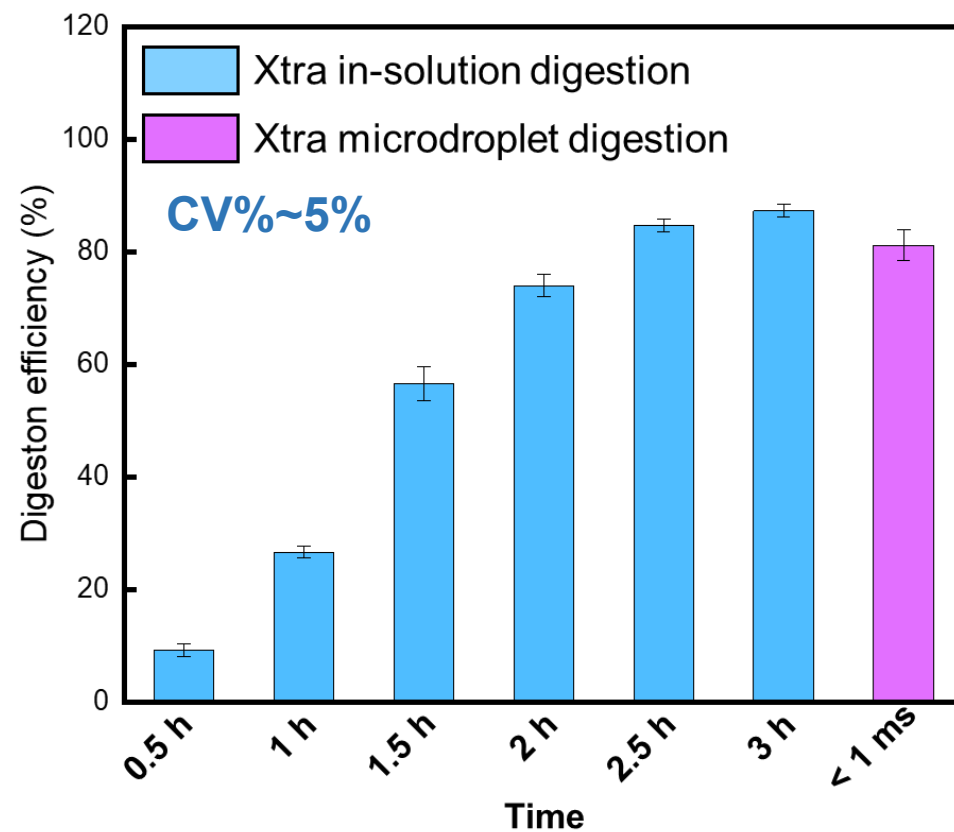
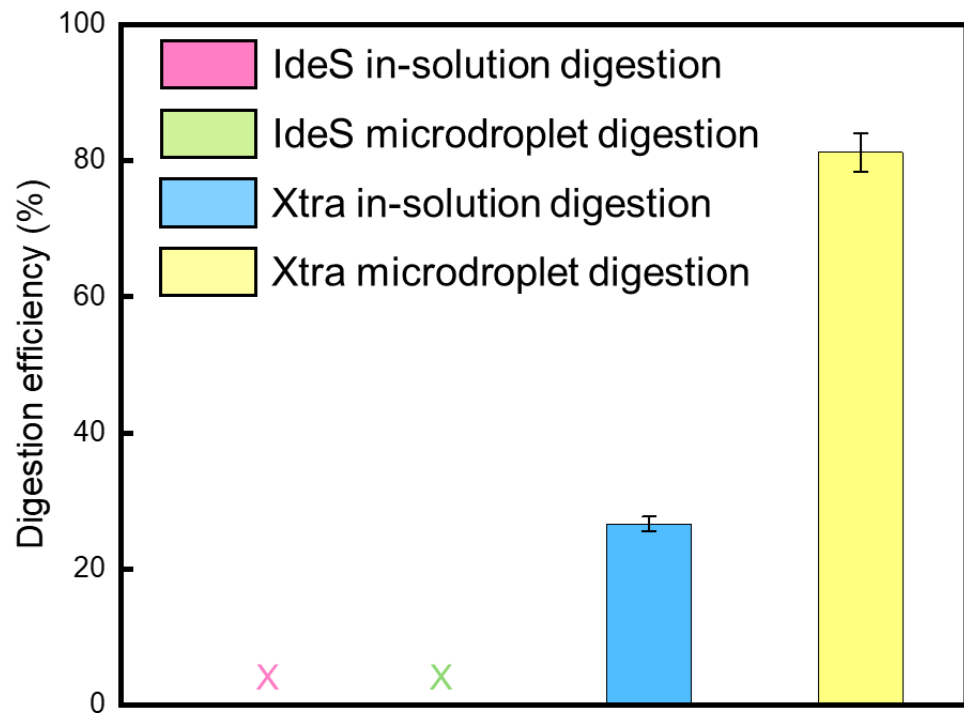
FAILED

FabRICATOR Xtra



PASSED

Comparing Digestion Efficiency of Anti-PD-L1 Antibody

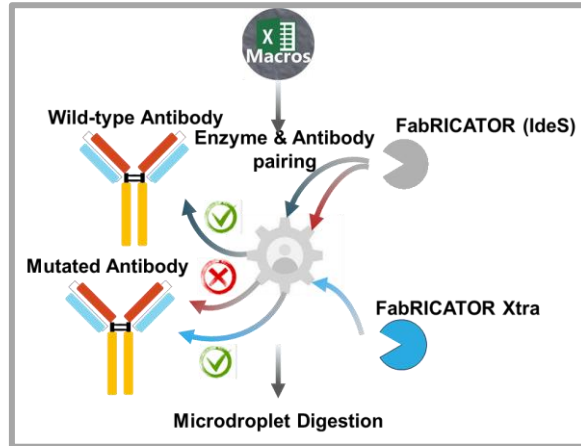


- IdeS failed digestion
- Xtra digestion efficiency: In-solution < Microdroplet

- 9×10^6 fold improvement in speed

Automated Enzyme-Antibody Selection and Microdroplet Digestion

enzyme and antibody pairing



Use script to create methods

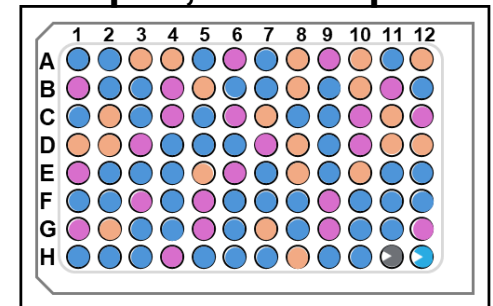
Import

Mass Hunter Worklist

Antibody 1 Method 1
Antibody 2 Method 2
▪
▪
▪
▪

programmed injections to
mix enzyme and antibody

Autosampler, 1.1 min/per sample



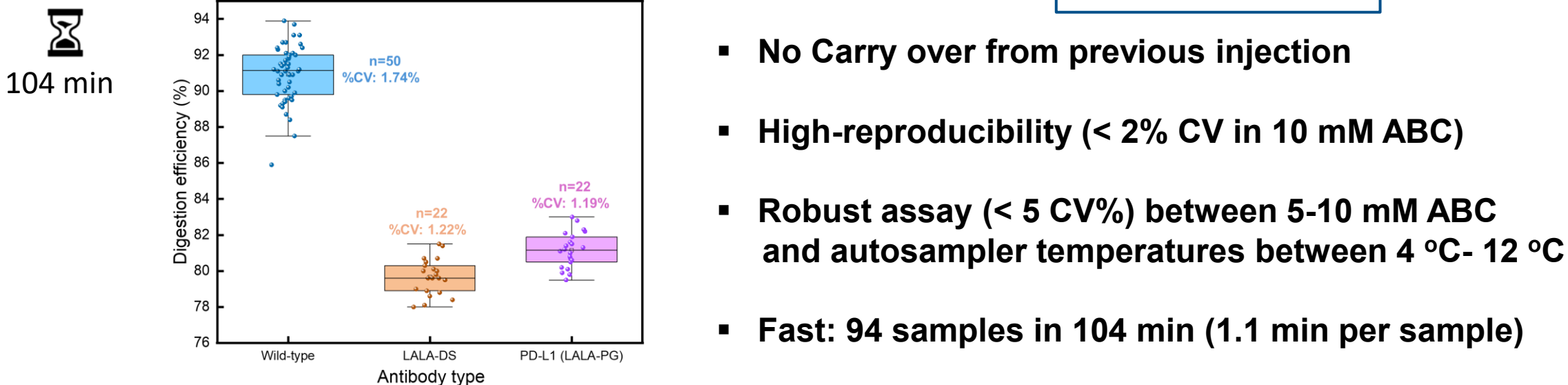
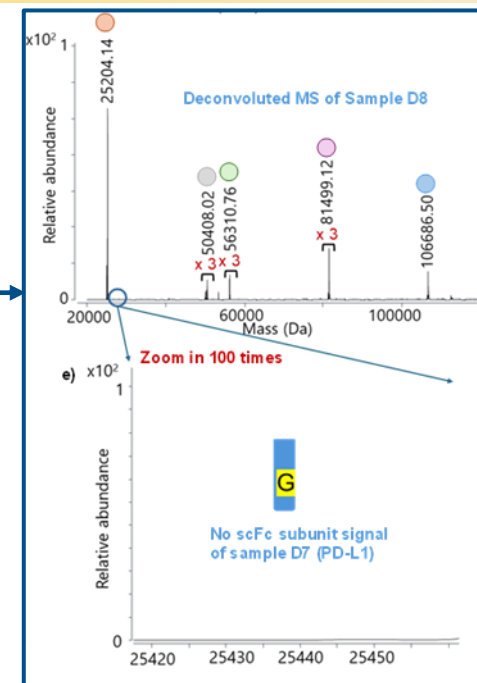
Microdroplet Digestion

FIA-MS

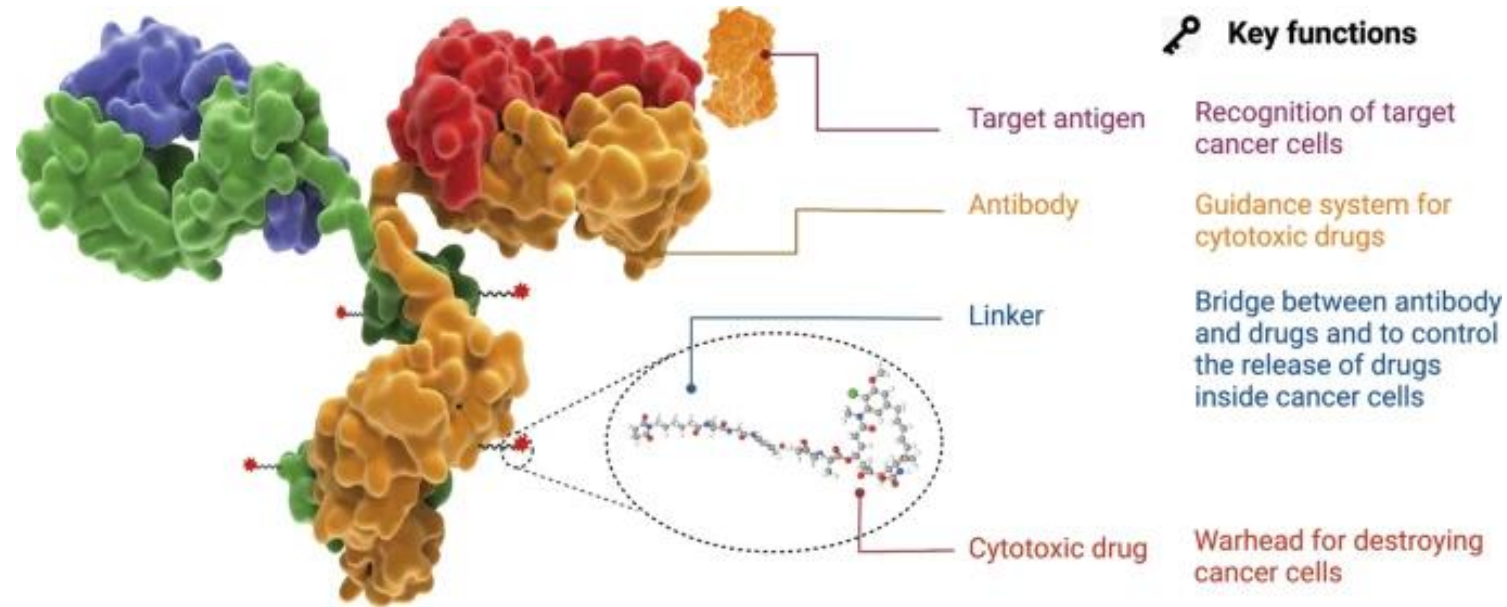
● Wild-type ● LALA-DS ● LALA-PG
● IdeS ● Xtra

High-Throughput Subunit Analysis of 96 Antibody Samples

	Wild-type: 			LALA-DS: 			PD-L1 (LALA-PG): 					
	1	2	3	4	5	6	7	8	9	10	11	12
A	92.3%	93.9%	80.5%	78.4%	89.2%	80.5%	93.1%	78.9%	82.2%	79.6%	91.2%	81.4%
B	79.8%	88.4%	92.7%	81.3%	78.0%	89.8%	91.8%	79.8%	93.7%	79.6%	81.6%	91.1%
C	85.9%	80.1%	90.9%	80.1%	87.5%	82.8%	79.5%	90.9%	90.2%	81.9%	79.0%	79.5%
D	80.0%	79.6%	81.0%	90.5%	90.9%	93.1%	82.3%	80.0%	91.5%	80.2%	78.8%	80.7%
E	80.7%	90.0%	92.0%	89.9%	80.3%	81.1%	92.6%	79.6%	91.1%	78.1%	90.6%	89.5%
F	89.3%	91.1%	81.5%	89.4%	83.0%	91.7%	89.6%	92.0%	79.9%	90.4%	92.1%	92.4%
G	80.8%	81.5%	89.1%	91.3%	82.1%	91.5%	78.6%	91.8%	80.6%	89.5%	88.7%	81.2%
H	92.4%	91.2%	91.9%	81.4%	89.7%	92.7%	91.4%	80.7%	91.5%	92.1%	IdeS	Xtra

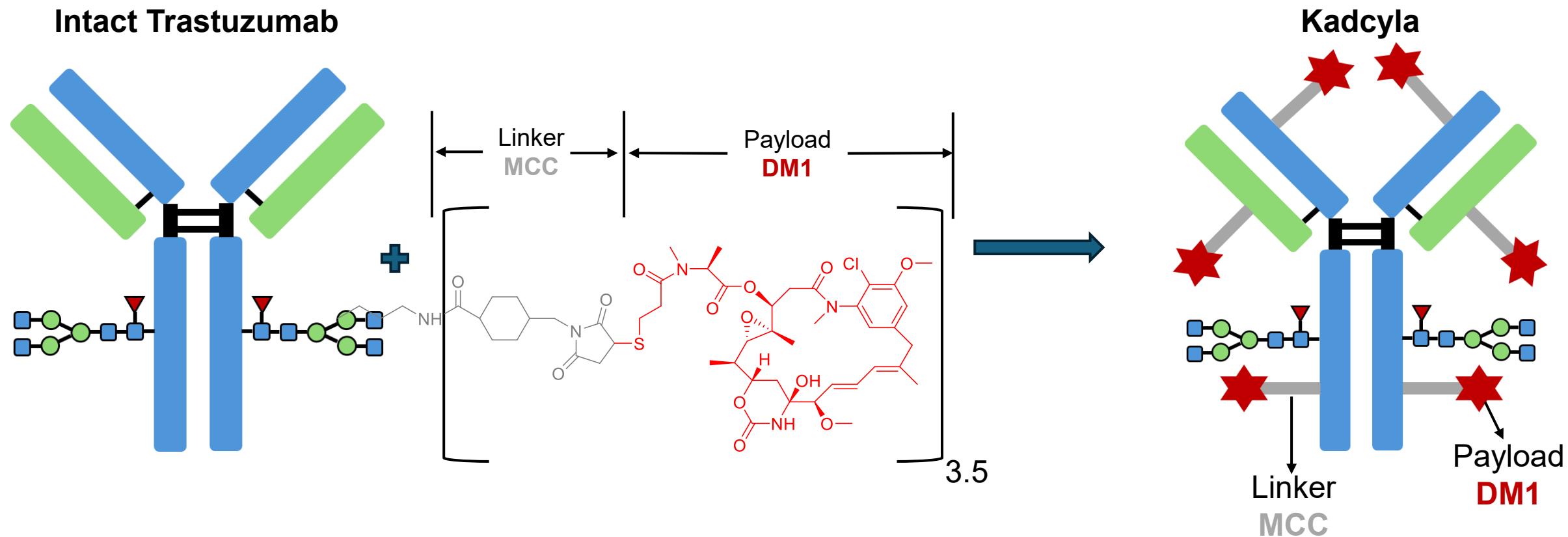


Rapid Determination of DARs in ADCs Using Ultrafast Microdroplet Digestion Technology



ADC = antibody (targeting) + drug (killing) + linker (control)

- Delivering the drug directly to cancer cells;
- The payload is usually extremely potent (active at very low doses) and this combination leads to stronger anti-tumor effects
- ADCs keep the drug inactive in circulation until it reaches the target

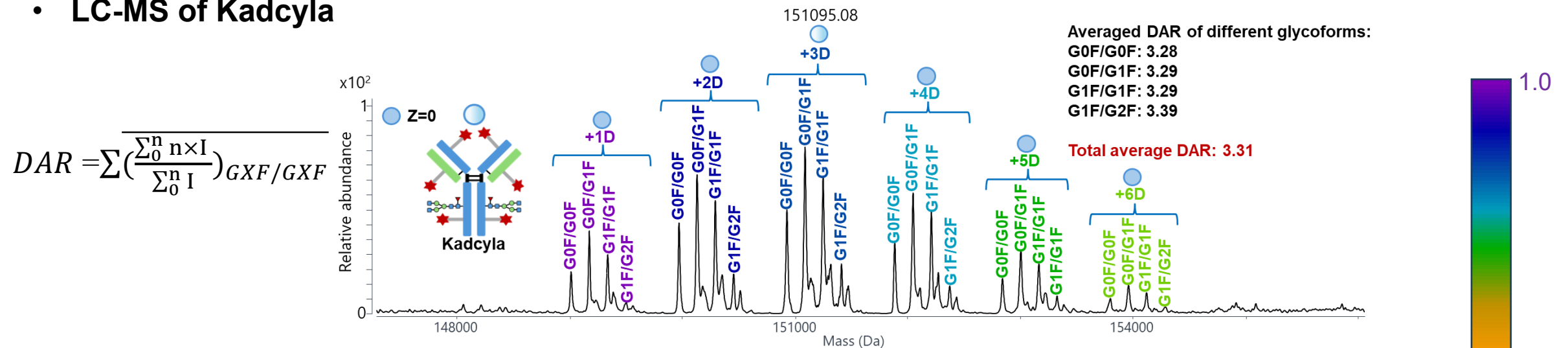


- Kadcyla is a targeted cancer medicine used to treat HER2 positive breast cancer

Theoretical DAR: 3.5

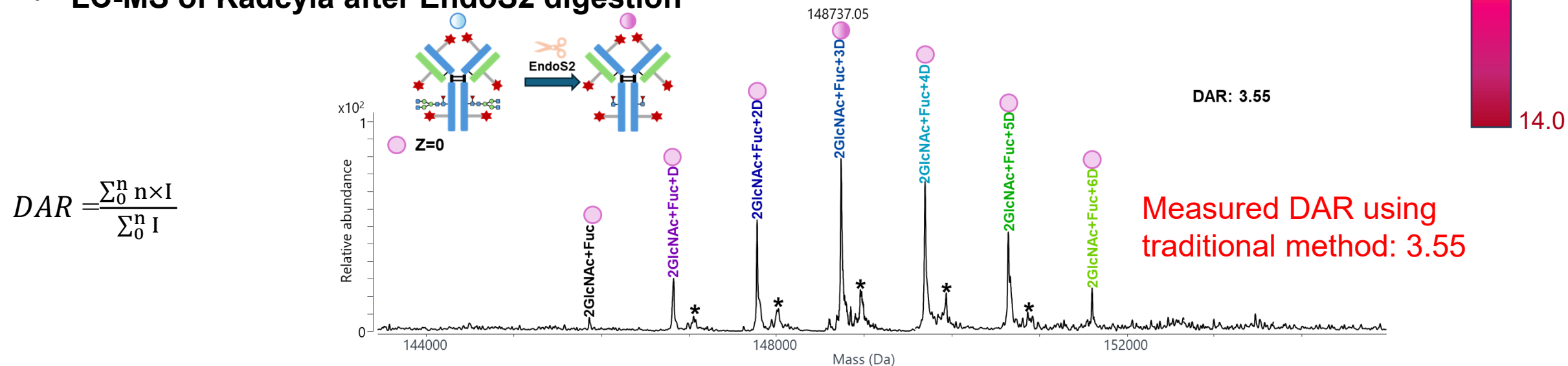
Glycoform Heterogeneity Affects DAR

• LC-MS of Kadcyra

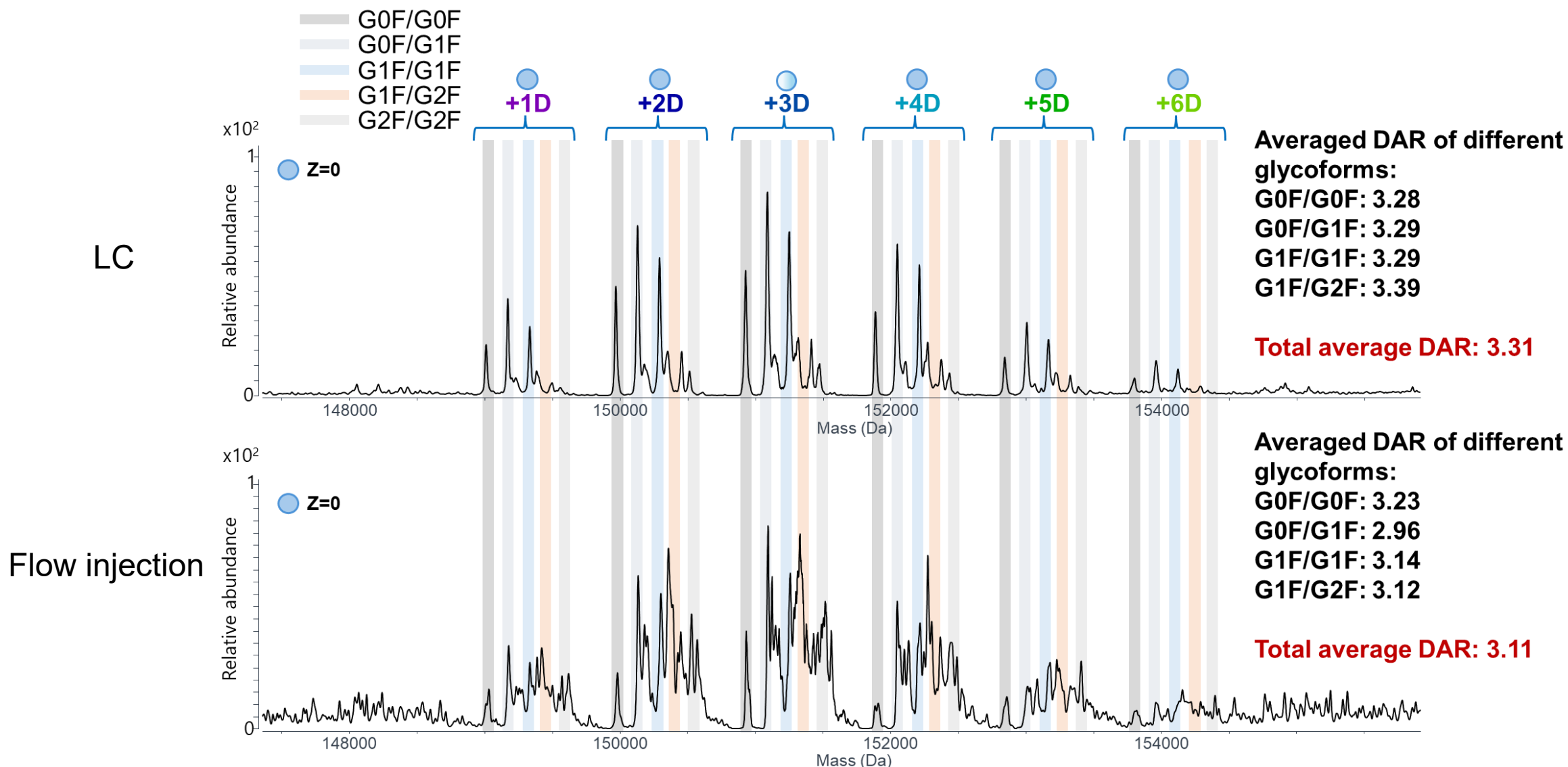


- Glycoforms may affect the DAR sensitivity
- LC separation needs long time

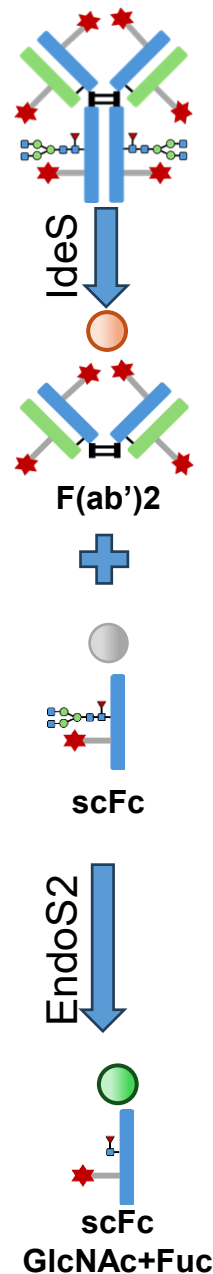
• LC-MS of Kadcyra after EndoS2 digestion



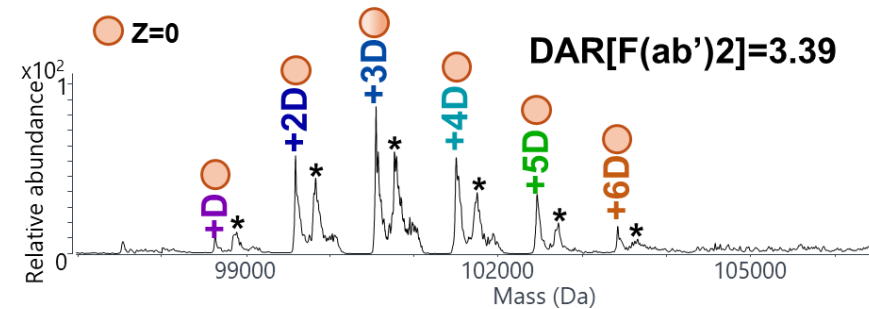
- Enzyme in-solution digestion and LC separation are time-consuming



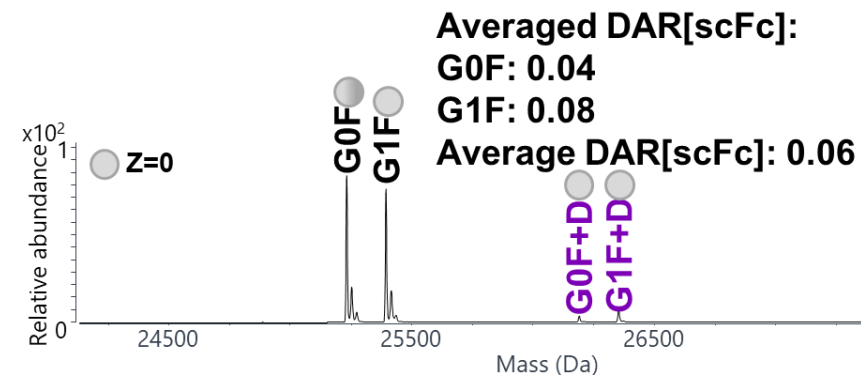
- Flow injection is necessary for the microdroplet reactions,
- DAR values calculation is challenging due to the quality of the spectra



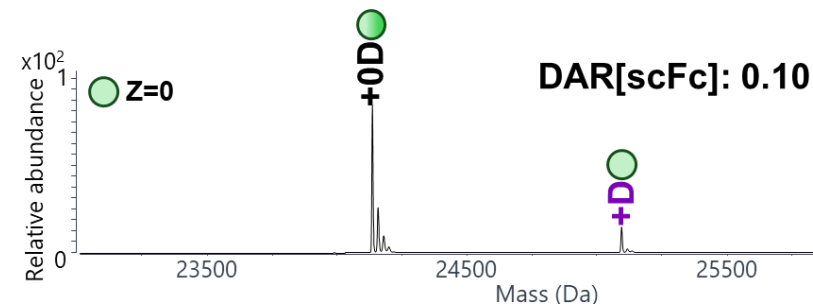
$$DAR = \left(\frac{\sum_0^n n \times I}{\sum_0^n I} \right)_{F(ab')_2} + 2 \left(\frac{\sum_0^n n \times I}{\sum_0^n I} \right)_{scFc} = 3.51 \text{ and } 3.68$$



- * --MCC linker without payload



DAR=3.51

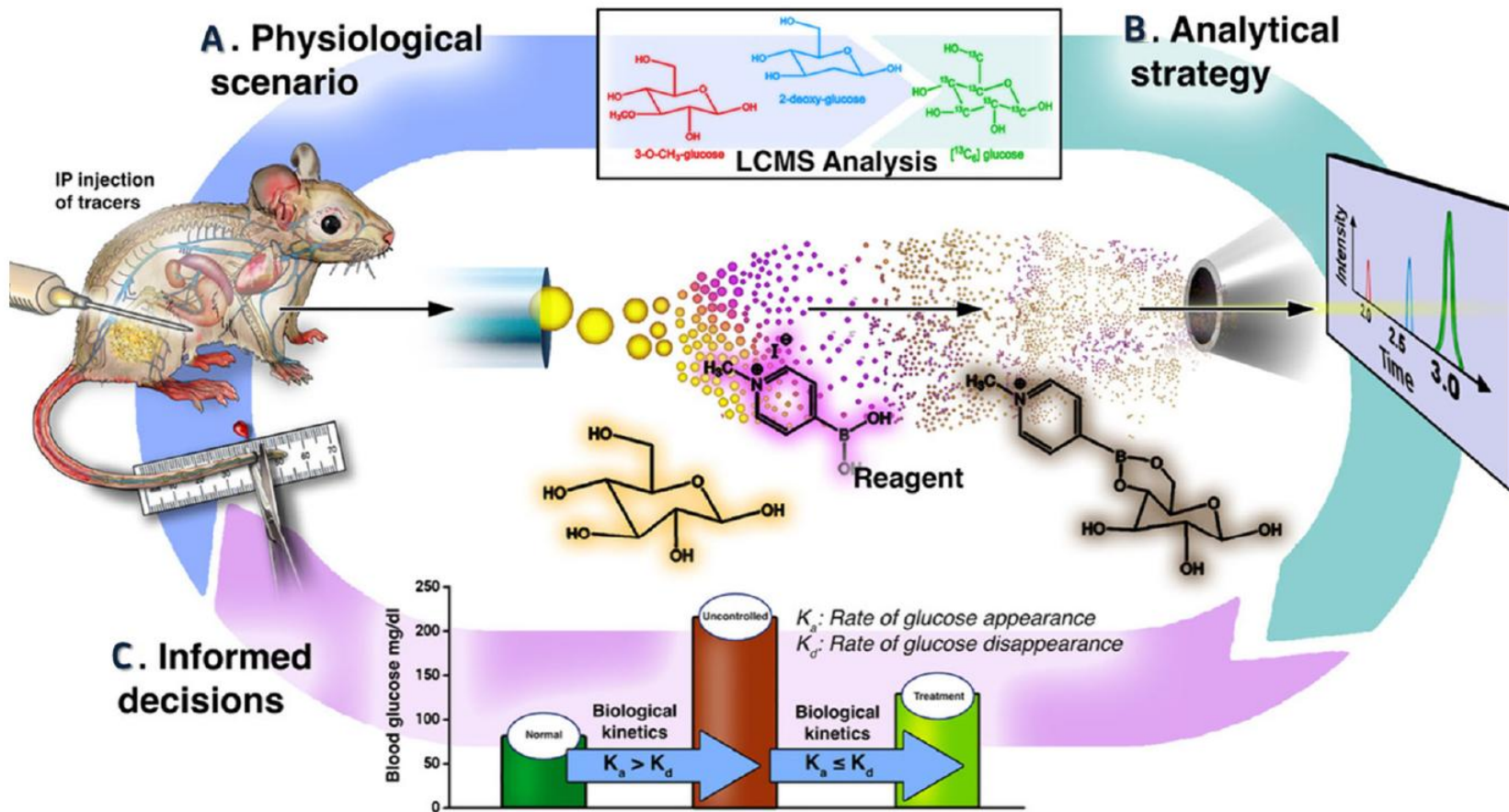


DAR=3.68

bioRxiv:

<https://www.biorxiv.org/cgi/content/short/2026.06.02.729562v1>

Microdroplet Mass Spectrometry Enables Rapid and Sensitive Measurement of Glucose Homeostasis for Elucidating Mechanism of Drug Action



Challenges for metabolite analysis

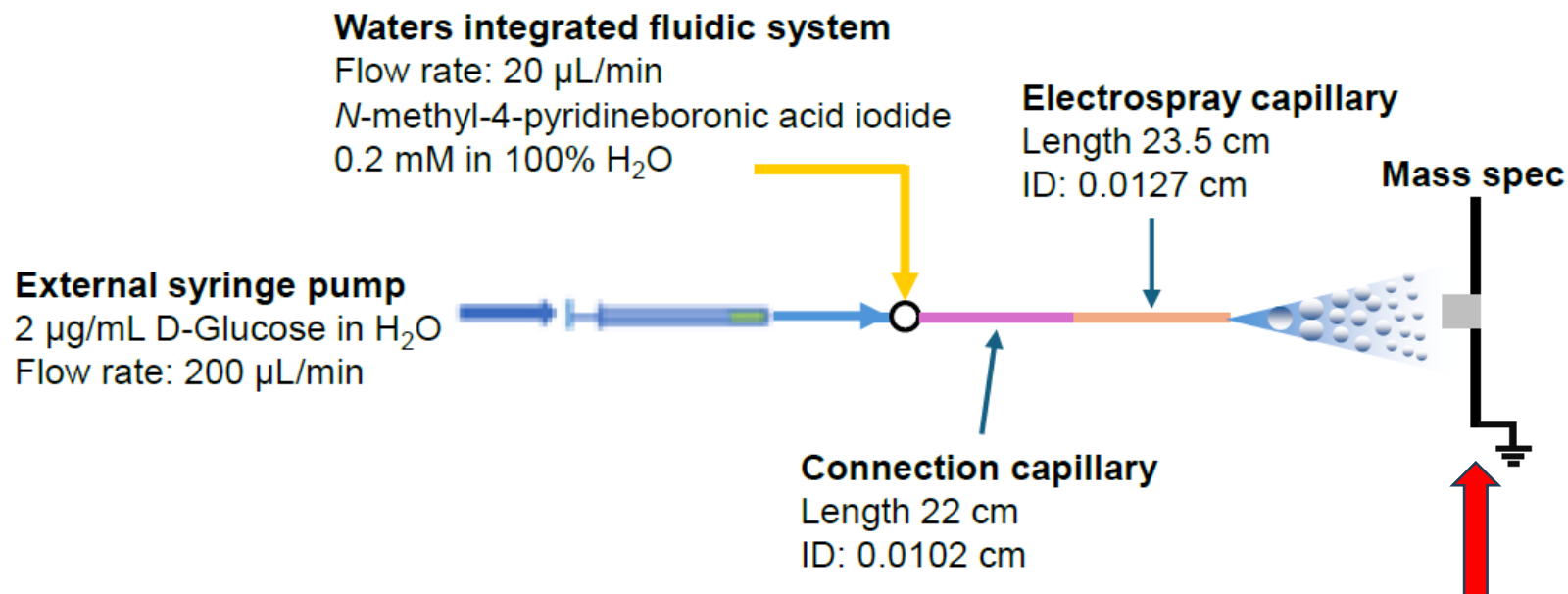
Small blood volume (5 μ M)

Low signal of glucose metabolite signal

Collaboration with Huifang Yao & Stephen F. Previs



a) Experimental setup for reaction scouting using TOF tune acquisition



Online derivatization during spray

In contrast, traditional GC/MS analysis with offline acetylation derivatization takes 4h for sample preparation

Huifang Yao, Dan Zhou, Sophie VanSickle, Haihong Zhou, David G. McLaren, Hao Chen* and Stephen F. Previs*, *Analytical Chemistry*, 2025, 97, 19480–19488

Conclusions

- Microdroplet enzymatic digestion of antibody, mutated antibody and ADCs can complete in less than 1 ms
- In combination with flow injection MS analysis, each sample can be analyzed within 1 min including data acquisition for 20s, data analysis for 40s.
- Microdroplet digestion is highly efficient > 80% and highly reproducible %CV < 5%
- High-Throughput antibody subunit analysis via microdroplets is possible: ~ 1.1 min per sample
- Microdroplets also enable one-pot TCEP reduction and enzymatic digestion > 80% efficiency
- Microdroplet also enables online LC/MS derivatization for small molecule analysis, streamlining the whole analytical processes

Acknowledgement

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Mengyuan Xiao, Yongqing Yang



**Johnson
& Johnson**



Harsha Gunawardena

- Agilent Technologies**

Jim Lau
Mike Knierman
Hui Zhao
Xi Qiu
Karen Luo
John Sausen



- Funding**



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