

synlogic

**Proteomic approaches for
characterization of live
biotherapeutic *Escherichia
coli* Nissle products from
fermentation to therapeutic
activity**

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CASSS Mass Spec 2023

Thursday, September 7th, 2023

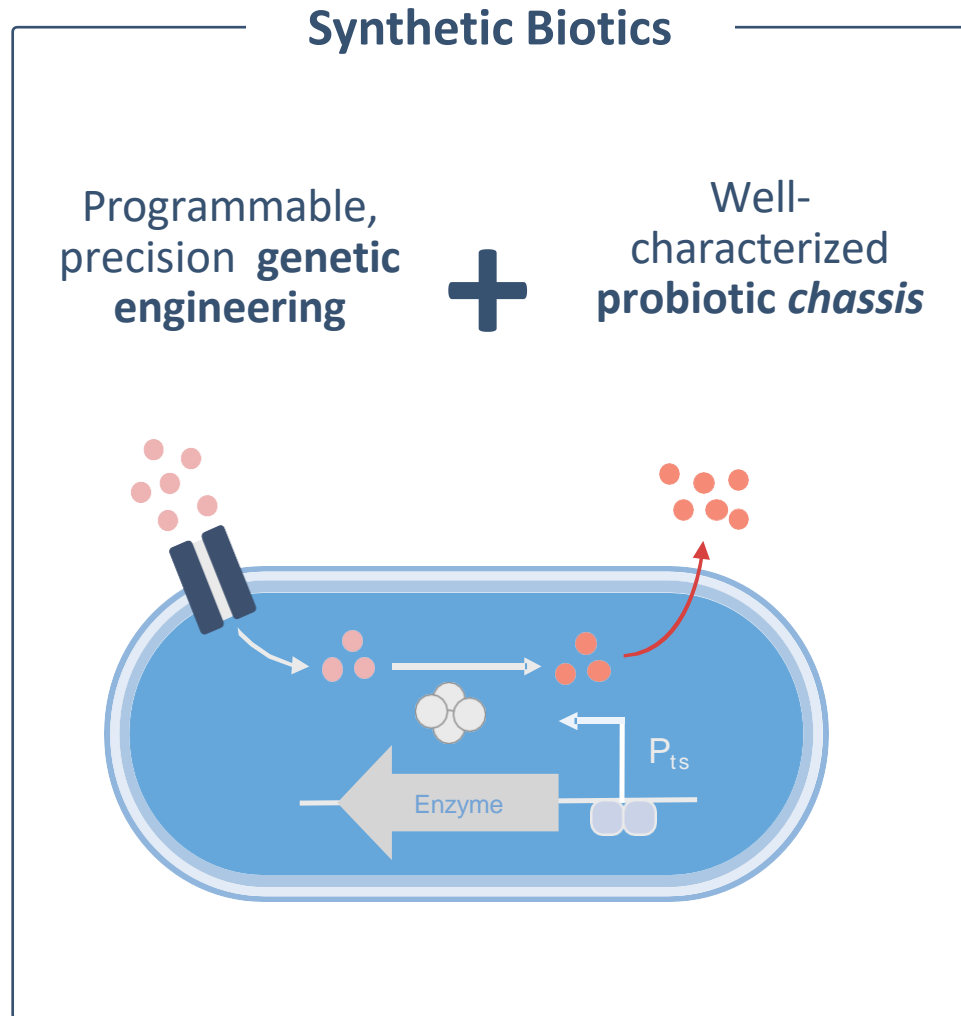


Synlogic

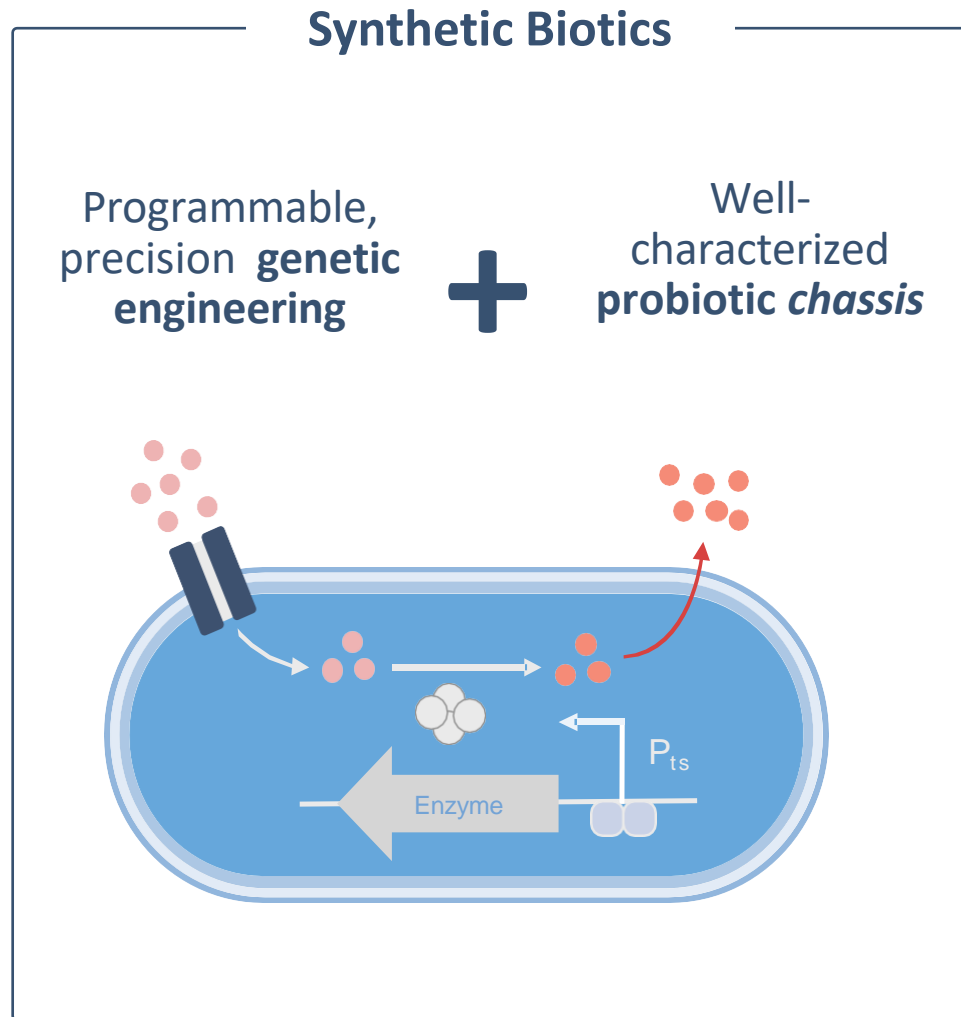
Designing first-in-class live
biotherapeutic products for
treatment of rare disease



A new paradigm of biotherapeutics



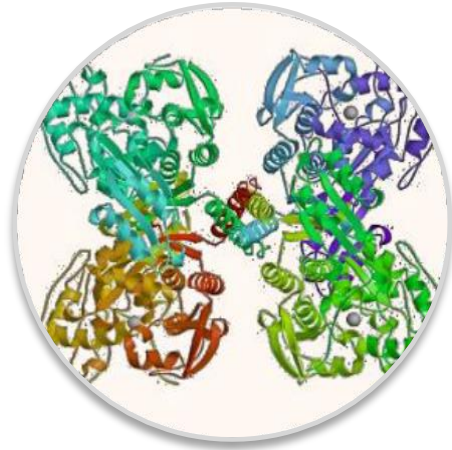
A new paradigm of biotherapeutics



Differentiated Drug Candidates

- Target validated biological pathways
- Orally-administered
- GI-restricted
- Non-colonizing with rapid GI clearance
- Aimed at treating rare and common, metabolic and immunological diseases

PKU burden includes extreme diet restrictions & neurocognitive risks



PKU is caused by defects in the enzyme that metabolizes Phe



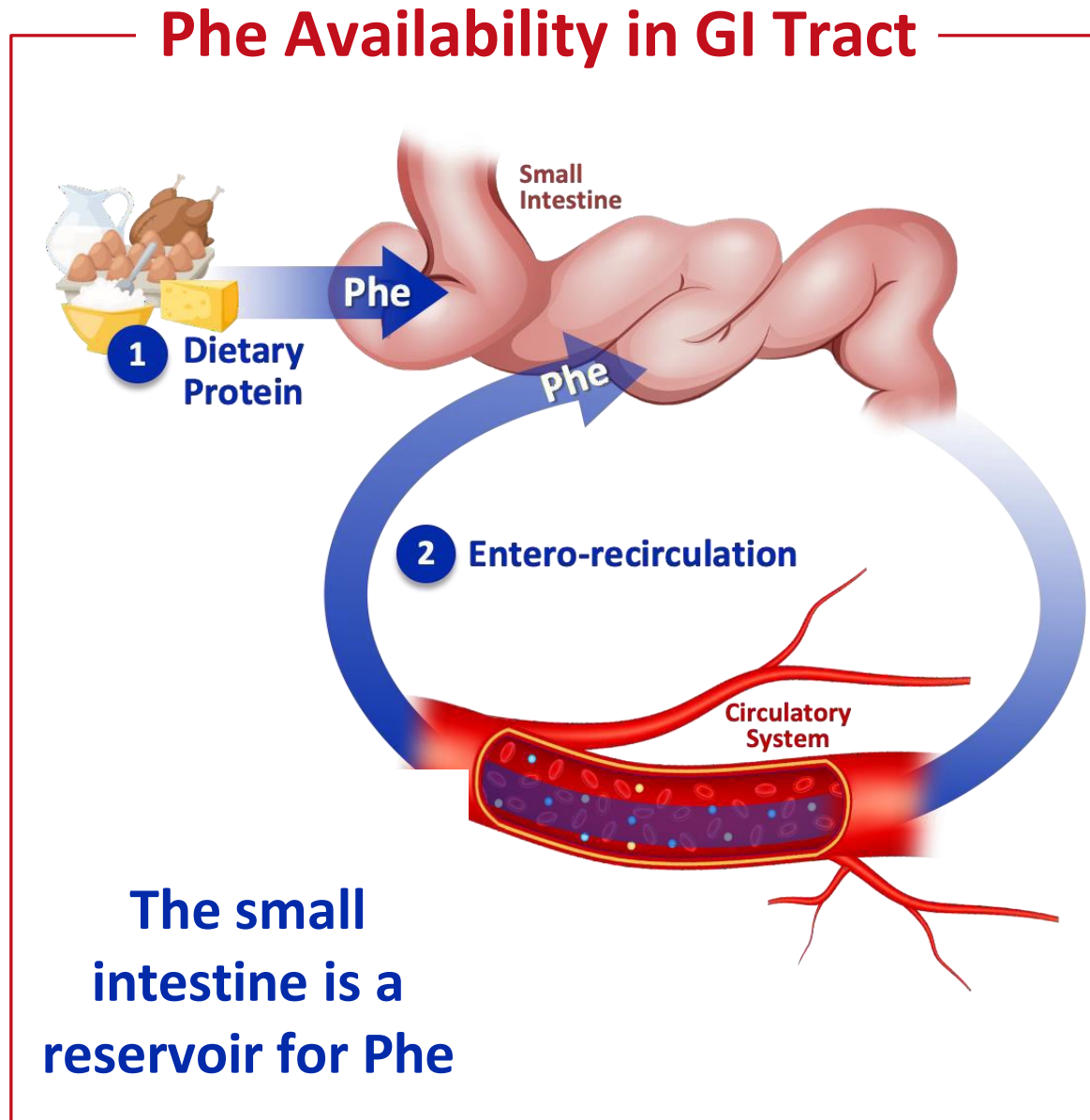
Phe build-up leads to neurological damage and defects



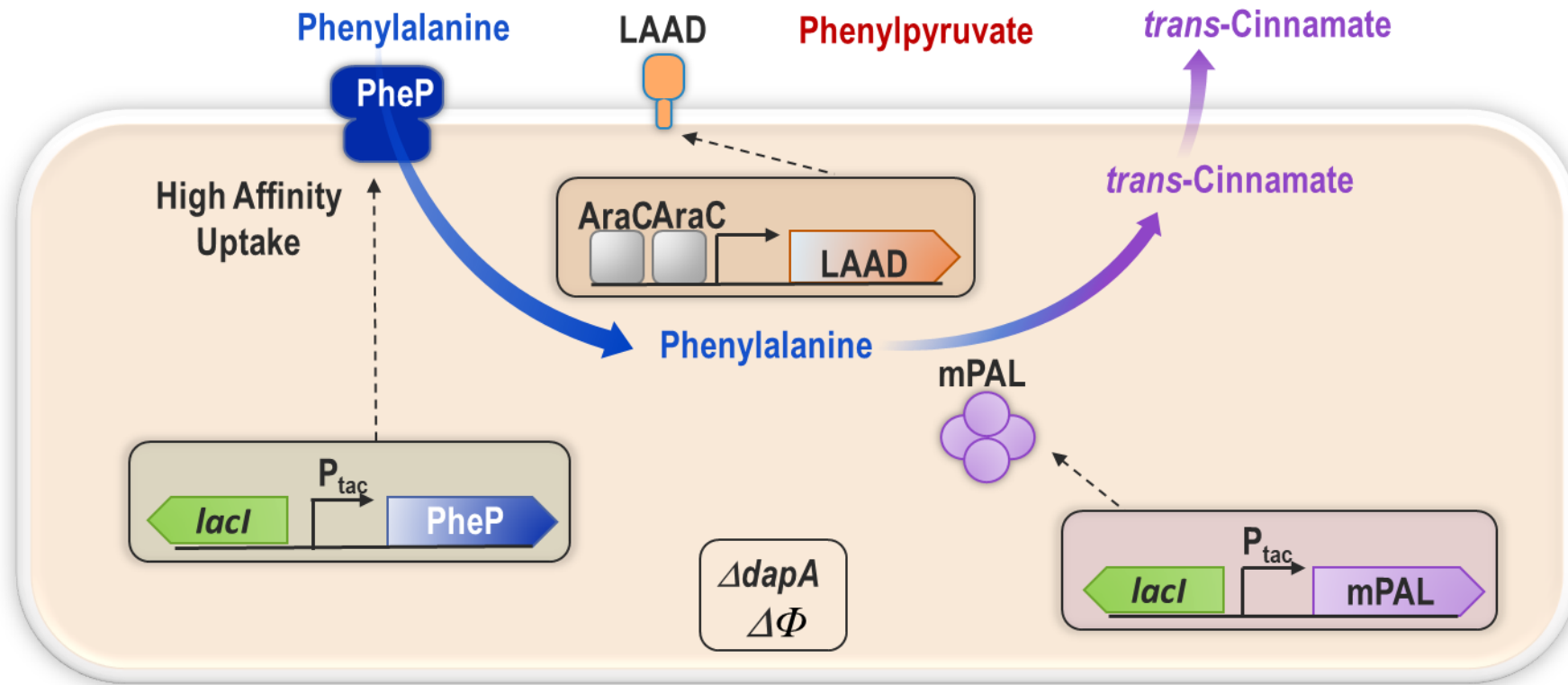
Risk intellectual disabilities, mental processing and social deficits, emotional problems^{1,2,3}

The key to risk reduction in PKU is lifelong control of Phe levels³

SYNB1934: Designed to lower plasma Phe via GI tract



SYNB1934 – A first-of-its-class EcN live biotherapeutic



E. coli Nissle chassis

Assessing product stability through proteomics

Quantifying product quality through viability



Quantifying product quality through viability

Colony Forming Units (CFU)



Quantifying product quality through viability

Colony Forming Units (CFU)



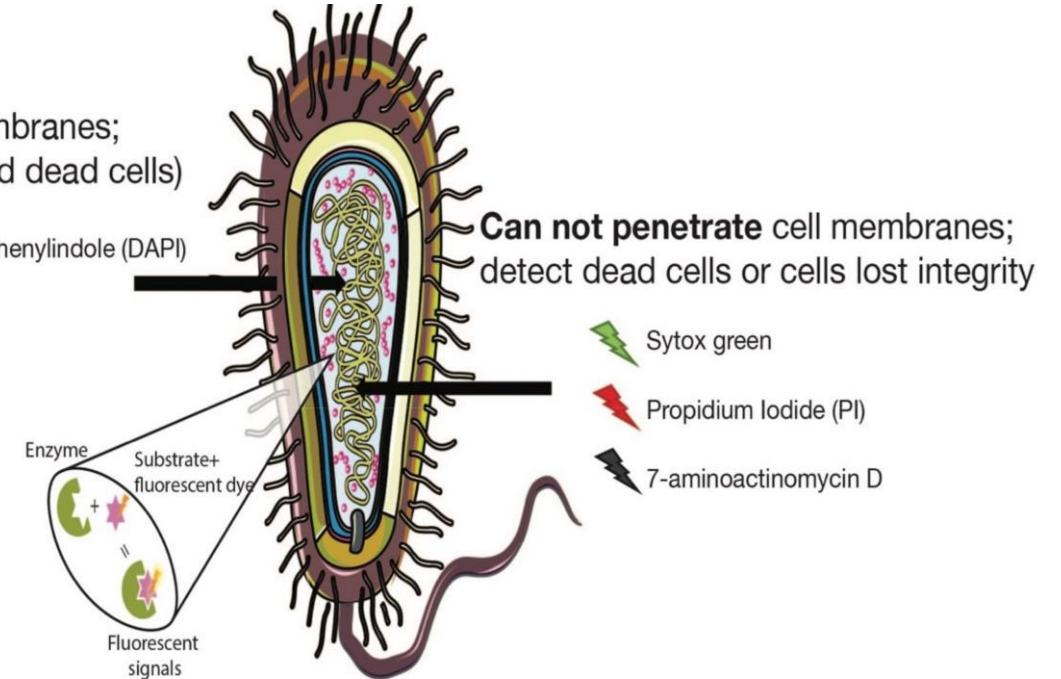
Fluorescent dyes

Penetrate cell membranes;
detect live cells (and dead cells)

- ⚡ 4',6-diamidino-2-phenylindole (DAPI)
- ⚡ Acridine orange
- ⚡ SYBR Green
- ⚡ Vybrant

Can not penetrate cell membranes;
detect dead cells or cells lost integrity

- ⚡ Sytox green
- ⚡ Propidium Iodide (PI)
- ⚡ 7-aminoactinomycin D

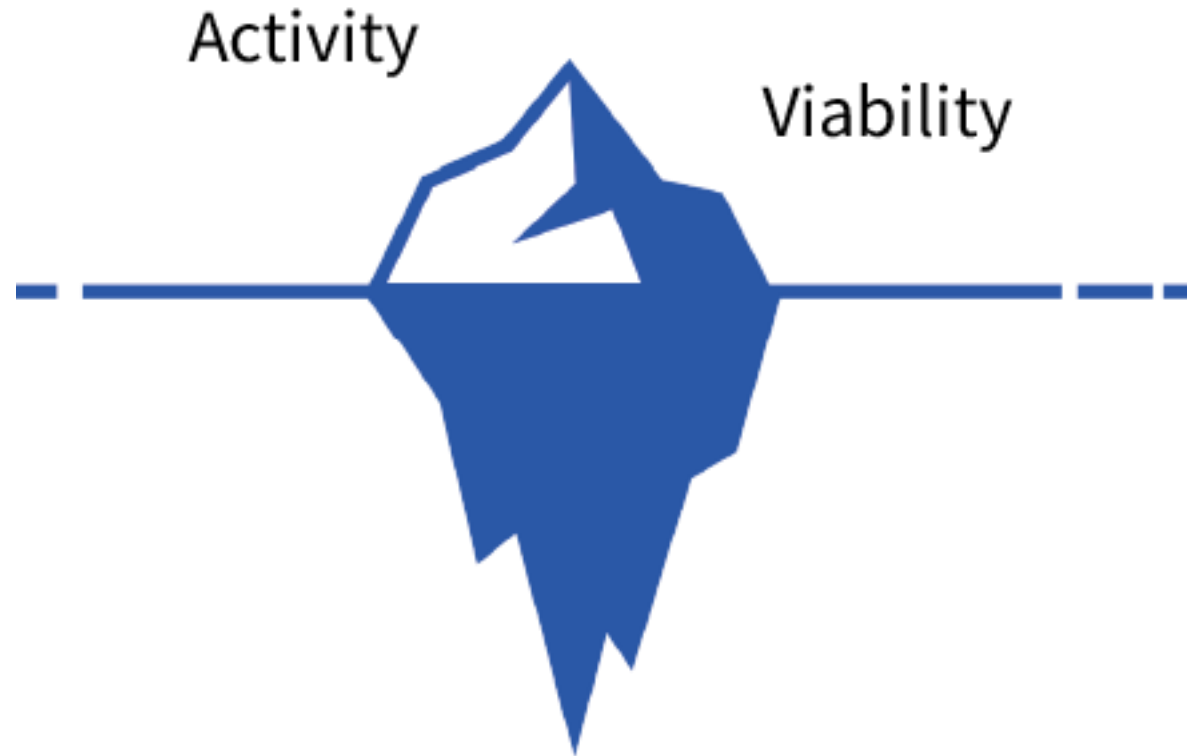


Enzyme activities in active cells:

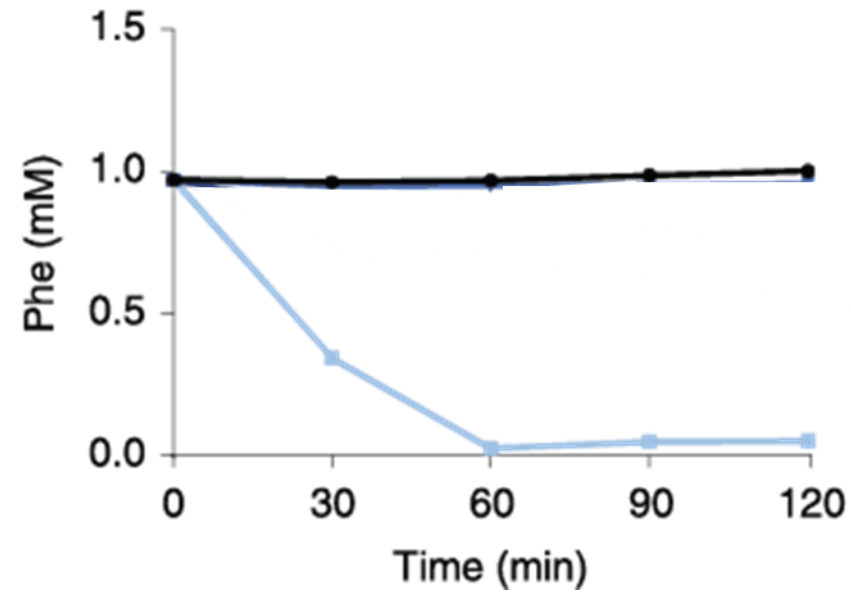
Esterase activity---diazonium salt ⚡

Dehydrogenase activity---5-cyano-2,3-ditoyl tetrazolium chloride ⚡

Quantifying product quality – beyond viability



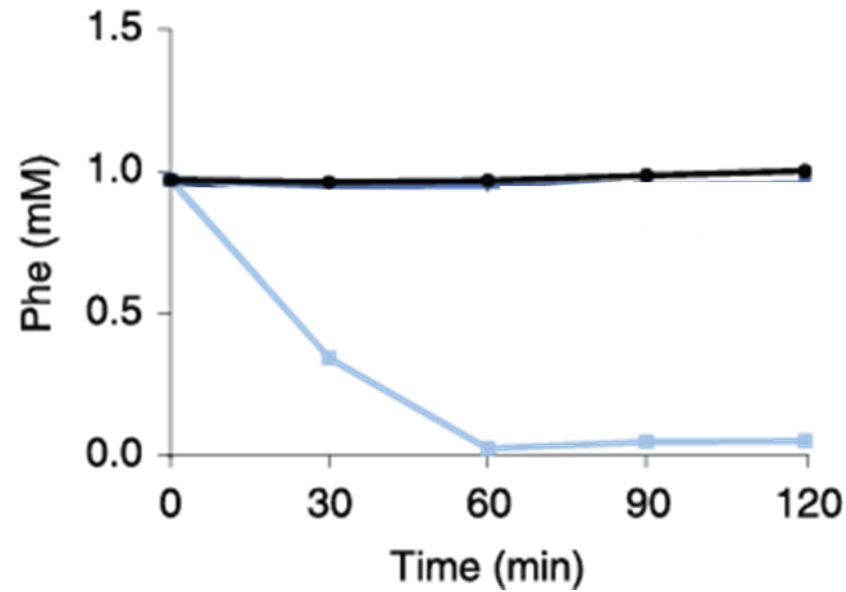
Quantifying product quality through **activity**



● EcN chassis

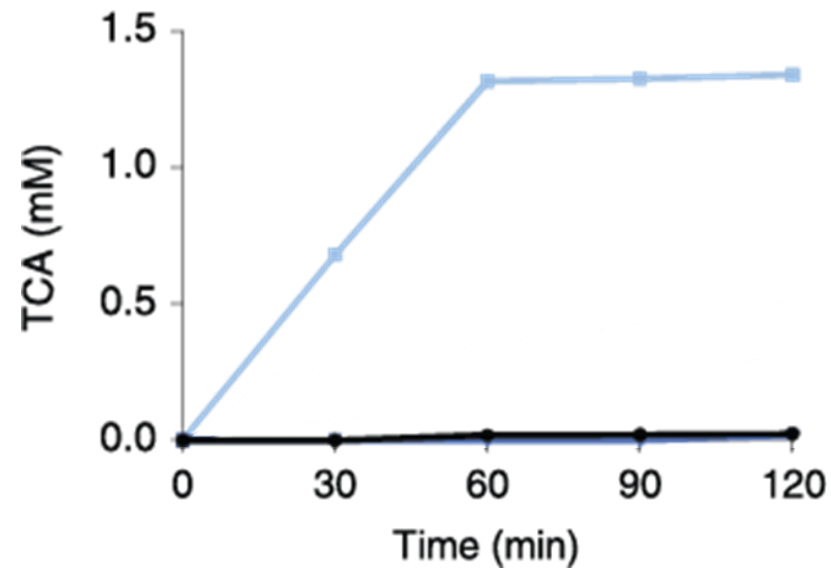
■ SYNB1934 (1st gen)

Quantifying product quality through **activity**

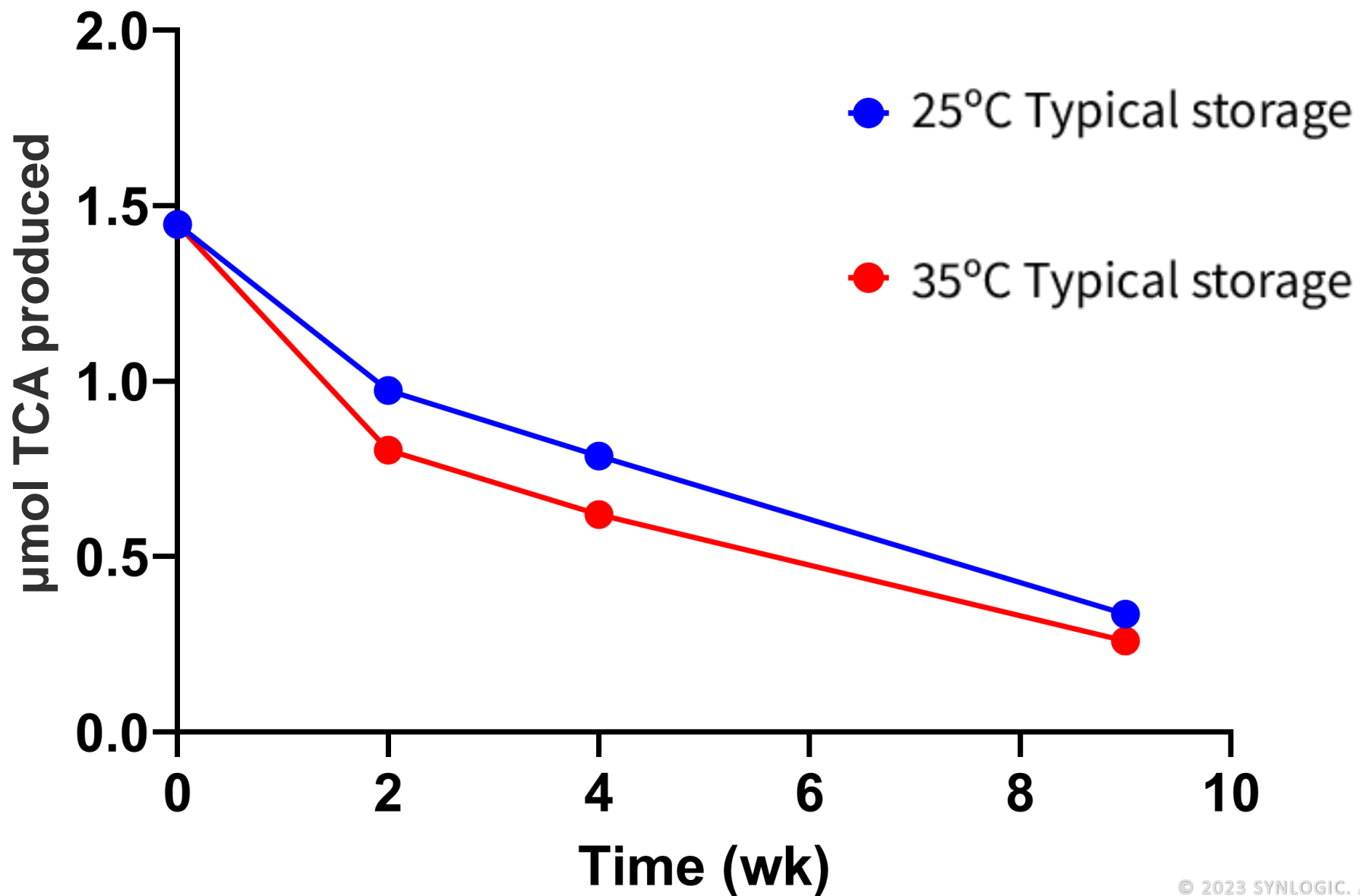


● EcN chassis

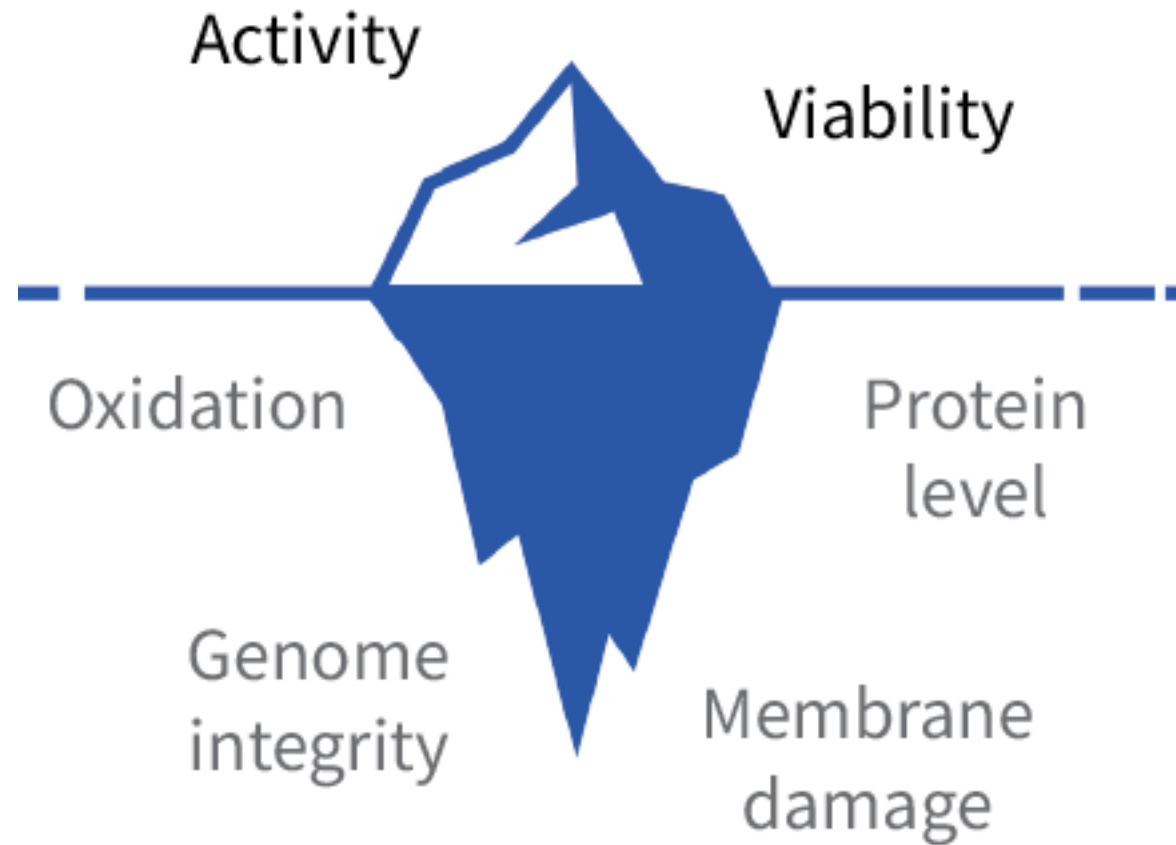
■ SYNB1934 (1st gen)



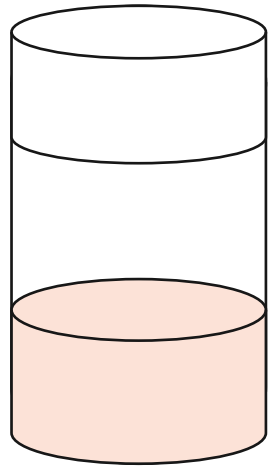
Product activity can drop over time – what else is at play?



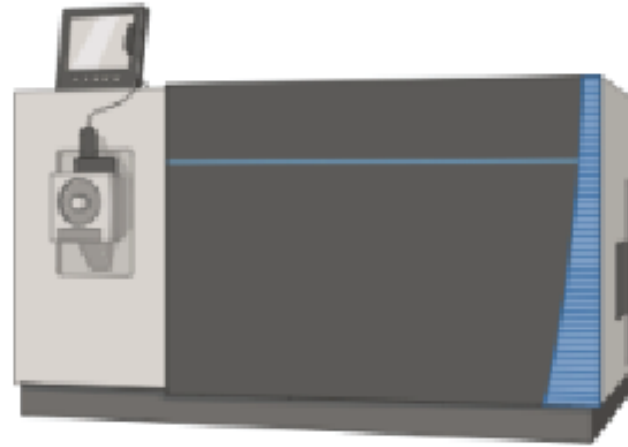
Quantifying product quality – what lies beneath the surface?



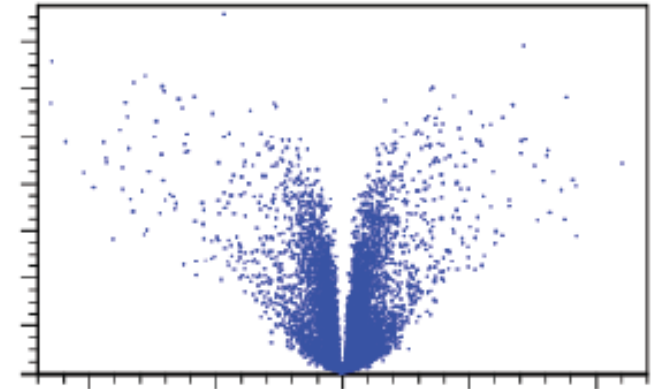
Quantifying peptide and protein-level disturbances with mass spec



Lyophilized EcN

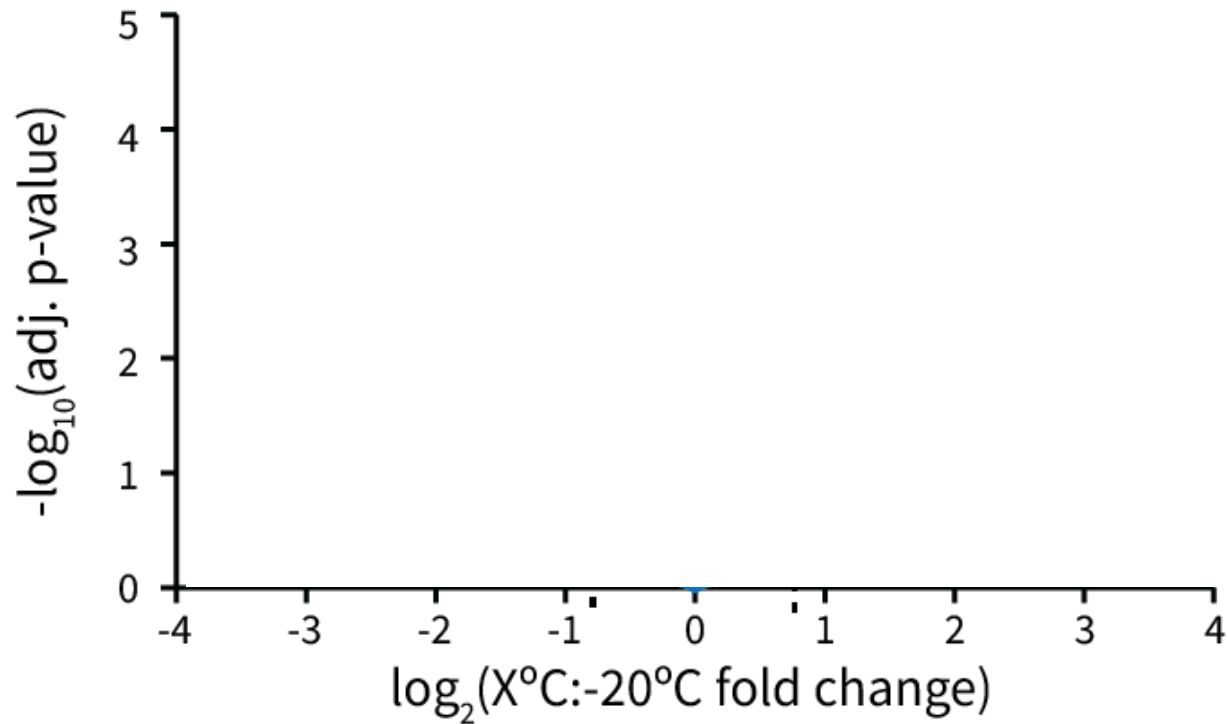


TMT-MS

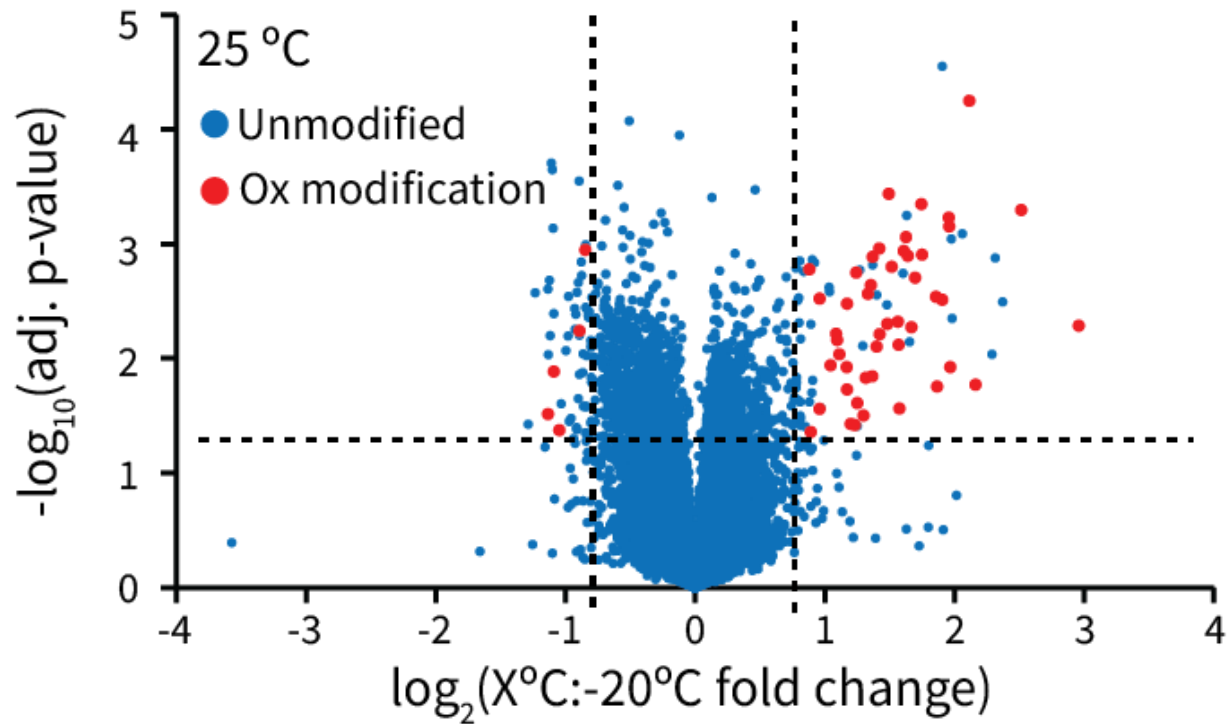


Quantification of protein levels and peptide PTMs

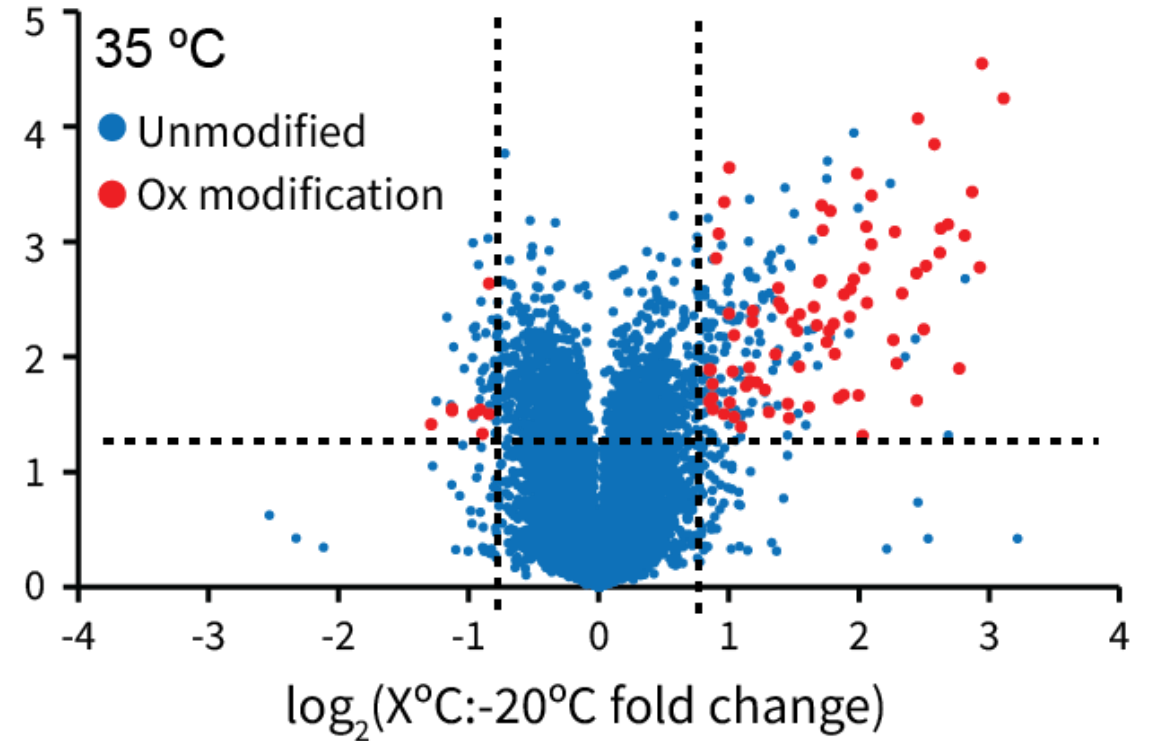
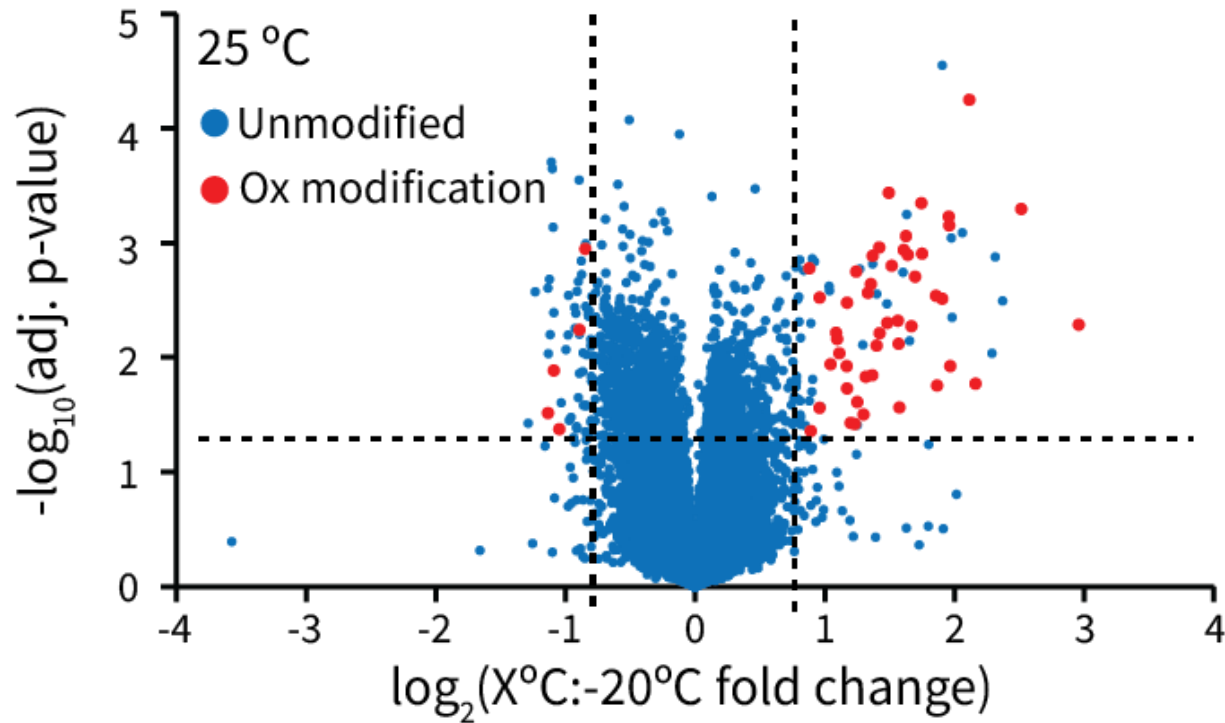
Peptides increase in oxidation and abundance with storage at high temperature



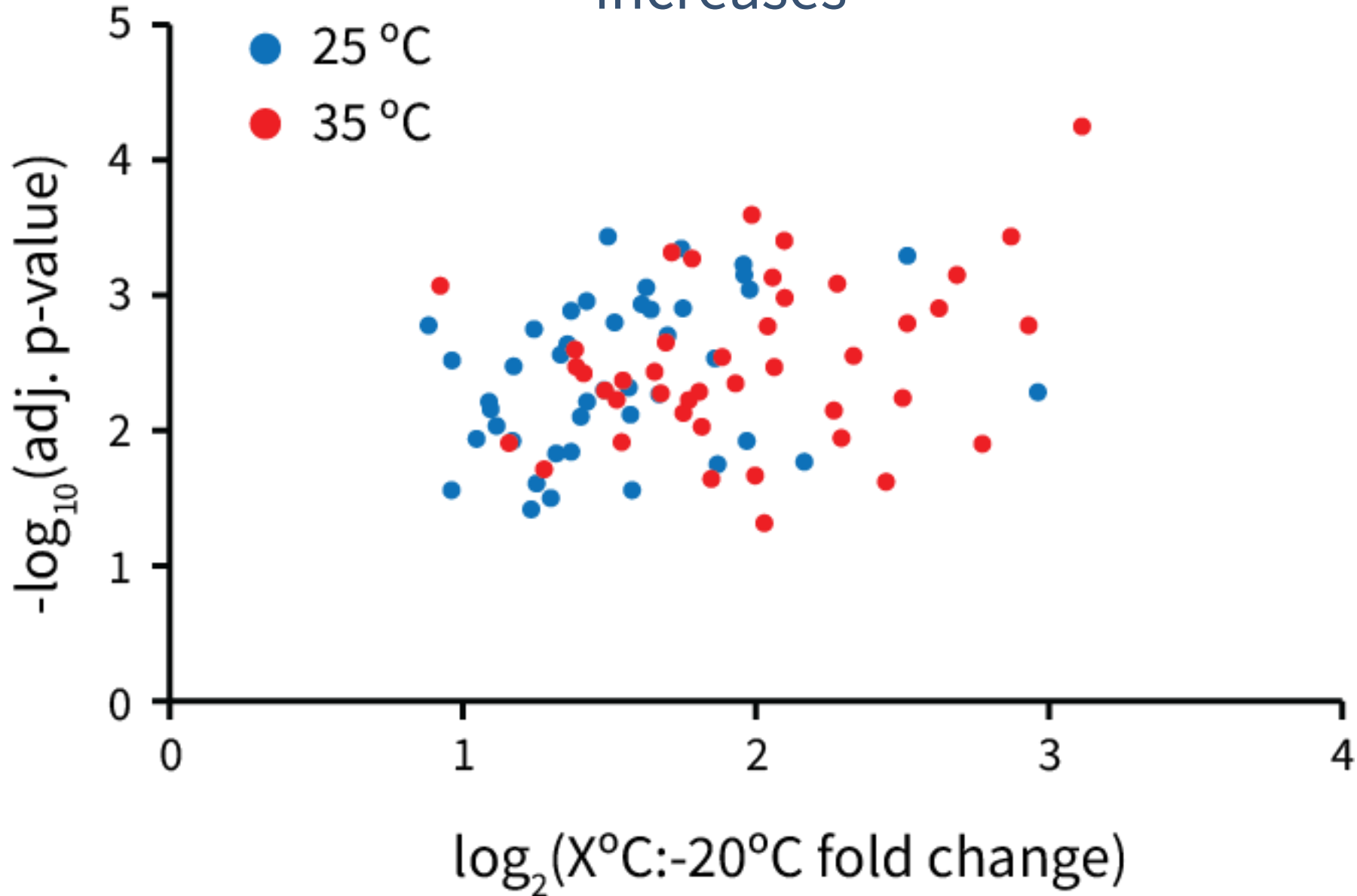
Peptides increase in oxidation and abundance with storage at high temperature



Peptides increase in oxidation and abundance with storage at high temperature



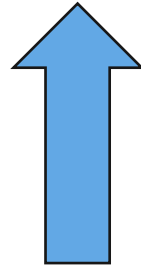
Oxidized peptides are more prevalent as temperature increases



Specific peptide quantification as a bellwether of oxidation

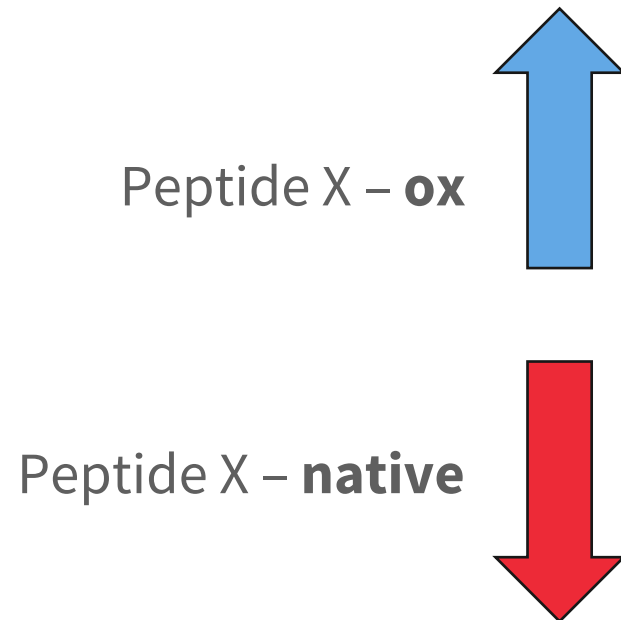
Peptides as biomarkers of oxidation

Peptide X – **ox**



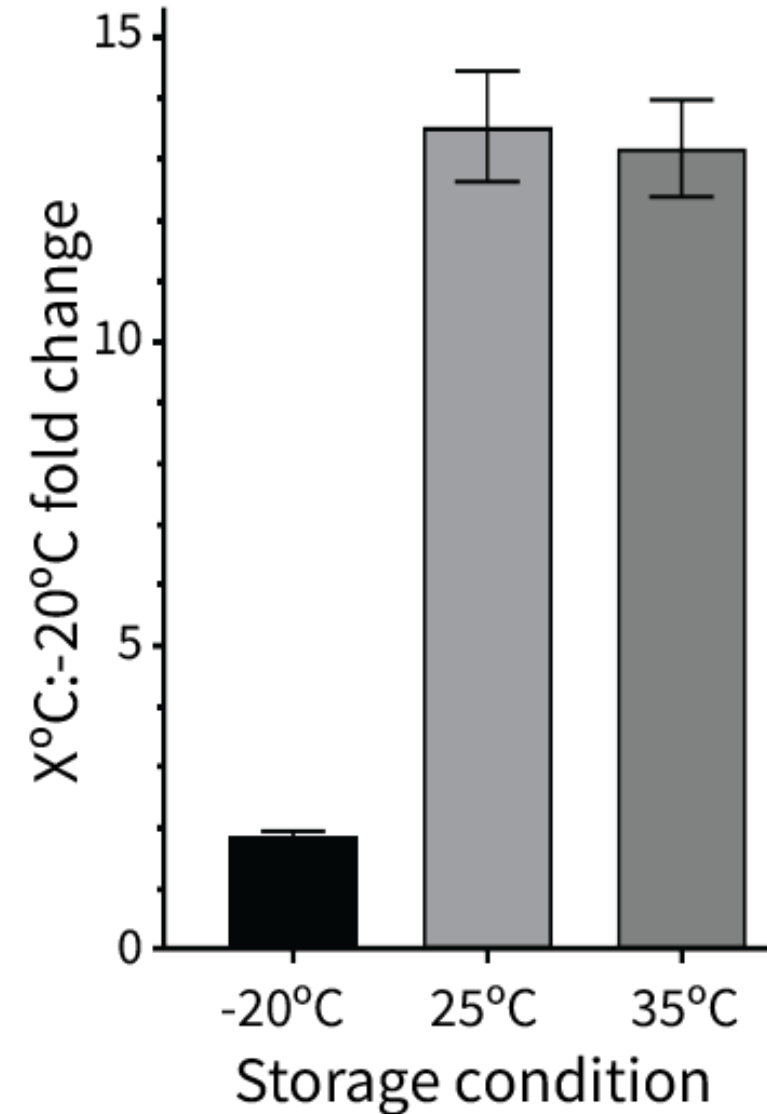
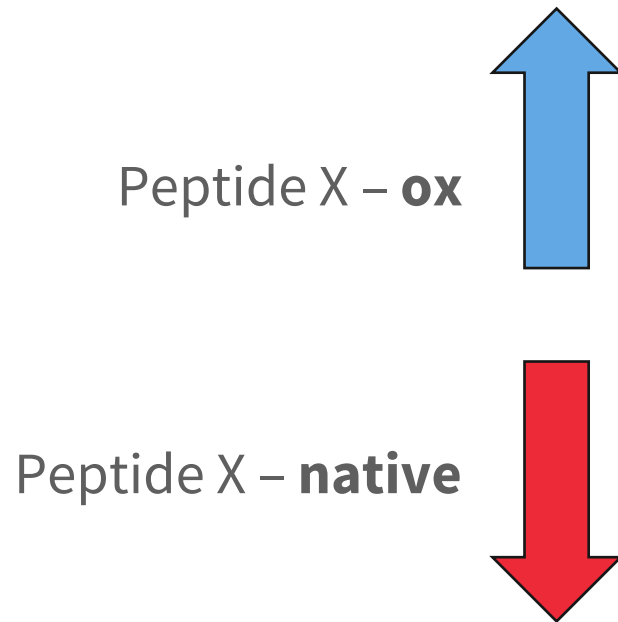
Specific peptide quantification as a bellwether of oxidation

Peptides as biomarkers of oxidation

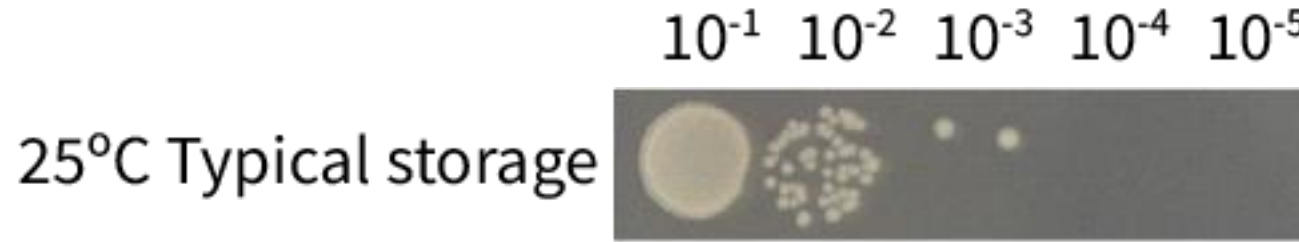


Specific peptide quantification as a bellwether of oxidation

Peptides as biomarkers of oxidation

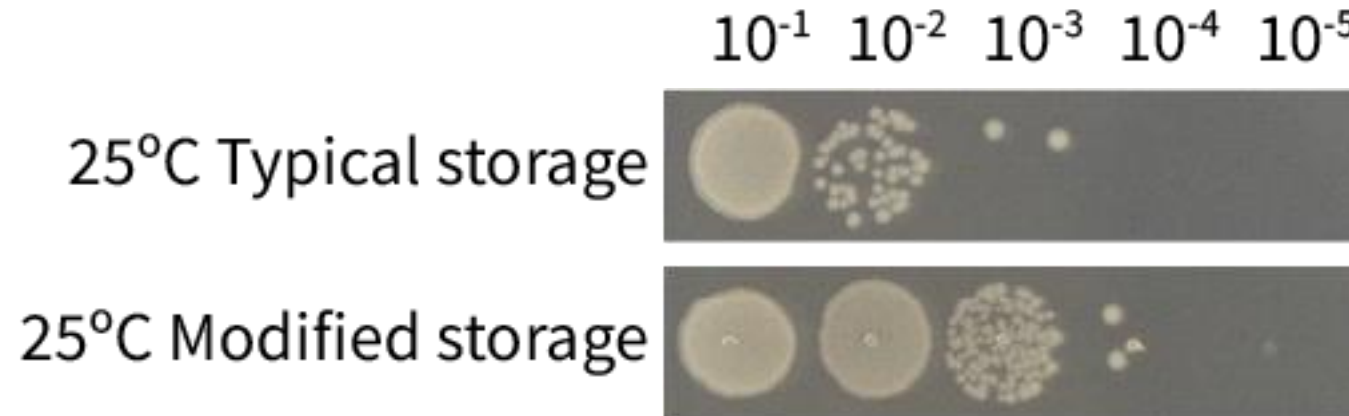


Proteomics-informed process changes increase product quality



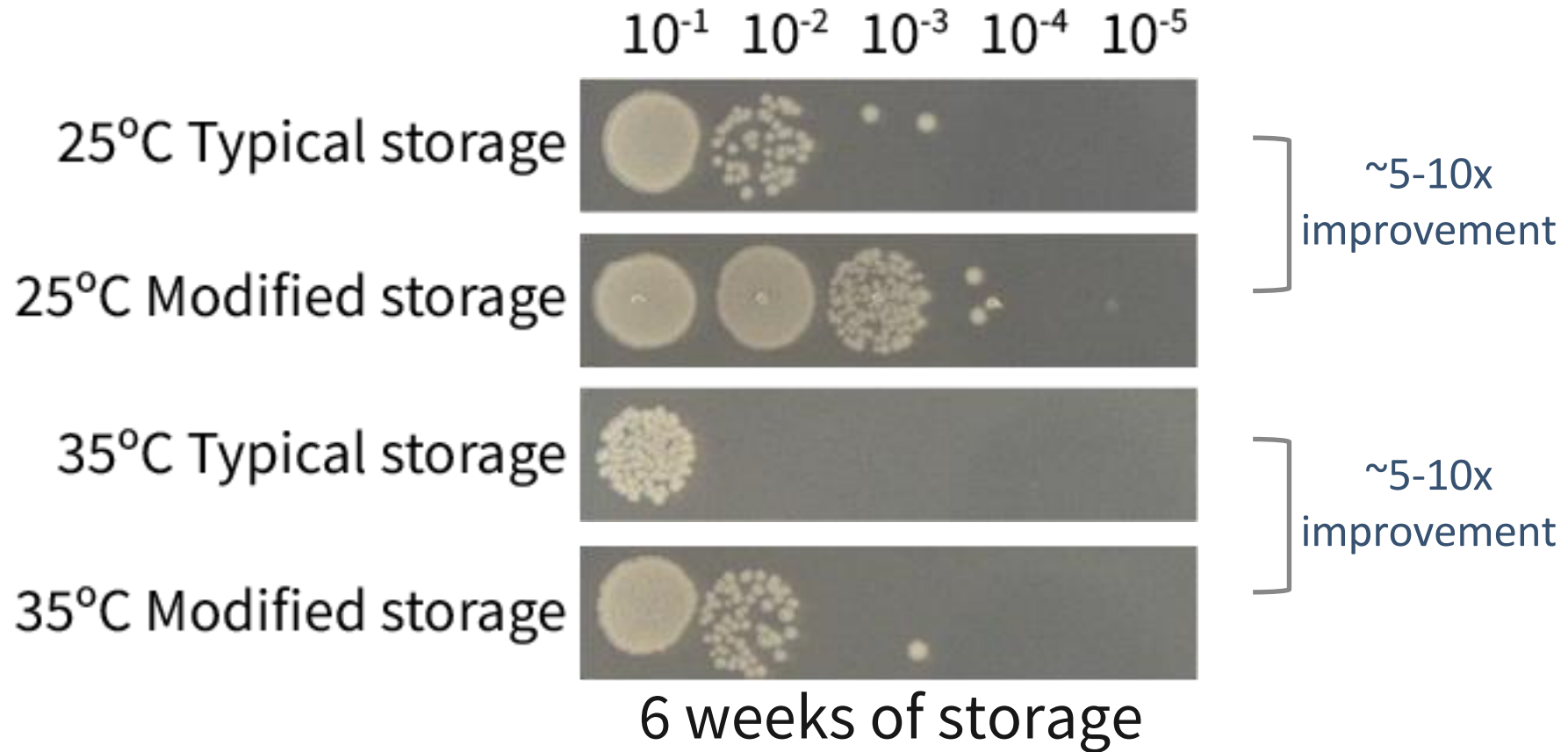
6 weeks of storage

Proteomics-informed process changes increase product quality

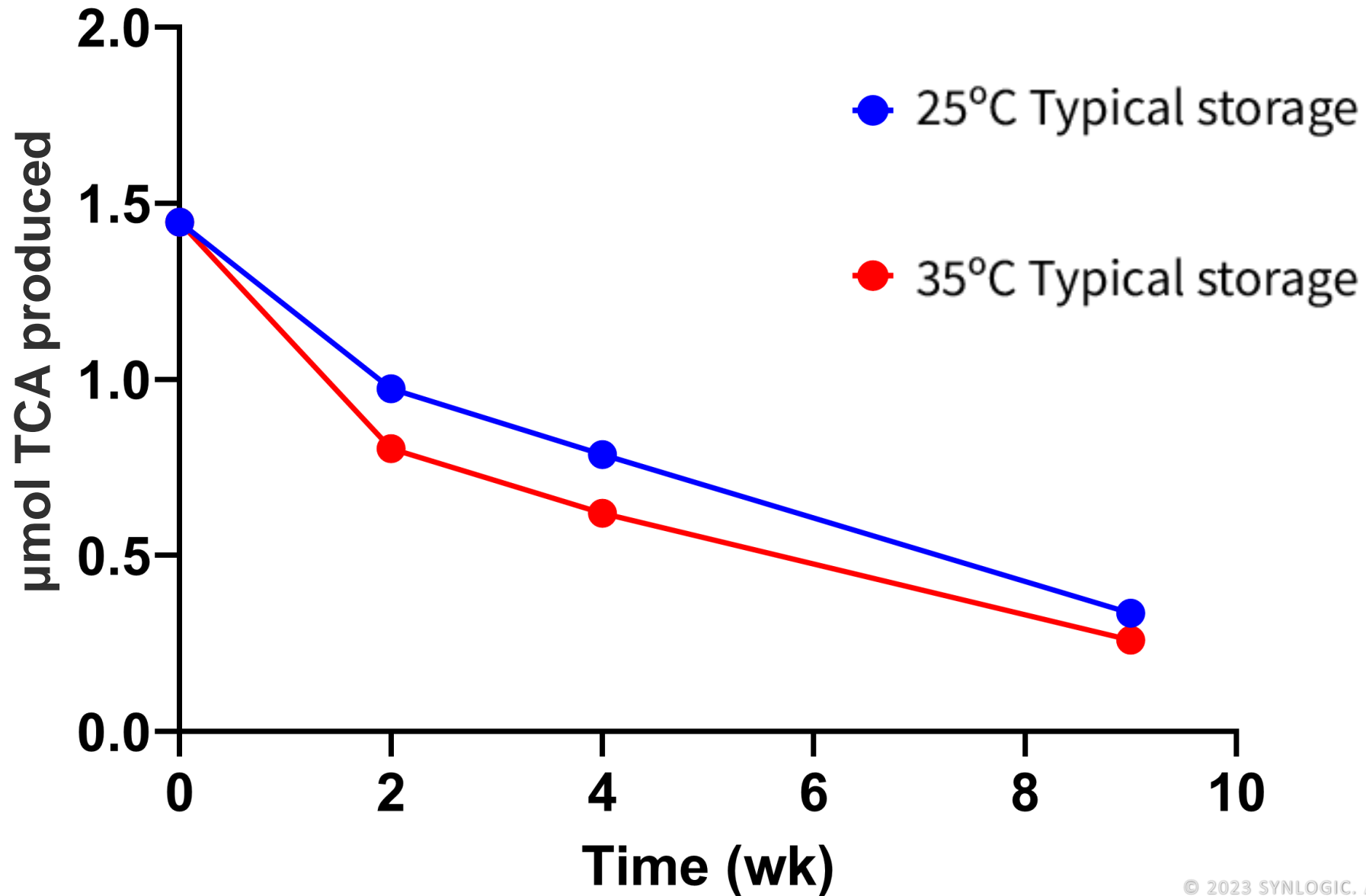


6 weeks of storage

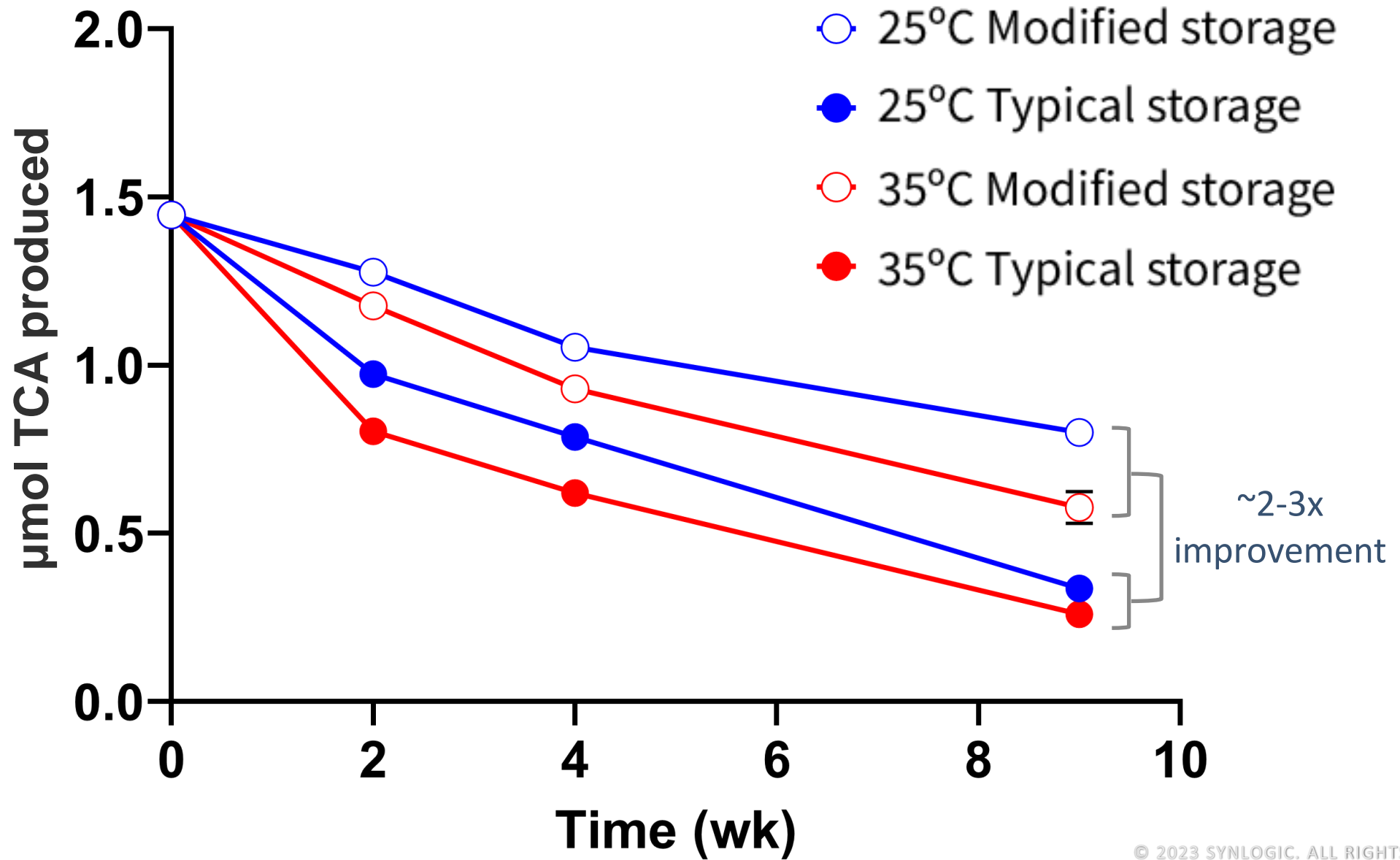
Proteomics-informed process changes increase product quality



Proteomics-informed process changes increase product quality



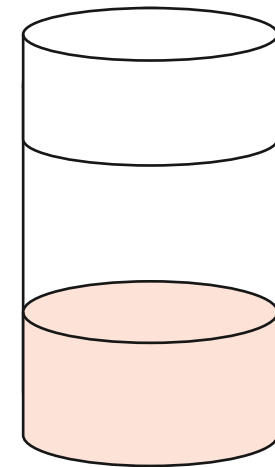
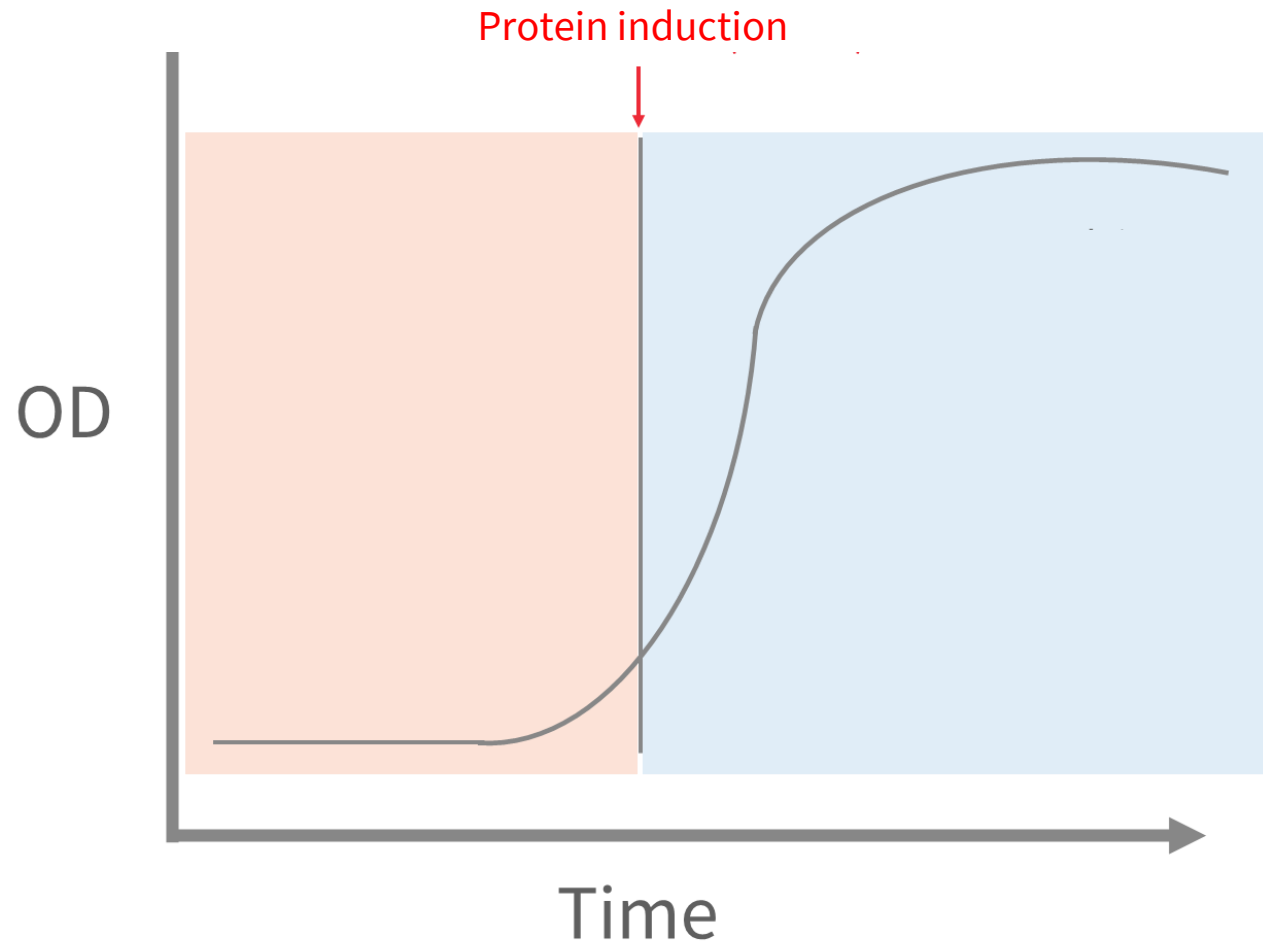
Proteomics-informed process changes increase product quality **and preserves activity**



**Beyond stability:
Informing
manufacturing
through proteomics**



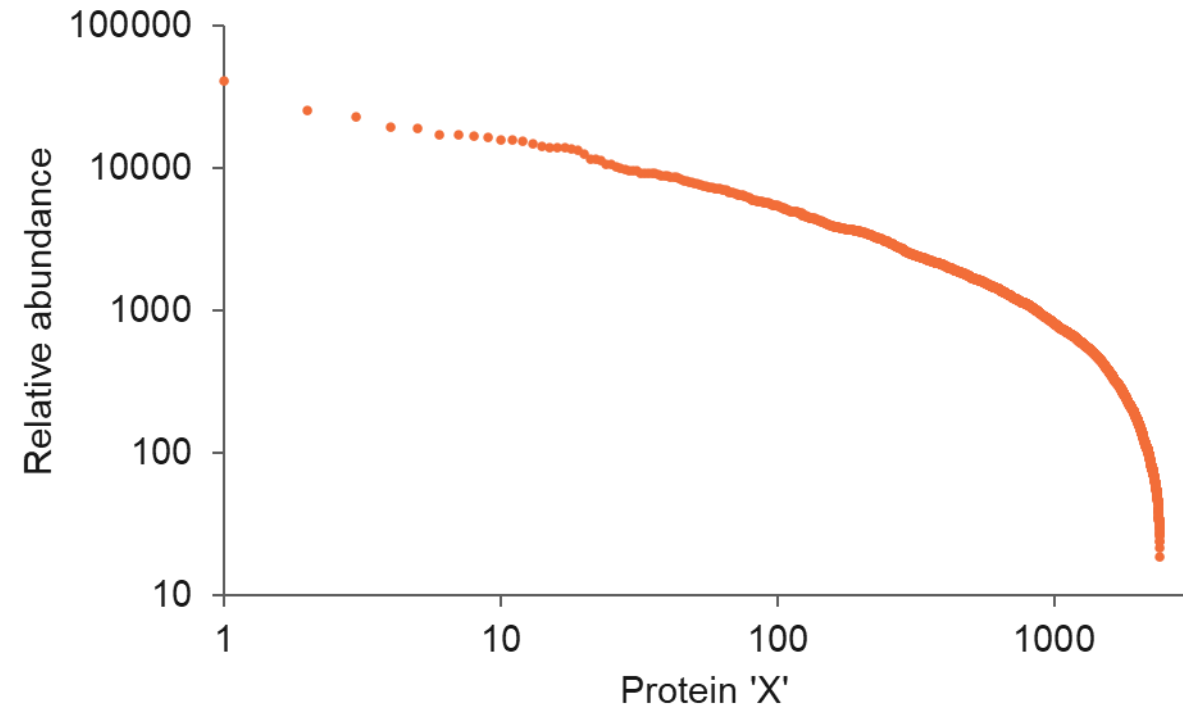
A brief overview of manufacturing bacterial products



Lyophilized EcN

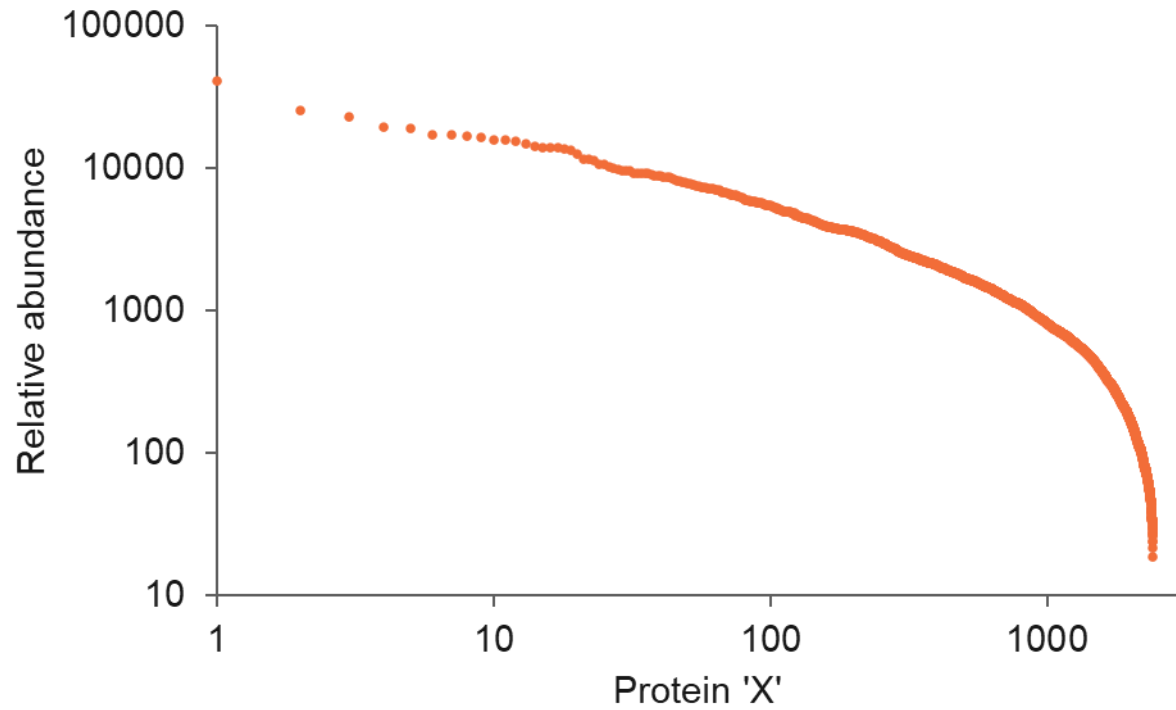
Assessing proteome-wide changes from manufacturing

Lot A: Replicate 1

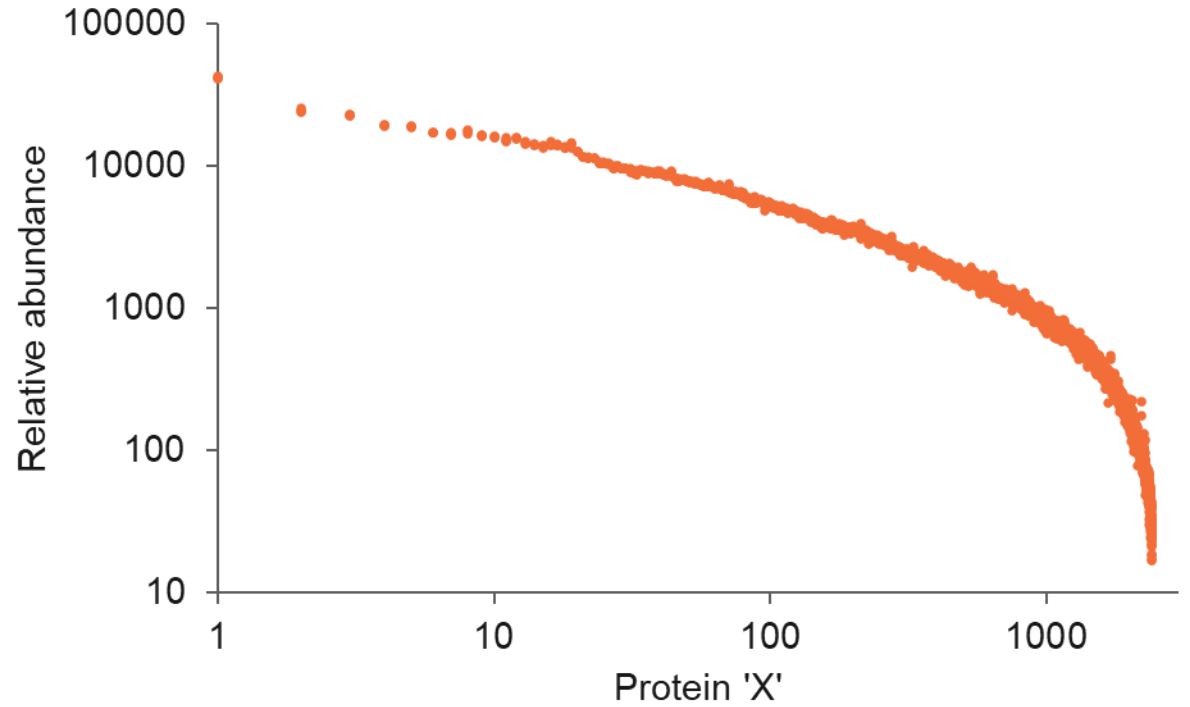


Mass spec provides window on protein expression levels within manufacturing lots

Lot A: Replicate 1

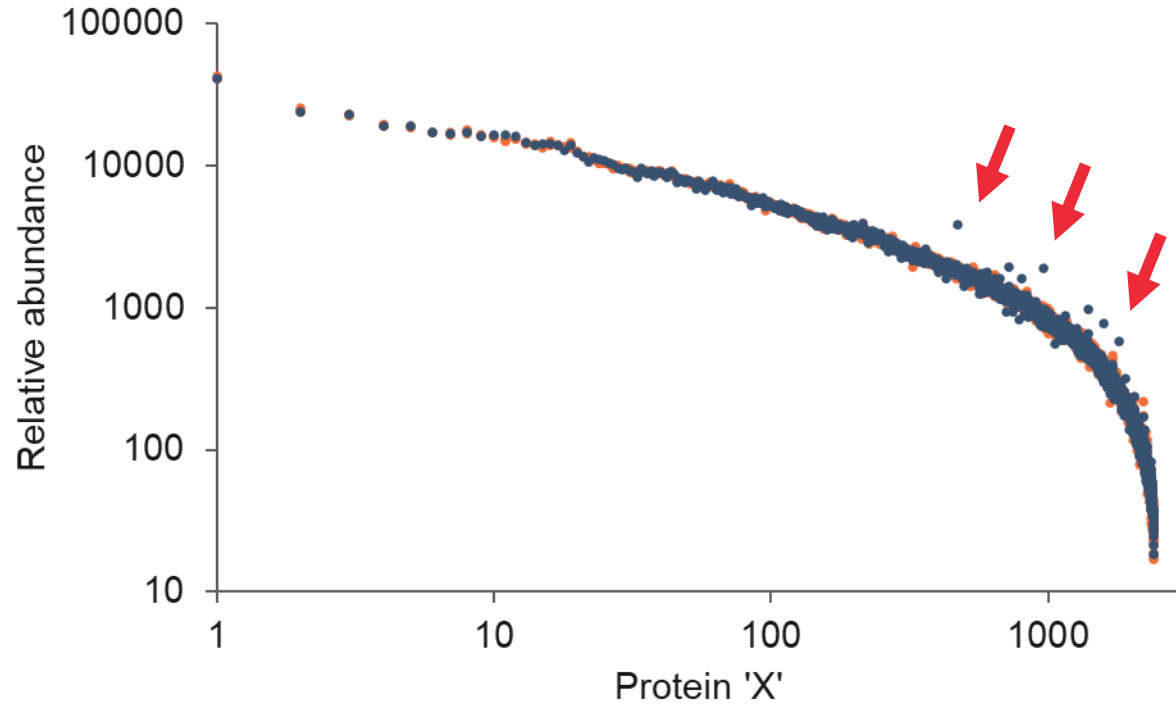


Lot A: Replicates 1-3

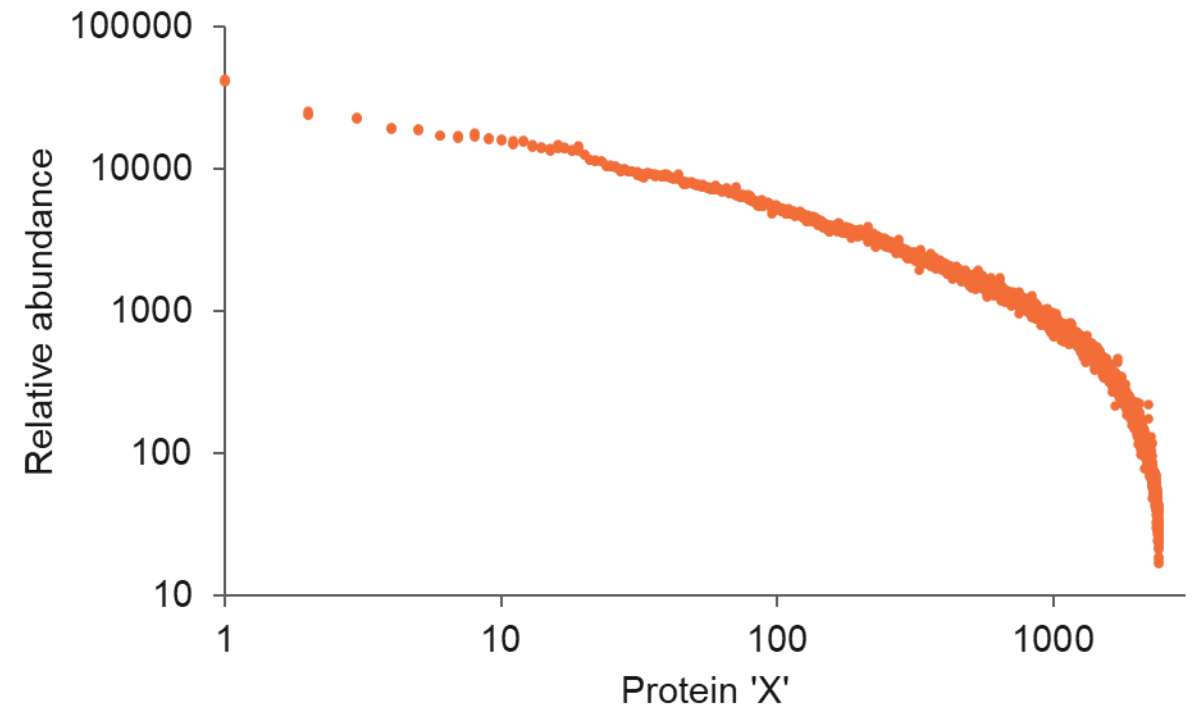


Proteomics can reveal minor and major changes between manufacturing lots

Lot A vs Lot B

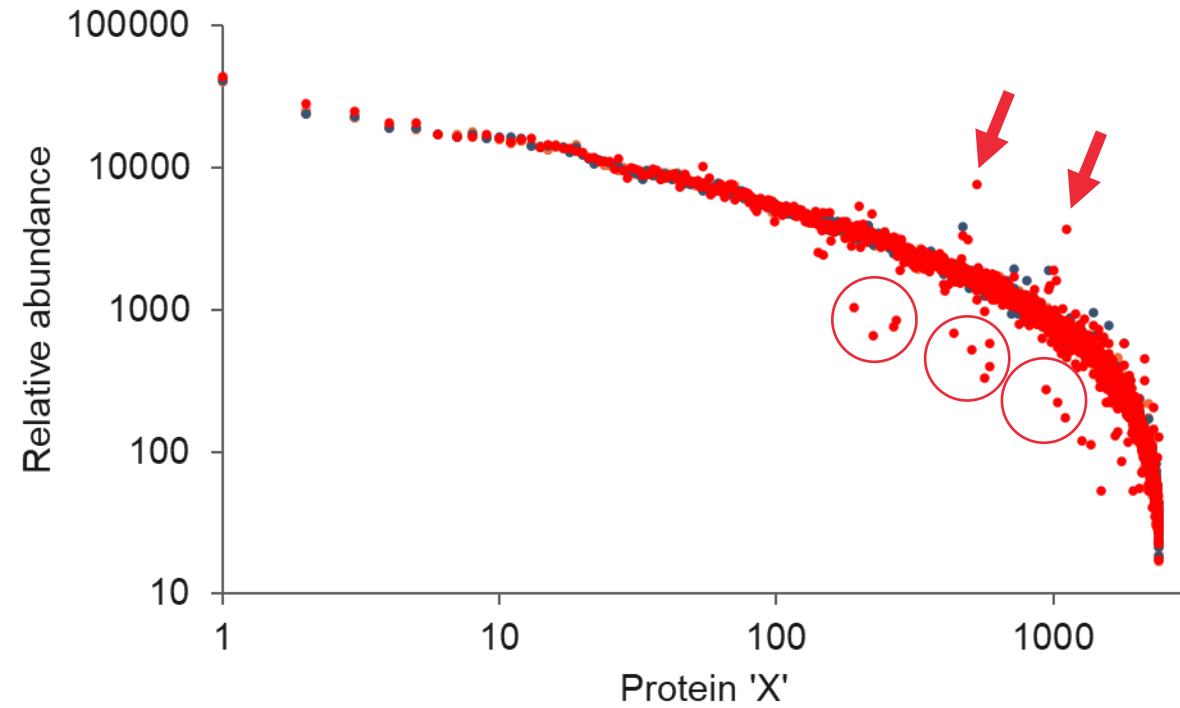


Lot A: Replicates 1-3

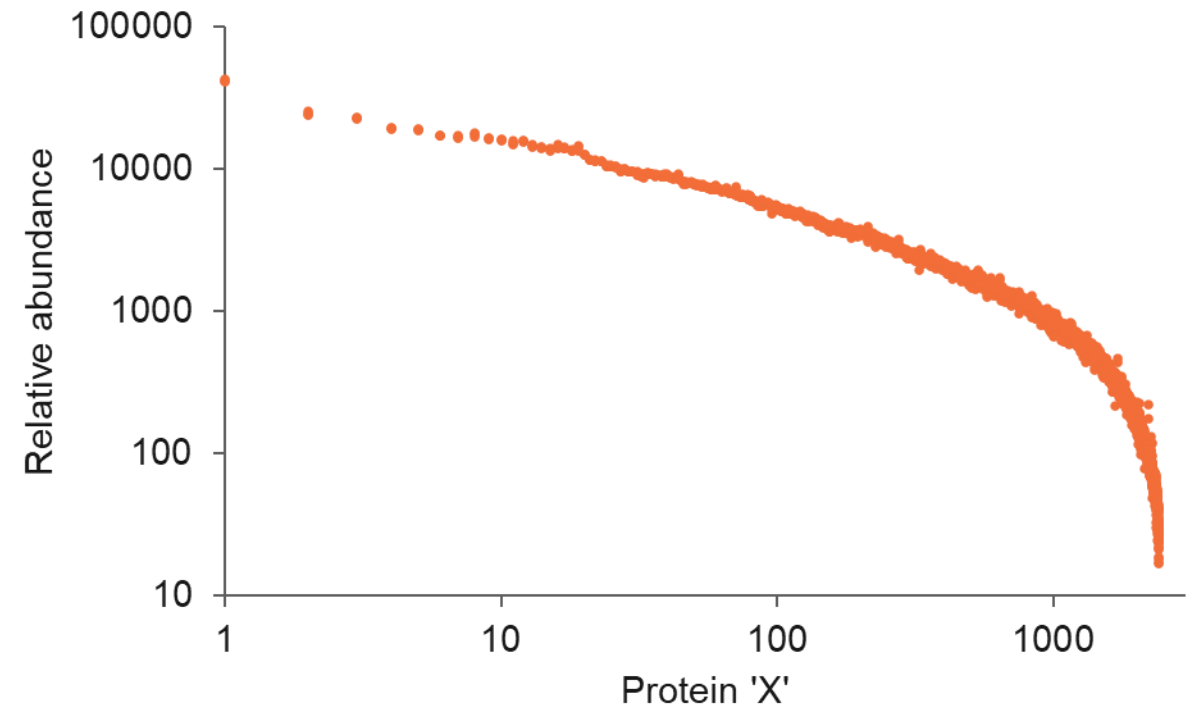


Proteomics can reveal minor **and major** changes between manufacturing lots – protein ID can reveal causes/fixes

Lot A vs Lot C

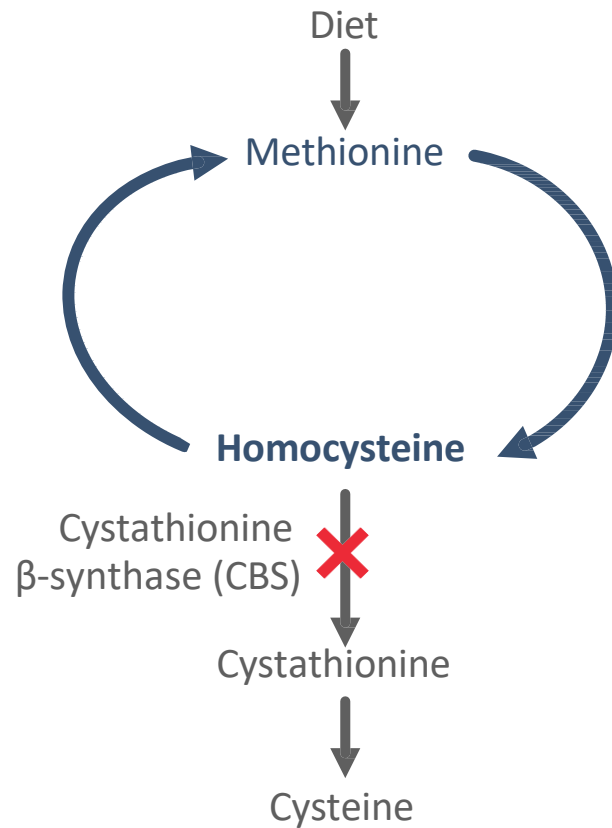


Lot A1: Replicates 1-3

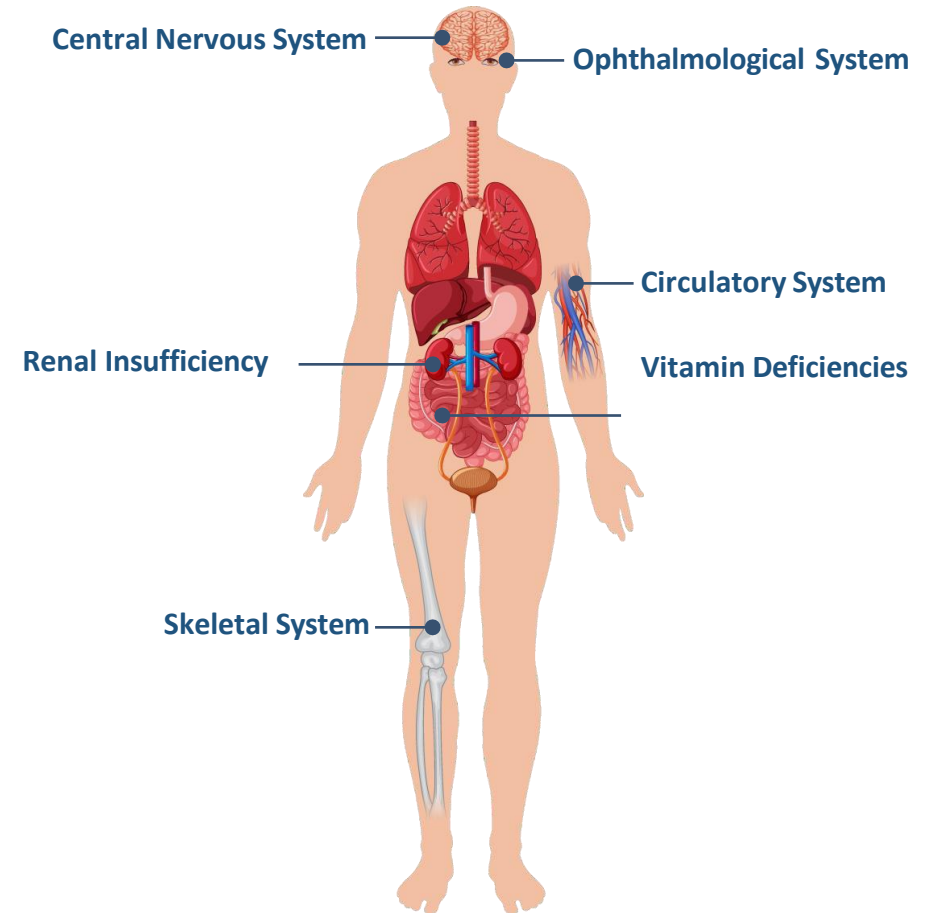


HCU: An Inborn Error of Metabolism with Multisystem Burden

CBS Deficiency Results in Accumulated Homocysteine¹

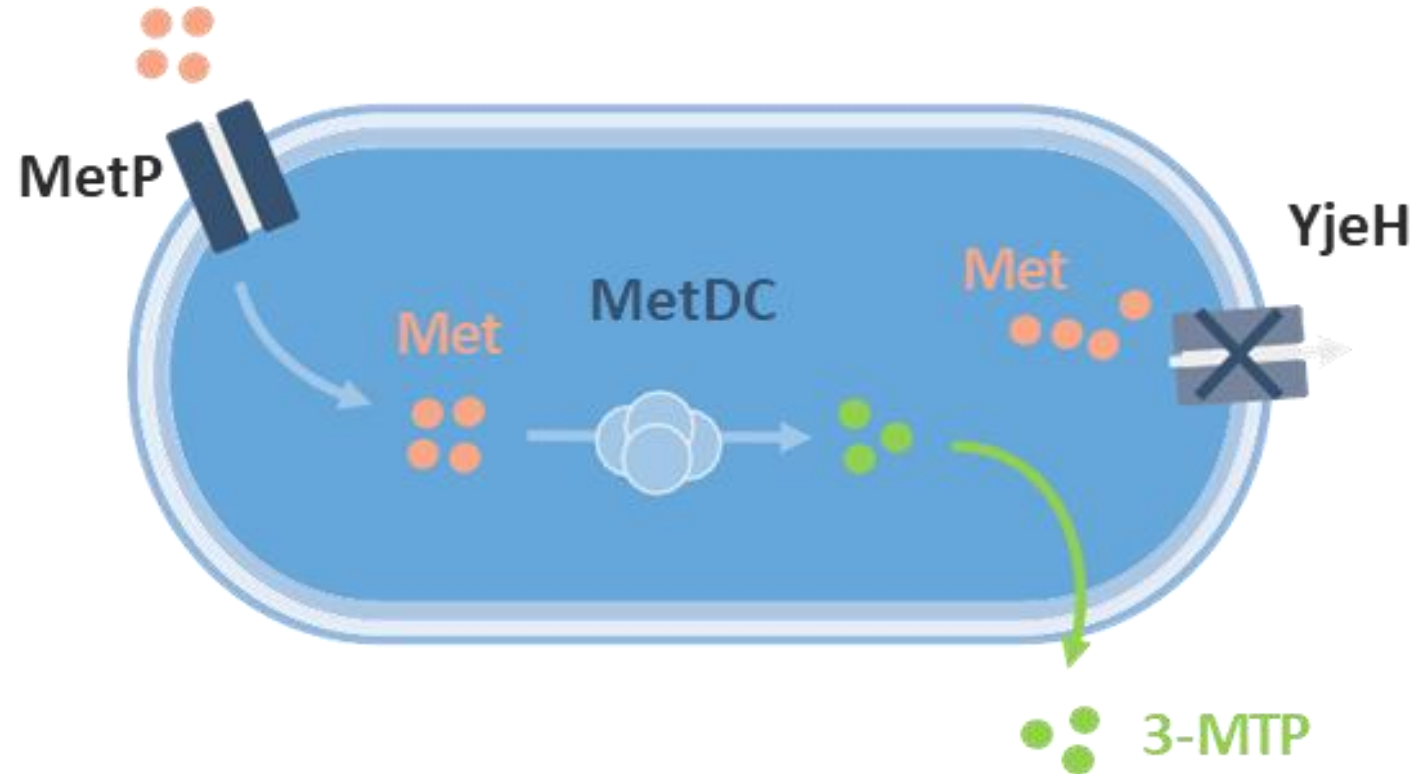


Multisystem Burden



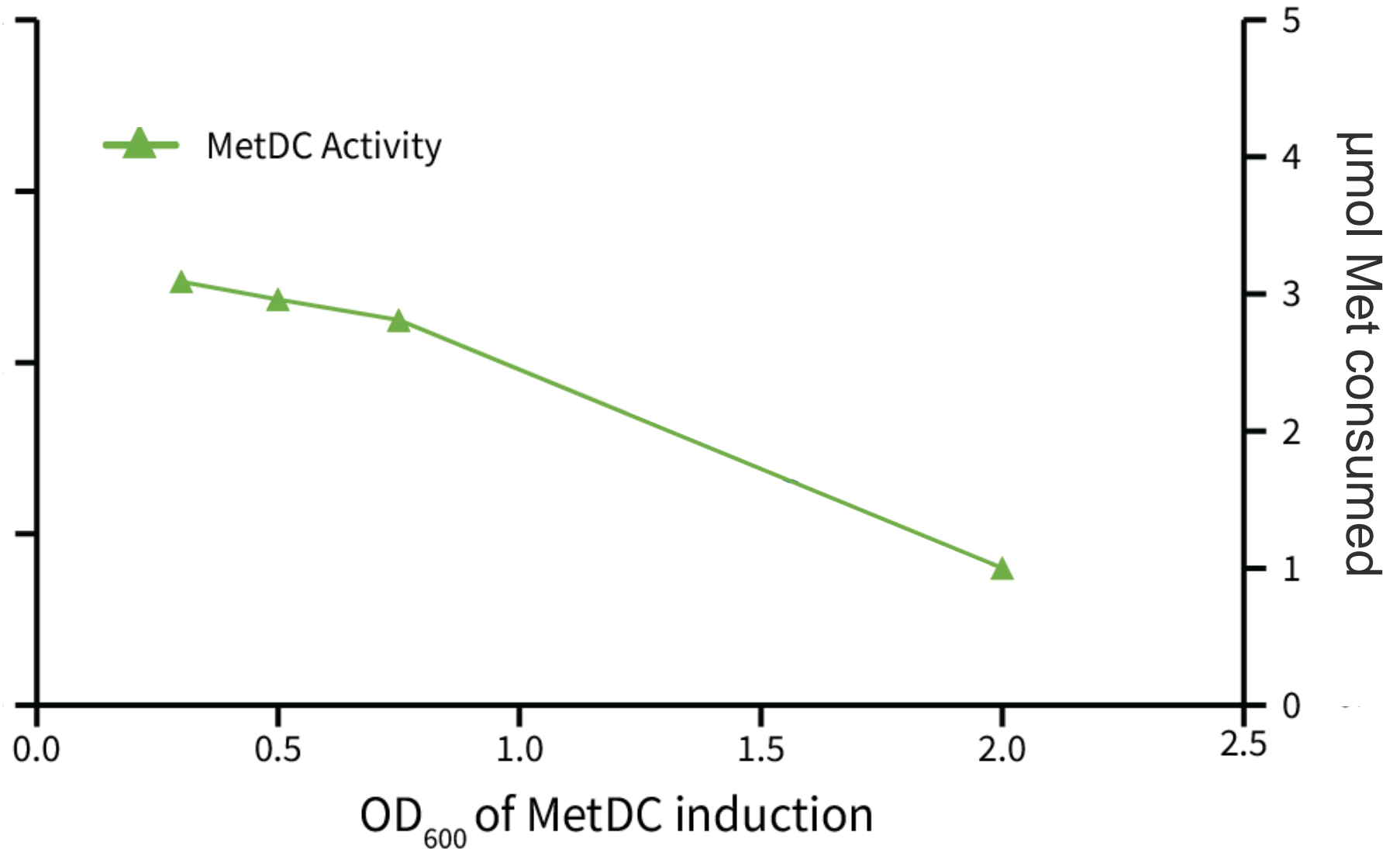
SYNB1353: A Methionine-consuming synthetic biotic

Methionine (Met)

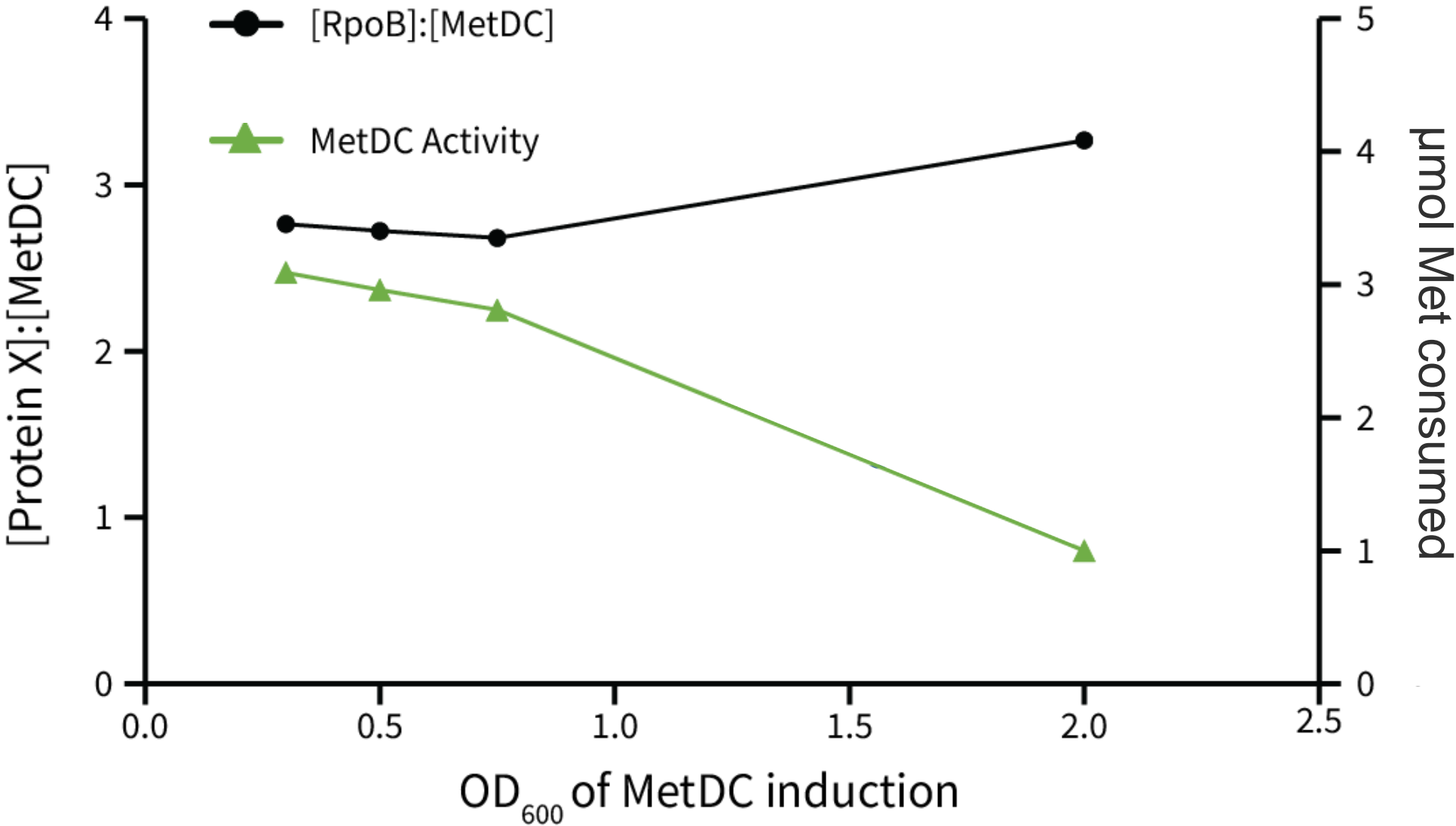


***E. coli* Nissle chassis**

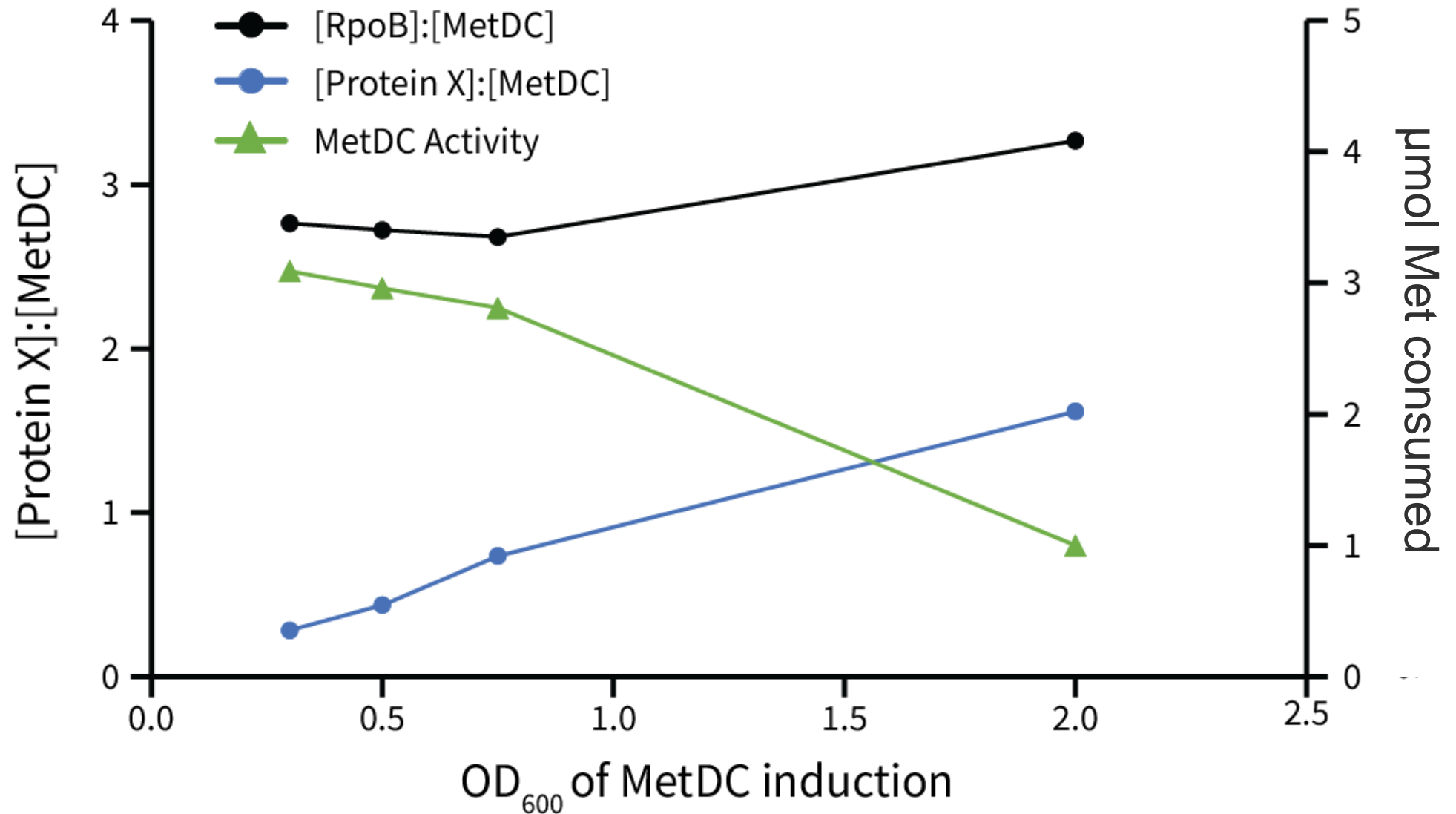
Altered fermentation conditions change product activity – why?



Proteomics show no significant change to MetDC levels



Proteomics **do** reveal expression of a putative competitor protein



Take-home messages

Metrics beyond viability
are needed to predict and
improve activity of cell-
based therapies

Peptide-level proteomics
reveal **abiotic**
mechanisms underlying
LBP activity

Protein-level proteomics
reveal **biotic** mechanisms
underlying LBP activity

Thank you!

CASSS organizers

The audience (you!)

Synlogic Therapeutics