

LC–MS–Based Next-Generation Proteomics for Identification and Control of High-Risk HCPs in Biotherapeutics and Vaccines

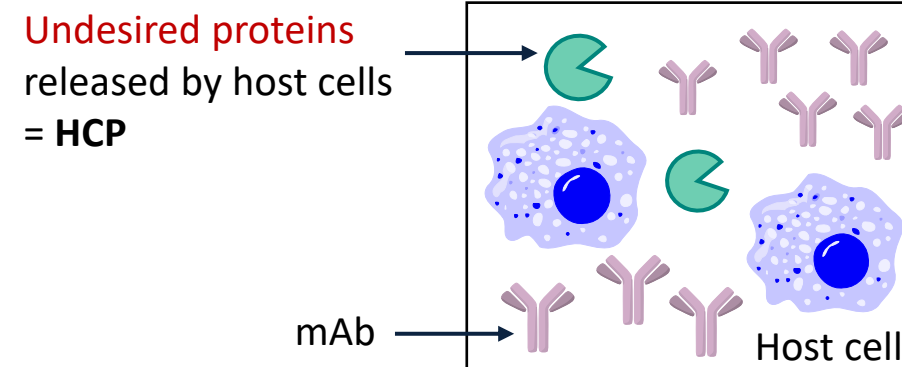
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High-risk HCPs threaten product safety and efficacy

- HCPs are process-related impurities derived from host organisms (e.g., Chinese Hamster Ovary cell (CHO) or E. coli)
- Certain residual high-risk HCPs have potential adverse effects for both safety and efficacy of bioproducts
 - Immunogenicity: PLBL2, RPI
 - Acute toxicity: MCP-1, TGFβ
 - Product stability: Cathepsin D, **HEXB**
 - **Excipient stability**: LPL/**PLA2G7**/SIAE/IAH1
- Compared to immunoassay ELISA, LC-MS based proteomics provides identification and relative quantification of individual and total HCPs



REVIEW

BioPhorum

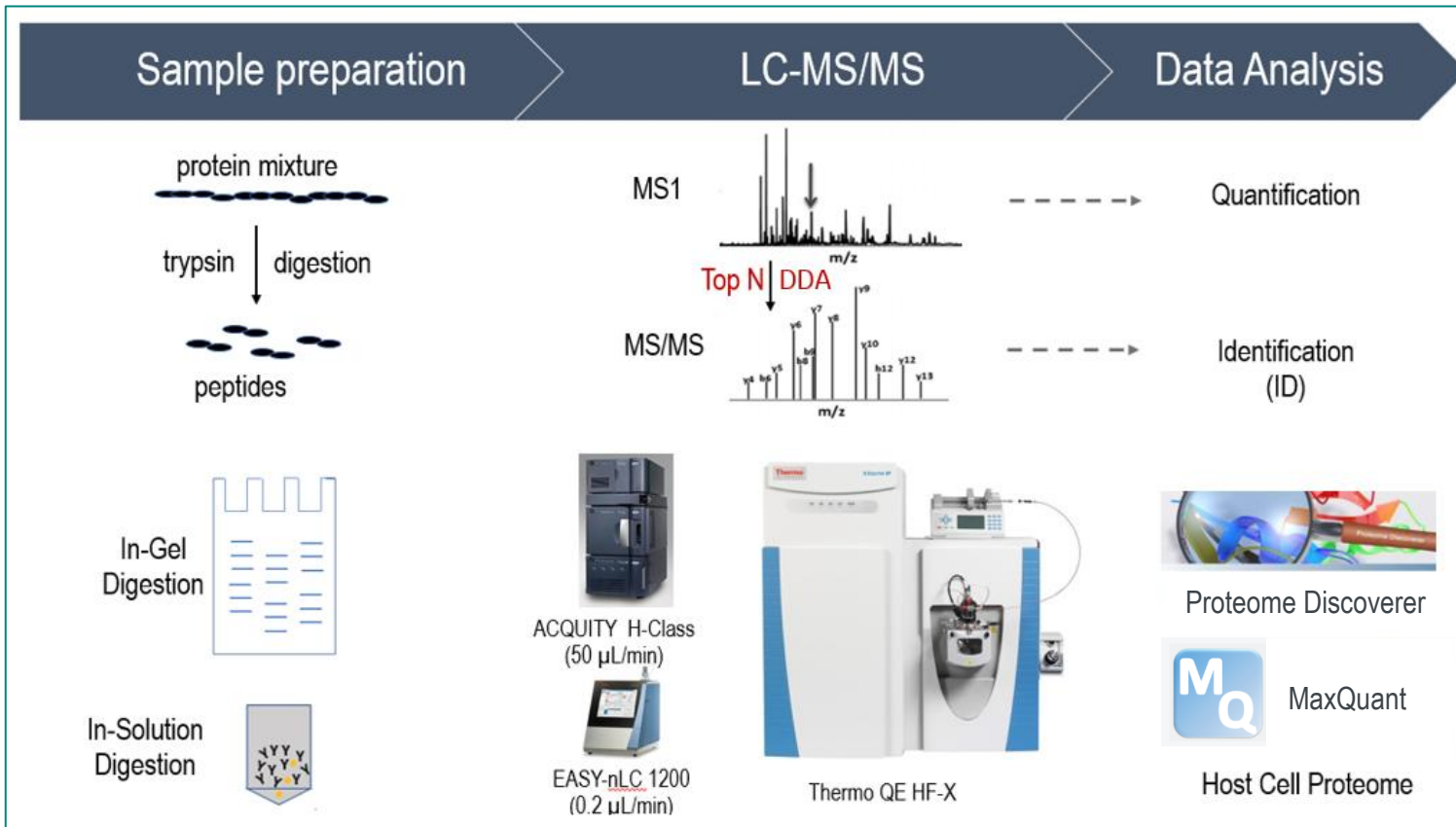
BIOTECHNOLOGY
BIOENGINEERING
WI

“High-risk” host cell proteins (HCPs): A multi-company collaborative view

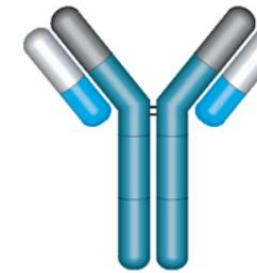
Marisa Jones¹ | Nisha Palackal² | Fengqiang Wang³ | Georgeen Gaza-Bulseco⁴
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Suli Liu⁸ | Emily Menesale⁸ | Nicole S. Schonenbach⁹ | Satish Sharma¹⁰ |
Pascal Valax¹¹ | Thomas Waerner¹² | Lei Zhang¹⁰ | Trish Connolly¹³

Jones M, et al., *Biotechnol Bioeng.* 2021;118(8):2870

TABP (Traditional Abundance-Based Proteomics): one LC-MS platform for HCP characterization across modalities with widely available instruments

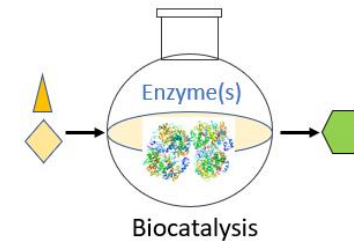


DDA: data-dependent acquisition

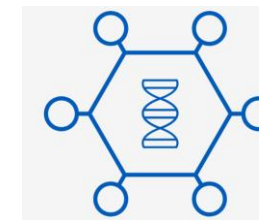


Biologics

- Gao X, et al., *Anal Chem.*, 2020;92(1):1007-1015
- Li X, et al., *Biotechnol Prog.*, 2021; e3128
- Li Z, et al., *Biotechnol Bioeng.* 2023;120(7):1902
- Osko JD et al., *Anal Chim Acta* 2024;1332



- Wang F, et al., *Biotechnol Bioeng.*, 2022;119(8):2088

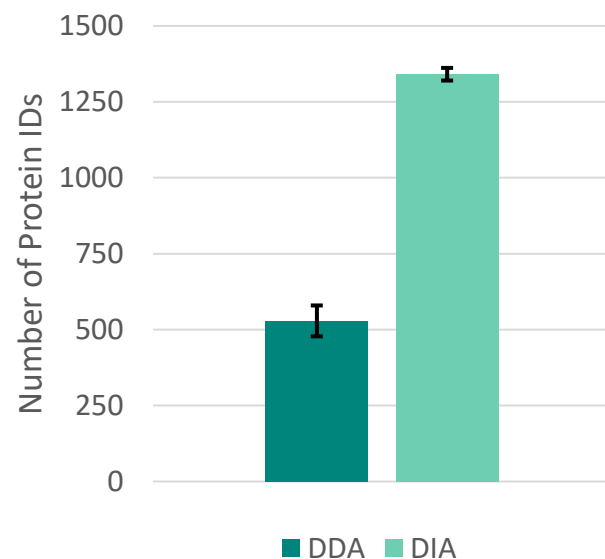


Vaccines

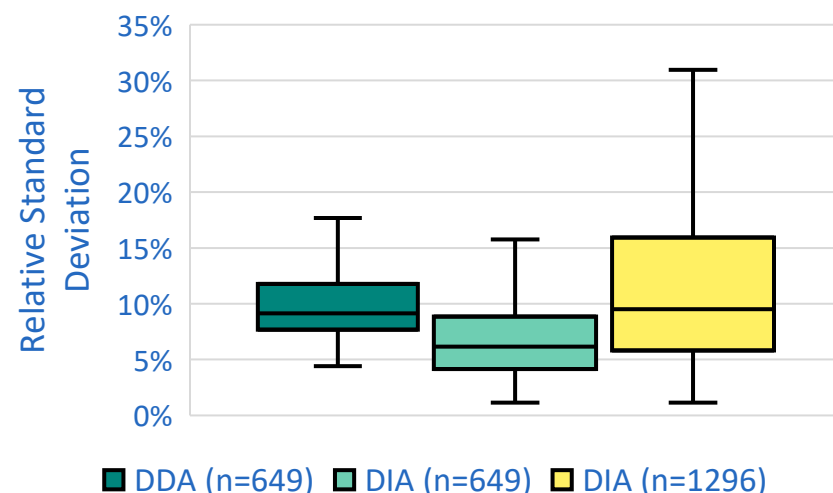
- Konstantinidis S, et al., *Biotechnol Bioeng.* 2024 ;121(8):2482.
- Gillespie PF, et al., *Vaccines.* 2024;12(12): 1373

DIA based proteomics improves assay performance with widely available LC-MS instruments

Protein Identifications



HCP Repeatability



- Acquity LC/ Q-Exactive
- Live virus vaccine

Assay Parameter	Results
Linearity	$R^2 > 0.98$
Linear Range	20-4000 ng/mL
Accuracy	73%
Repeatability	Median RSD < 12%
Intermediate Precision	Median < 15%

- Ongoing effort pushing the LC-MS based proteomics assay in replacement of ELISA as the **primary characterization assay** for advanced vaccine modalities, such as live virus vaccines
- LC-MS based proteomics assay is qualified as an **extended characterization assay** for vaccine **PPQ** and **GMP** batch testing

High-throughput (HT) DIA: Balancing throughput and sensitivity for process development

- Standardization
- Throughput
- Robustness

Evosep One LC



TimsTOF Pro2 MS

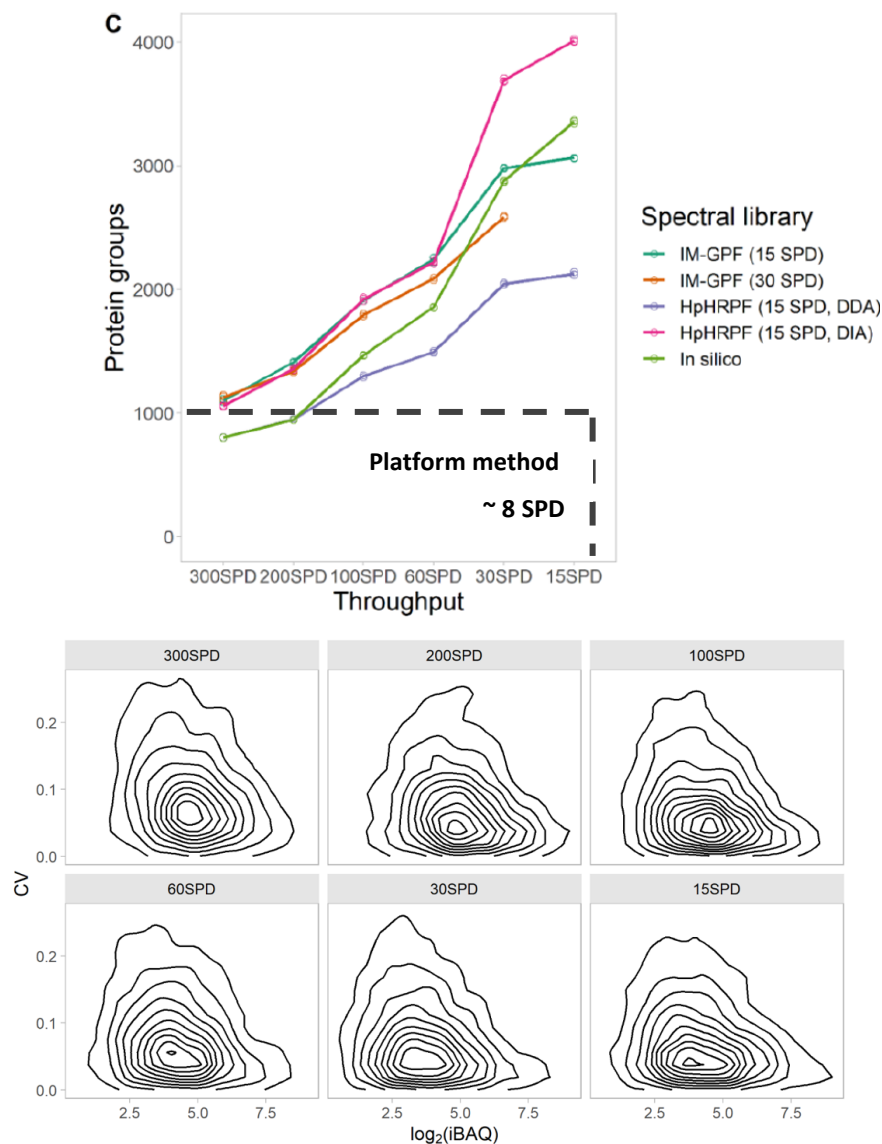


- Sensitivity
(**single cell**)
- Scan speed
(~200 Hz)

Astral MS



HT-DIA: ~1800 HCPs at 100 runs per day



High throughput

Up to **100** runs in **24 h**

High data quality

Sample pool median CV ~ **6 %**

Sample pool Pearson's r > **0.97**

Deep profiling/Sensitivity

HCCF:

~ **1800** HCPs quantified

(double HCP # vs in-house platform method)

PAP:

~ **500** additional HCPs identified

(vs in-house platform method)

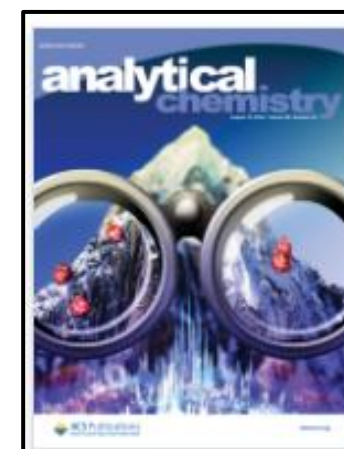
IM-GPF: ion mobility-coupled gas-phase fractionation

HpHRPF: high pH reversed-phase fractionation

SPD: samples per day

HCCF: harvested cell culture fluid

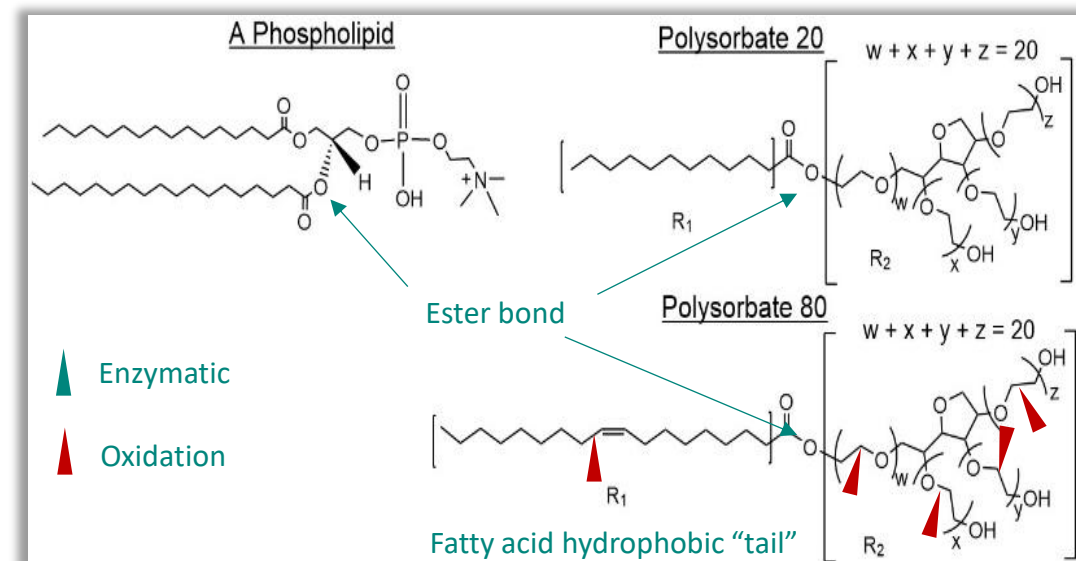
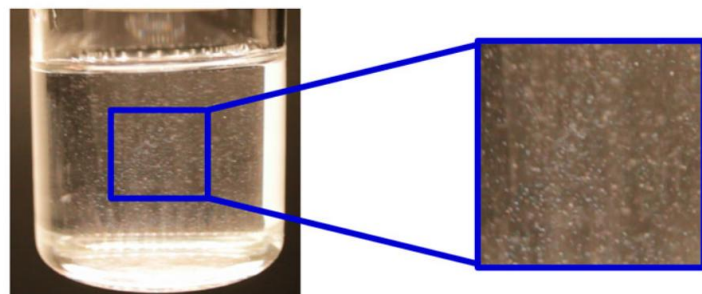
PAP: protein-A pool



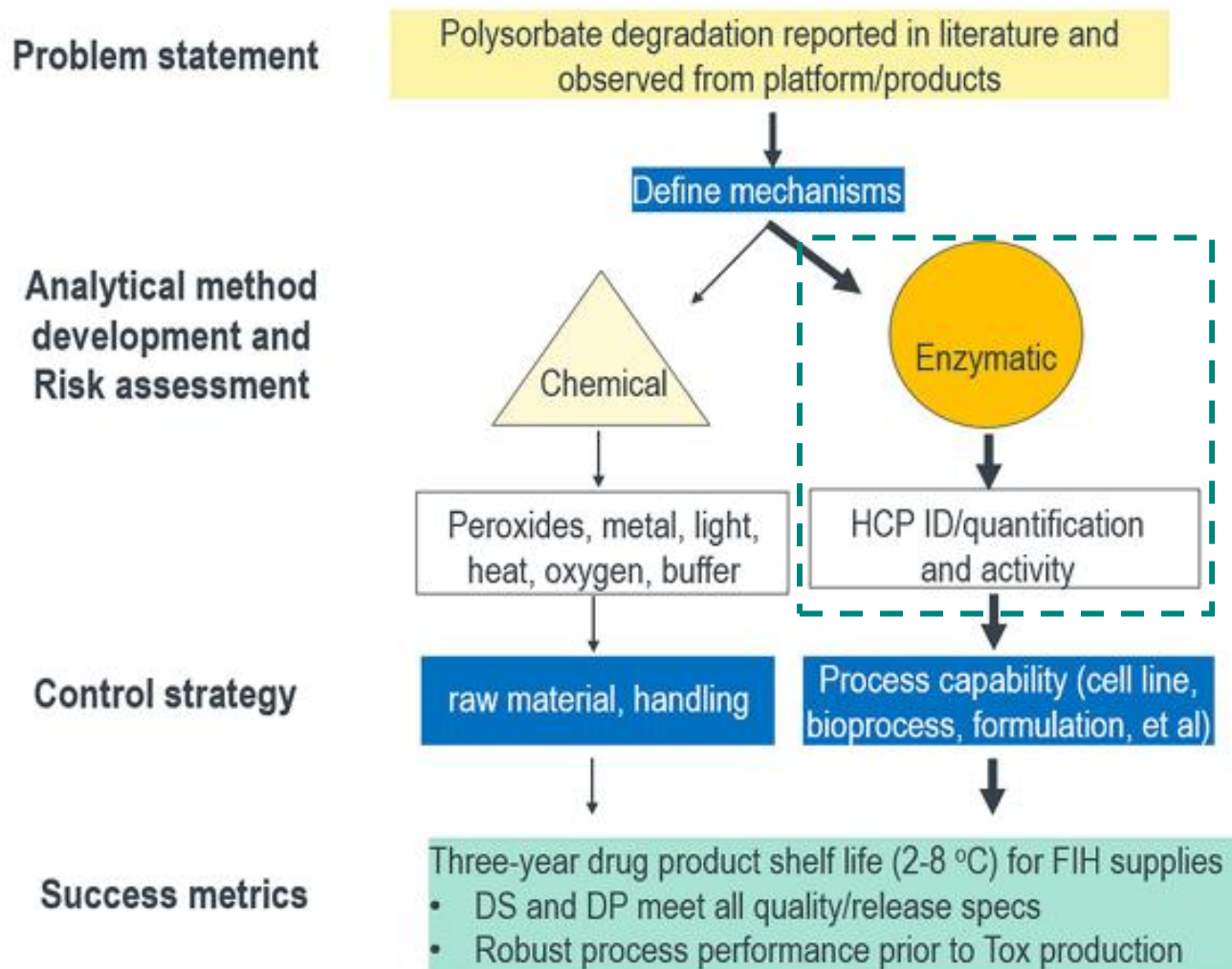
Tsukidate T, et al., **Anal Chem.** 2024;
96(32):12999

HCP and Polysorbate Degradation

- Polysorbates (PS or Tween, 20/80) are widely used in biopharmaceutical protein formulations to maintain protein stability
- PS is susceptible to degradation by oxidation and **enzymatic hydrolysis**, especially with liquid formulation
- PS degradation by high-risk host cell Polysorbate-Degrading Enzymes (**PSDEs**), such as lipases, can lead to **subvisible or visible particle formation**, potentially reducing product **shelf life**
- An increased industry-wide challenge for
 - 1) **New modalities** without affinity purification
 - 2) **Higher titer** for cell culture
 - 3) **Higher concentration** for formulation



A staged strategy for identification and control of PS degradation



Sensitivity and activity gaps limit PS degradation investigation

Sensitivity

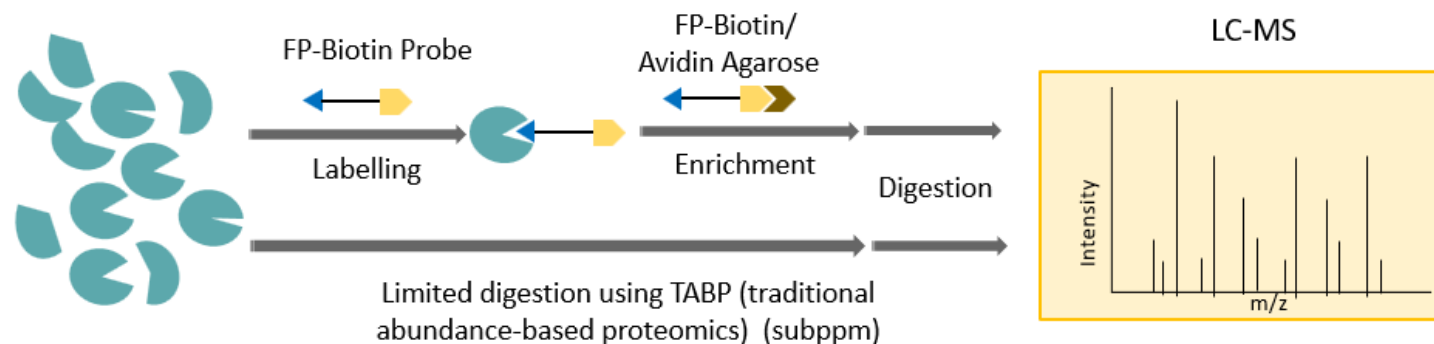
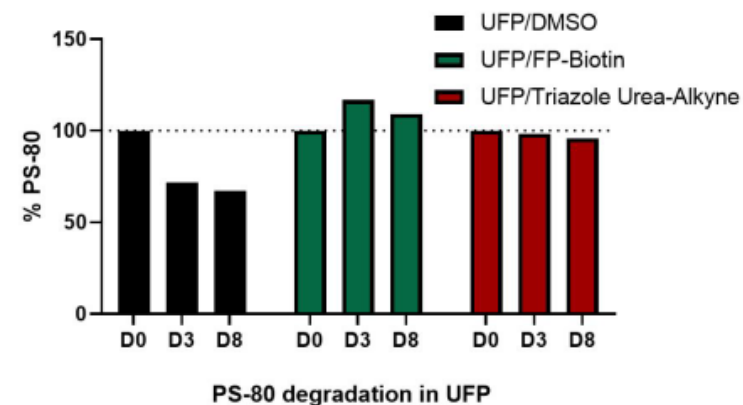
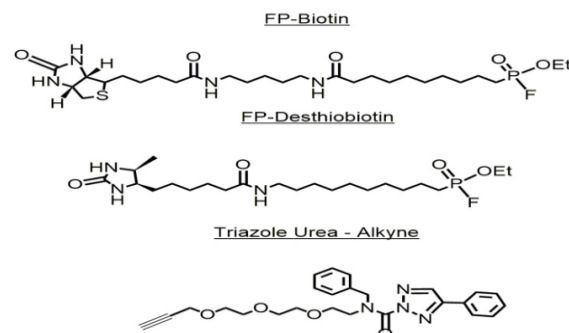
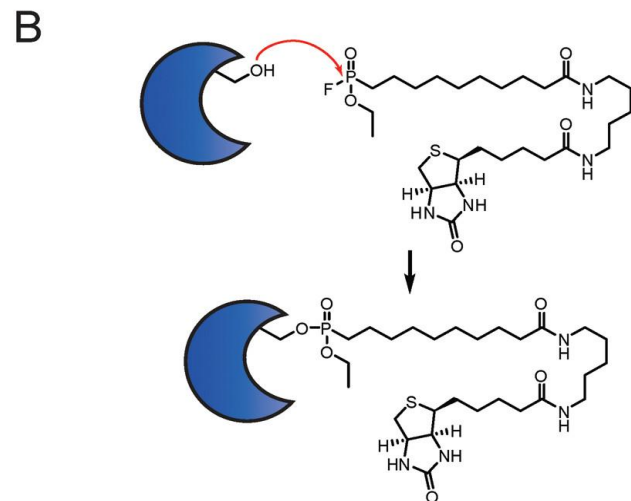
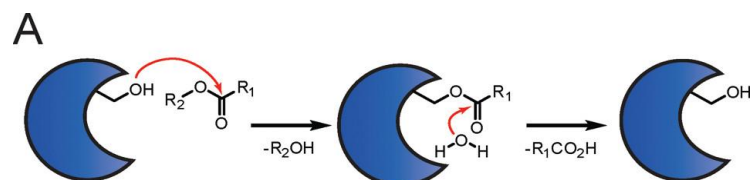
- ppb levels of enzymes and high background from antibody (>6 orders of magnitude)

Activity

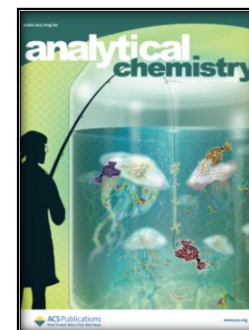
- Enzyme abundance and activity is not a linear relationship

ABPP (Activity-based protein profiling) for PSDE (Polysorbate-Degrading Enzymes)

A defining characteristic of Polysorbate-Degrading Enzymes: **nucleophilic serine** in their active site

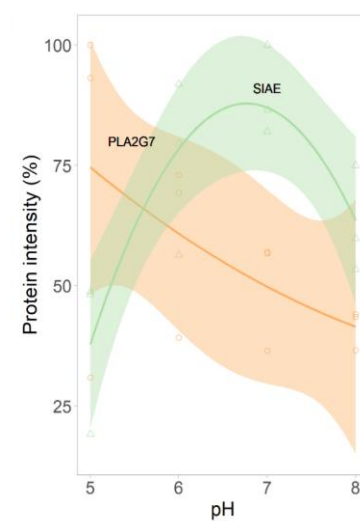
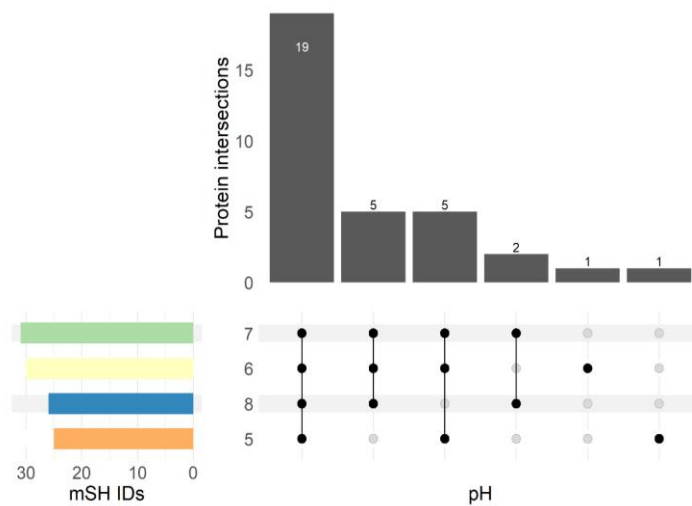
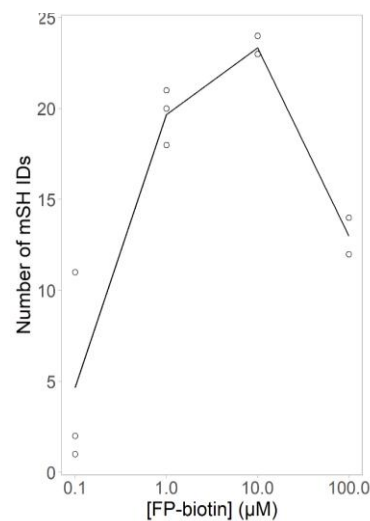
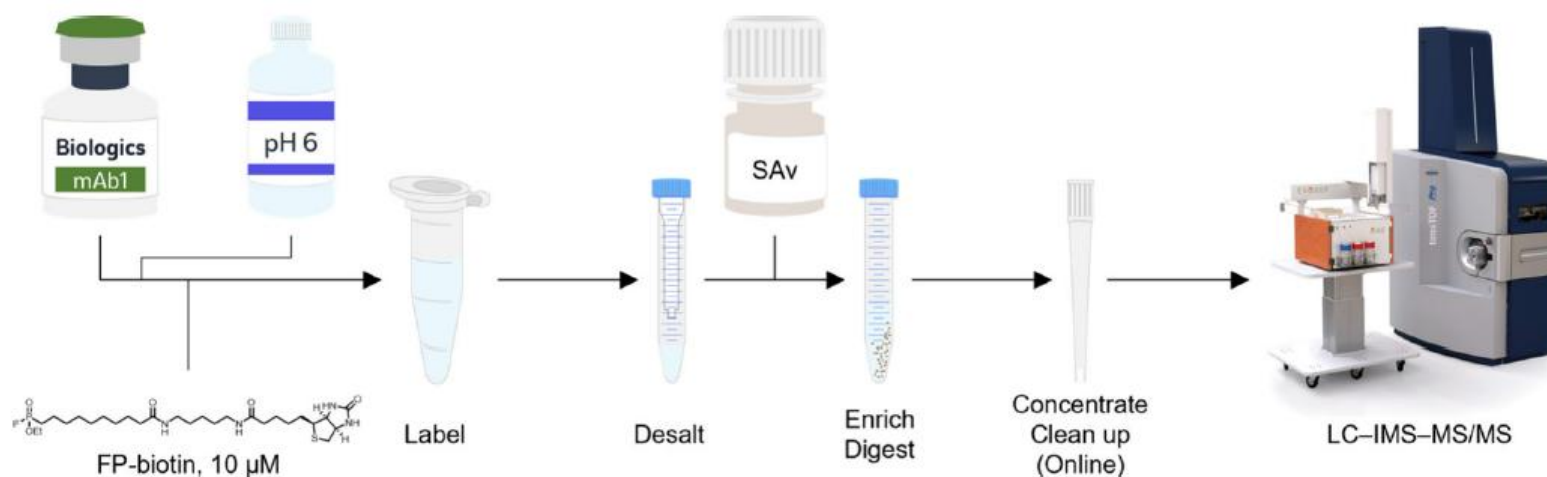


Long, JZ, Cravatt BF, **Chem Rev.** 2011;111(10):6022

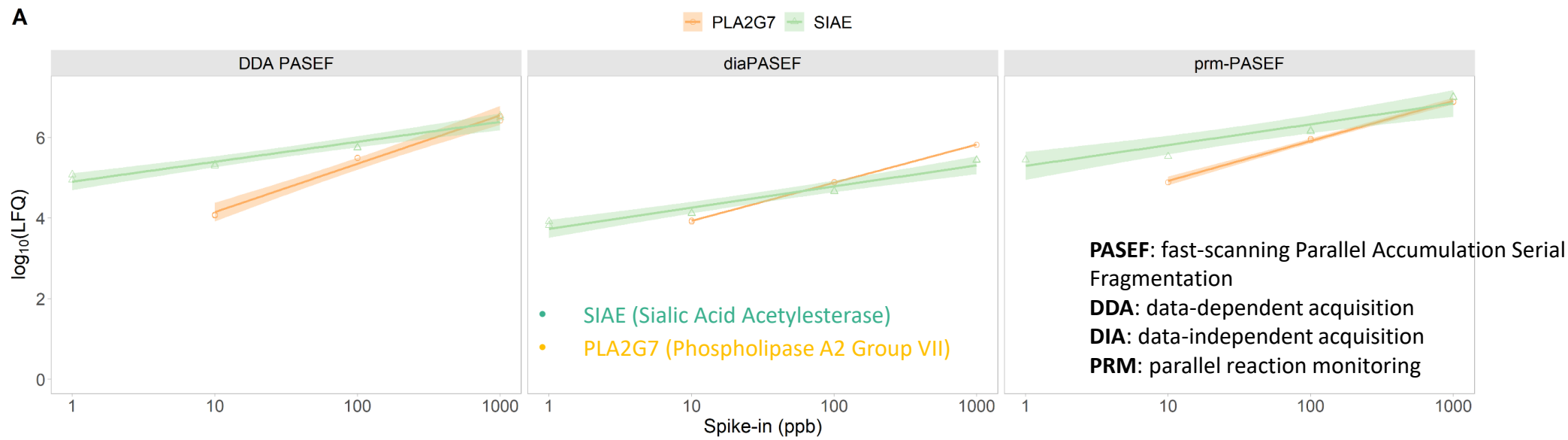


Li X, et al., **Anal Chem.**, 2021;93(23):8161

ABPP 2.0: Systematic optimization of ABPP for identification of PSDEs

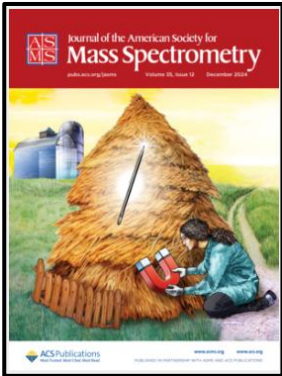


ABPP 2.0: Identification of PSDEs down to 10 ppb



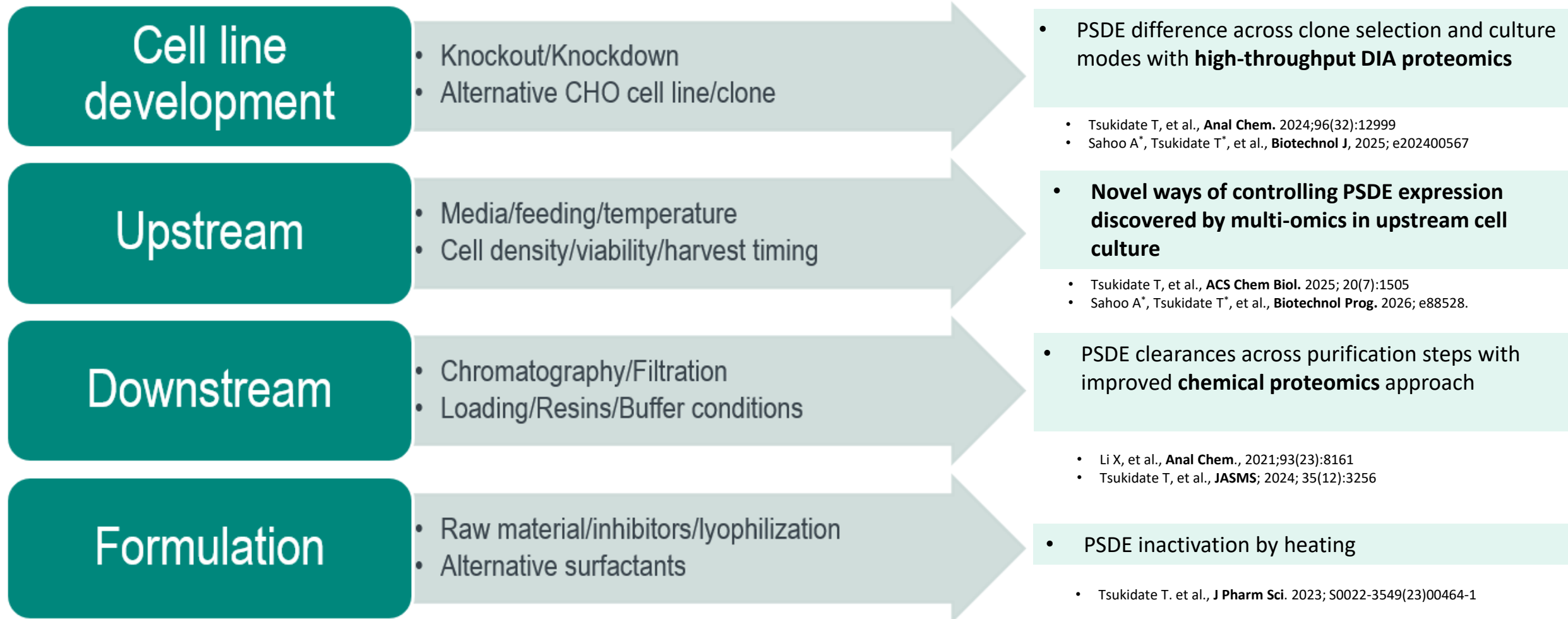
Material	ABPP	Input (mg)	PSDE IDs
mAb UFP	2.0	5	IAH1; SIAE
mAb UFP	1.0	20	SIAE

UFP: ultrafiltration permeate
IAH1: Isoamyl acetate-hydrolyzing esterase 1 homolog
SIAE: Sialic Acid Acetylesterase



Tsukidate T, et al., **JASMS**; 2024; 35(12):3256

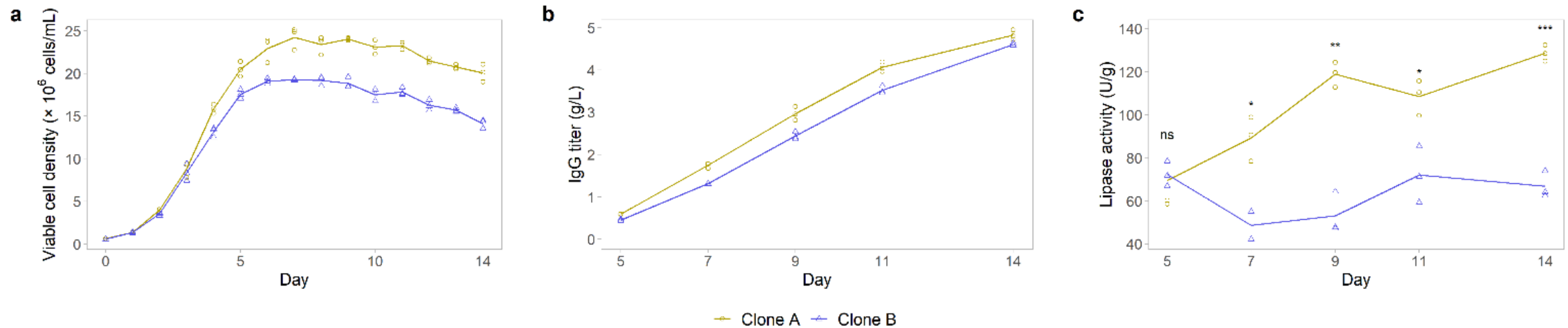
LC-MS proteomics enables end-to-end control of PSDE/HCPs



Controlling PSDE expression and secretion with multi-omics

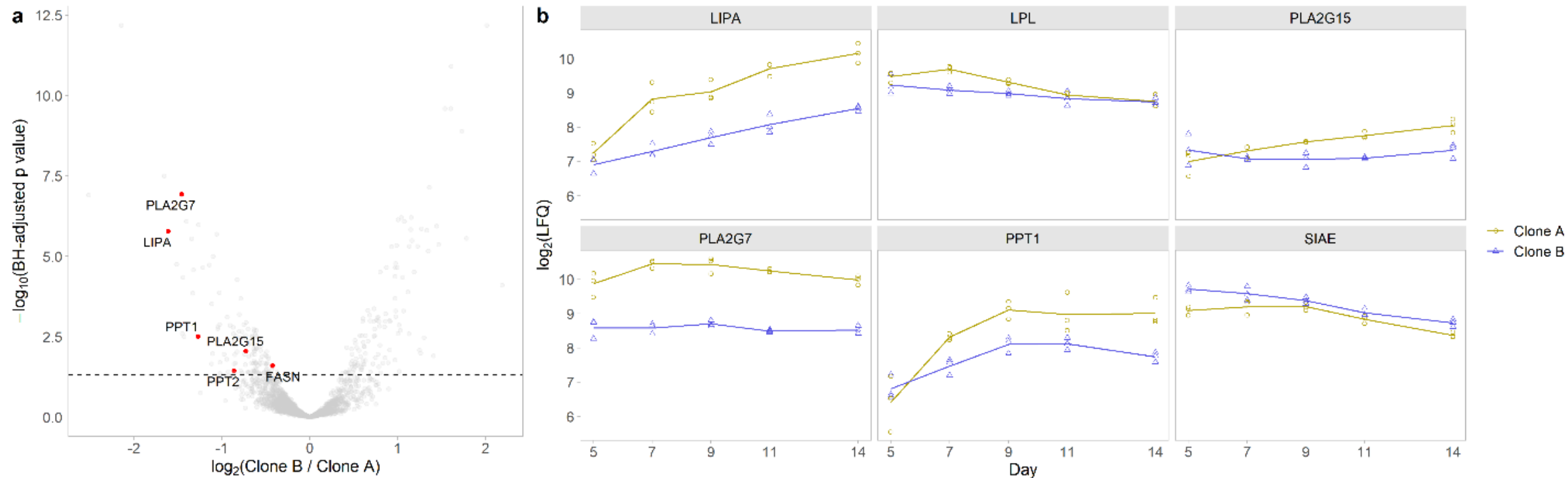
Can PSDE/HCP expression and secretion be modulated on the molecular level and improve PS stability?

Clone A shows far higher lipase activity than equally productive Clone B



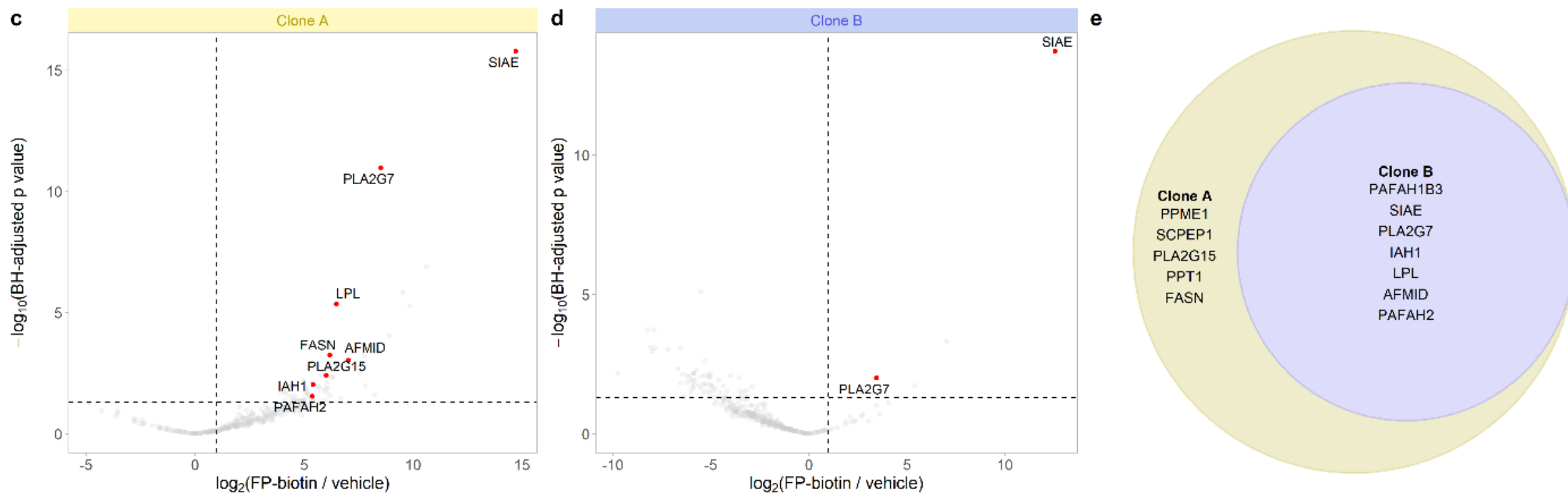
- Two most productive clones from a mAb based biologic with fed-batch culture
- Lipase activity: Clone A >>> Clone B

HT-DIA proteomics: several PSDE HCPs more abundant in Clone A



- Harvest from days 5, 7, 9, 11 and 14
- **Proteomics** (diaPASEF, 30 SPD, ~**2000** HCPs quantified in HCCF)

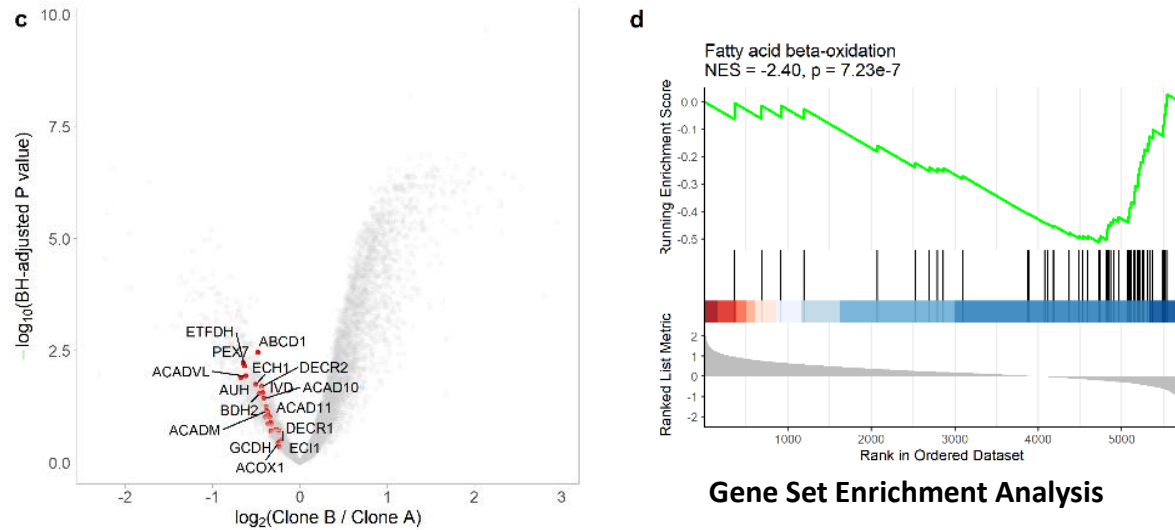
ABPP: the same PSDE HCPs are also more active in Clone A



- Harvest from days 5, 7, 9, 11 and 14
- ABPP tracks dozens of PSDEs

Multi-omics reveals fatty acid metabolism upregulated in Clone A

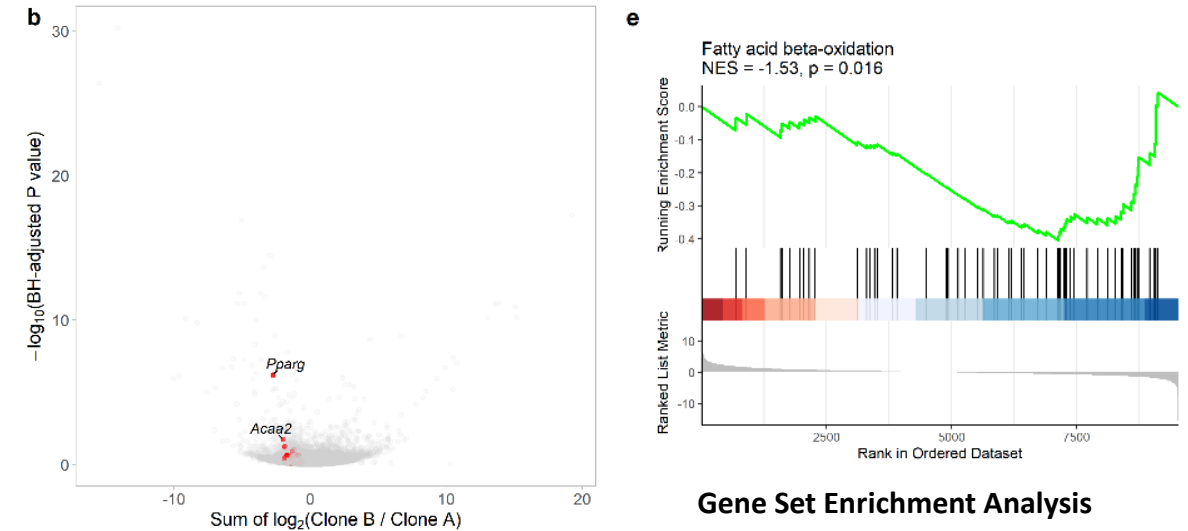
Proteomics



- 6,103 **intracellular** proteins quantified in CHO cells by DIA proteomics across 30 samples
- PSDEs are not only released more but also produced more by **Clone A** than **Clone B**

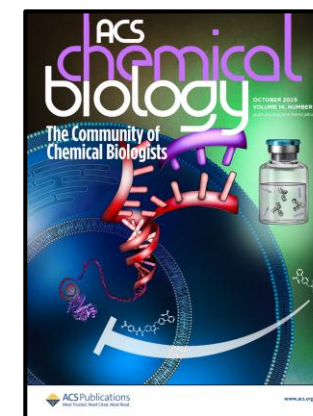
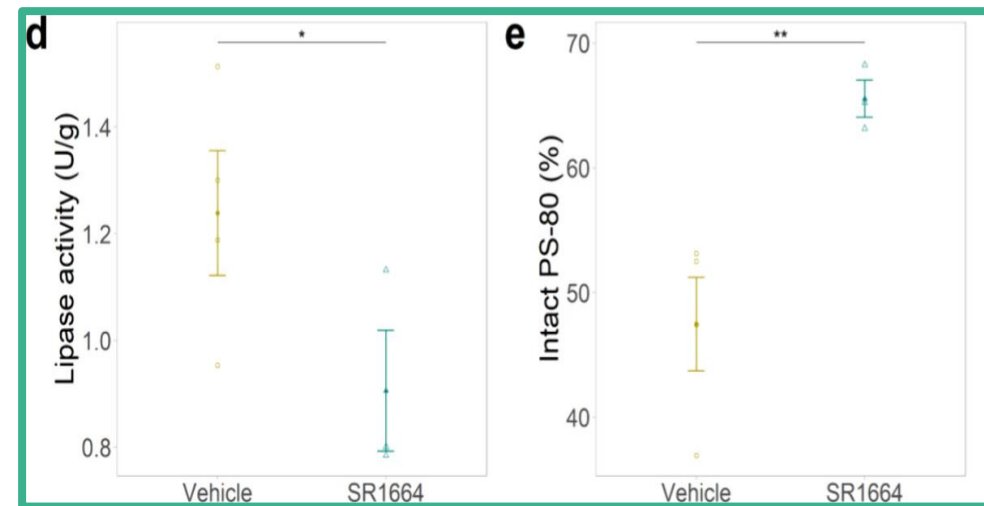
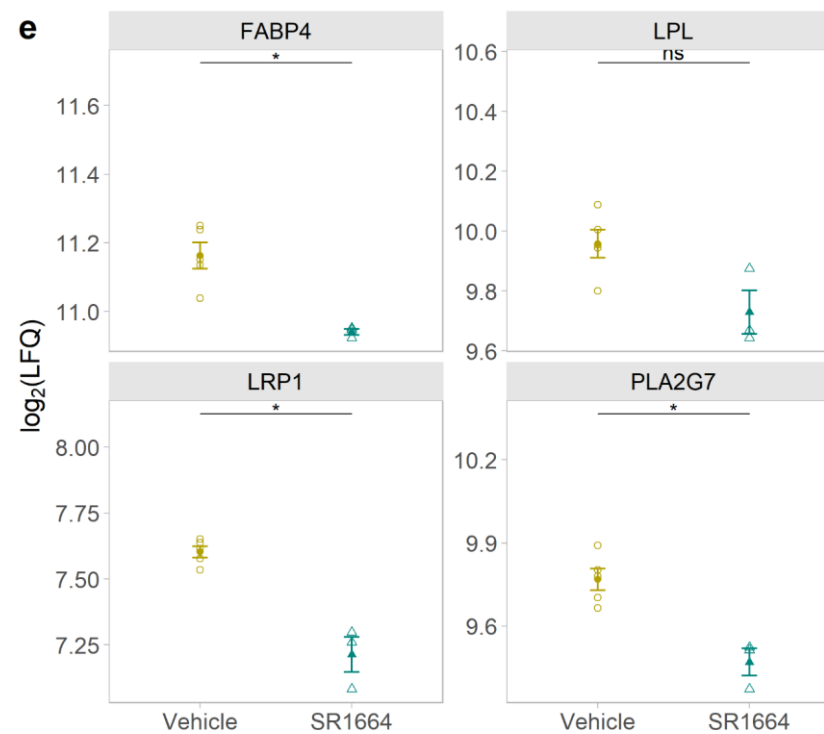
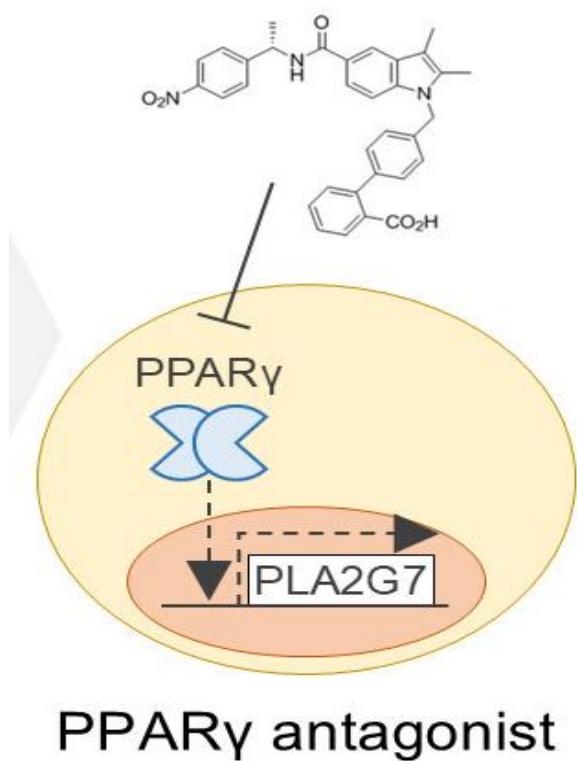
Clone A VS Clone B	PLA2G7	LPL	PLA2G15	LIPA	PPT1
Extracellular (HCCF/HCP)	>>	=	=	>	>
Intracellular (cell lysate)	>	=	=	=	=

Transcriptomics



- 12,105 mRNA in 11 bioreactors at six time points by RNA-Seq
- Correlation between the cumulative sum of mRNA and protein was **0.66**

PPAR γ antagonist cuts PSDE levels and activity, improving PS stability

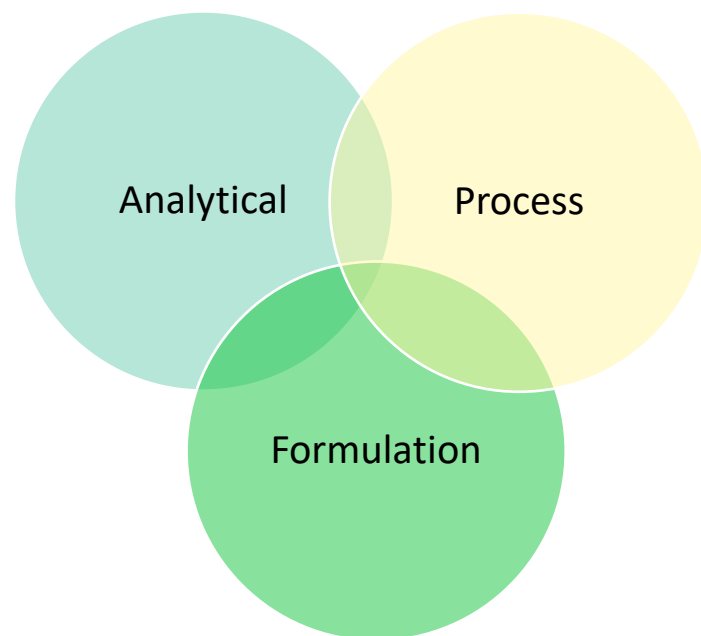


Summary

- LC-MS proteomics has been widely used for HCP characterization assay for process development, and it has been a **qualified extended-characterization assay** for vaccine PPQ and GMP batch testing, moving beyond ELISA in process development and manufacturing
- Advances in next-generation proteomics including high-throughput/**HT proteomics** and **ABPP** enable robust process development and risk assessment for the control of high-risk HCPs
- Integrated **multi-omics** (extracellular/intracellular **abundance** and **activity** proteomics, and transcriptomics) traced clone-level PSDE differences to fatty acid metabolism, and a PPAR γ antagonist reduced PSDE abundance and activity — a **pathway-engineering** complement to KO/KD approaches

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Thank you

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