CASSS - MASS SPECTROMETRY 2024
Session: VIII Multi-omics in Process Development

Targeted Metabolomic Analysis of mAb producing CHO cells: Impact of Bioprocess Conditions on CHO Cell Metabolism and Lactate runaway

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Upstream Development and Analytical Development and Attribute Science,
Biologics Development

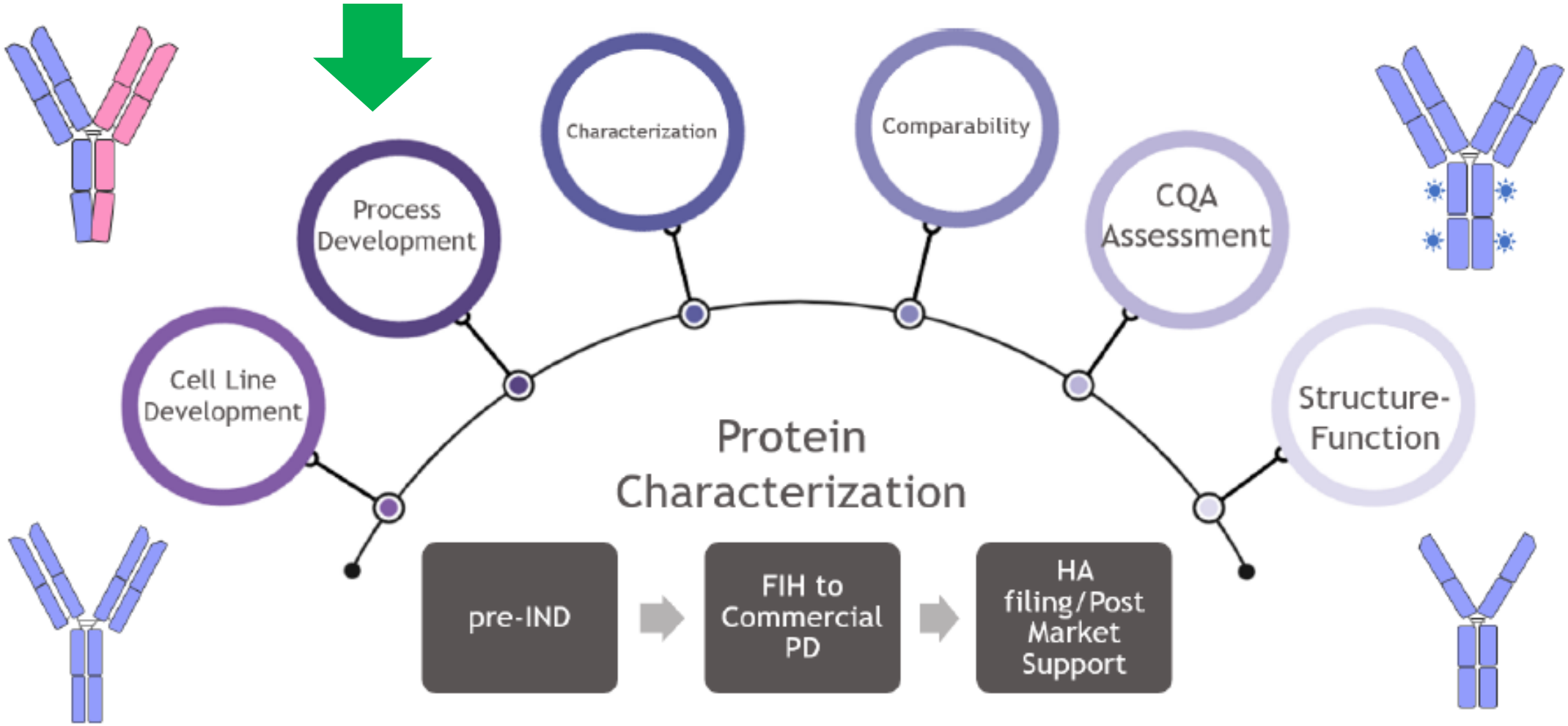
September 10-13, 2024

Bristol Myers Squibb™

Presentation Outline

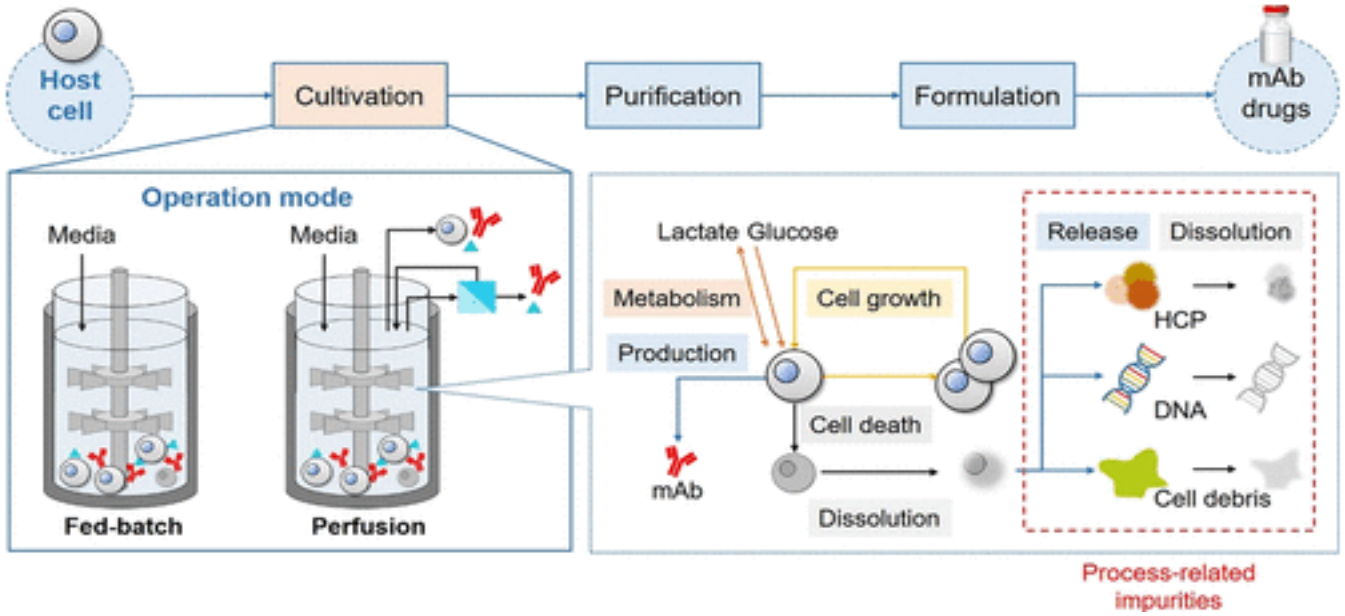
- Complexity of Bioprocess and Analytical Techniques- Omics
- Target Metabolomics Method Development
- Bioprocess Lactate runaway Case study
- Targeted Metabolomics Analysis – Results
- Conclusion and Next step

Mass spectrometry (LCMS) Plays an Integral Role in Biologics Development to Commercialization



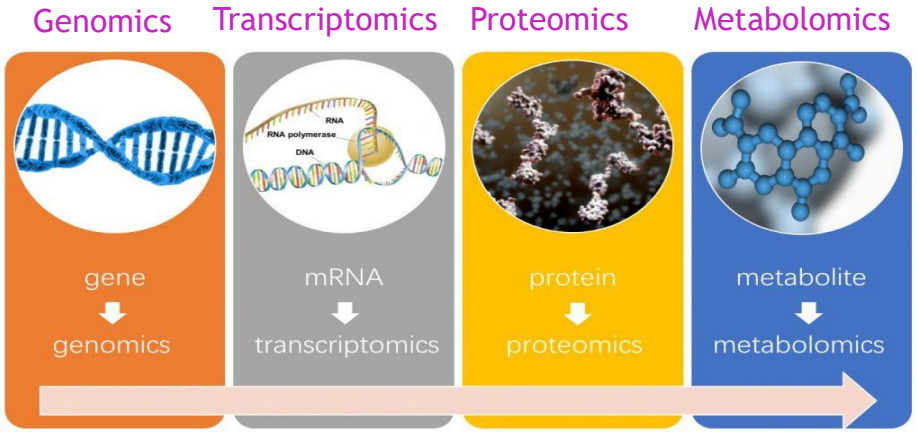
Challenges

Bioprocess: Complex



Analytical: Mass spectrometry: Complex

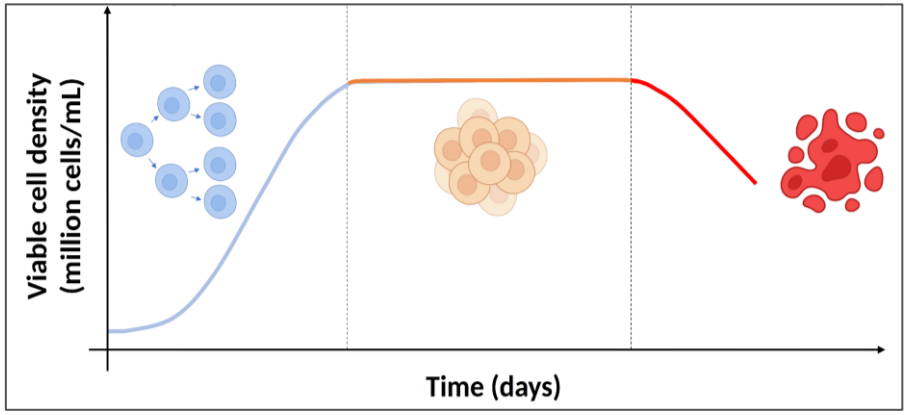
Omics: Highly Complex



Discovery Metabolomics (omics)	Target Metabolomics (omics)
Low -Throughput	High-Throughput
Large data sets, Relative Quant.	Small data set, Absolute Quant
Complex Analysis	Less complex
Generating Hypothesis	Hypothesis Driven

Bioprocess (CHO Cell Culture): Complexity and the Problem

The Problem:



- **Exponential Phase**
  - Glycolysis active
  - High Growth
  - Lactate Production
- **Stationary Phase**
  - TCA active
  - High Qp
  - Lactate Reutilization
- **Death Phase**
  - Low Qp
  - High Lactate/Ammonia

The Problem: Phase Specific-Flux, disruption or inhibition in metabolism

- ➢ Metabolite accumulation: Lactate or Ammonia
- ➢ Impact Product Yield and Product Quality

The Problem: Clone Selection to Scaleup- Phase Specific

- ➢ Poorly understood
- ➢ Not easy to control

The Complexity & Analytical Challenge:

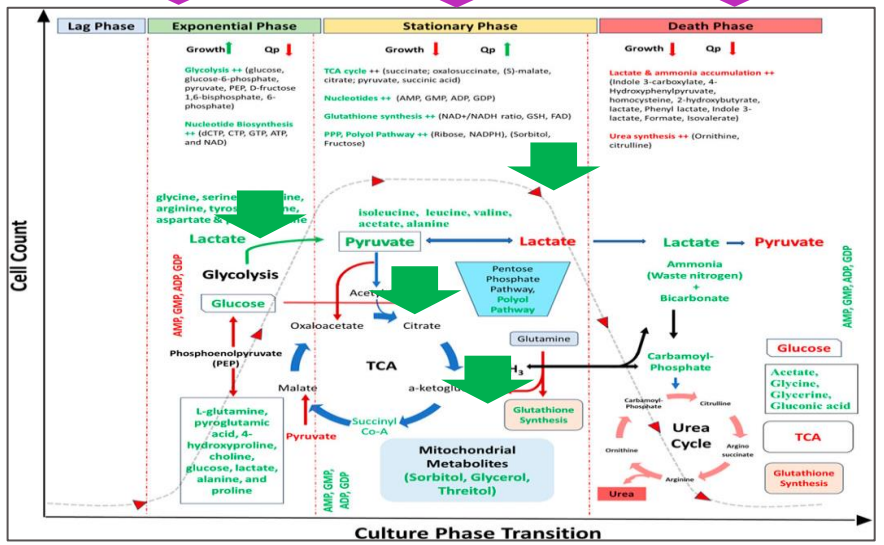
CHO Cell Culture: Feed (e.g.: glucose or amino acid)

& Biochemical and Engineering Parameters

CHO Cell Culture Phase Transition & Metabolism

Pathway: Glycolysis, TCA Cyle, Pentose Phosphate Pathway, ETC, ROS

Location: Mitochondria and Cytoplasm



Reference: 1. Advancements in CHO metabolomics: techniques, current state and evolving methodologies Front. Bioeng. Biotechnol., 26 March 2024, Sec. Bioprocess Engineering, Volume 12 - 2024

2. Metabolic Profiling of CHO Cells during the Production of Biotherapeutics Cells. 2022 Jun; 11(12): 1929

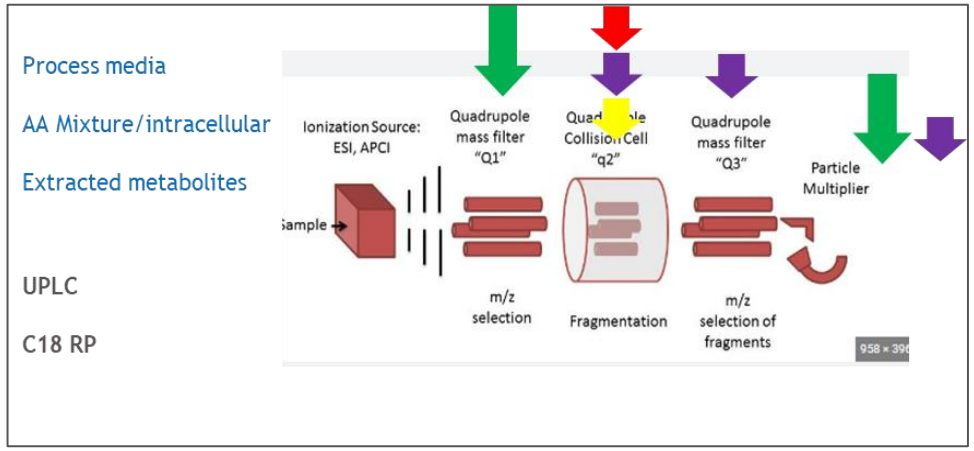
Analytical: Target Metabolomics and Pathway Analysis

How to analyze and follow the process for developing control strategies ?

- Bioprocess parameters (DO, CO2, pH), Glucose, Lactate, Amino acids (Glutamine, Glutamate): Offline or On-line Analysis
- Extra cellular and Intracellular Metabolite and Pathway Analysis: Off-Line LCMS or Untargeted or Targeted Omics Analysis

- Targeted LCMS Metabolomics: Multiple reaction monitoring (MRM) is the most common method for quantitation of metabolites by Triple Quad based LC/MS/MS Analysis.
- MRM: ions are selected to make it through the first quadrupole and into the collision cell. These ions are referred to as the precursor, or parent, ion. These ions are fragmented in the collision cell and the fragmented ion (daughter ion) with precursor selectively detected and quantified.

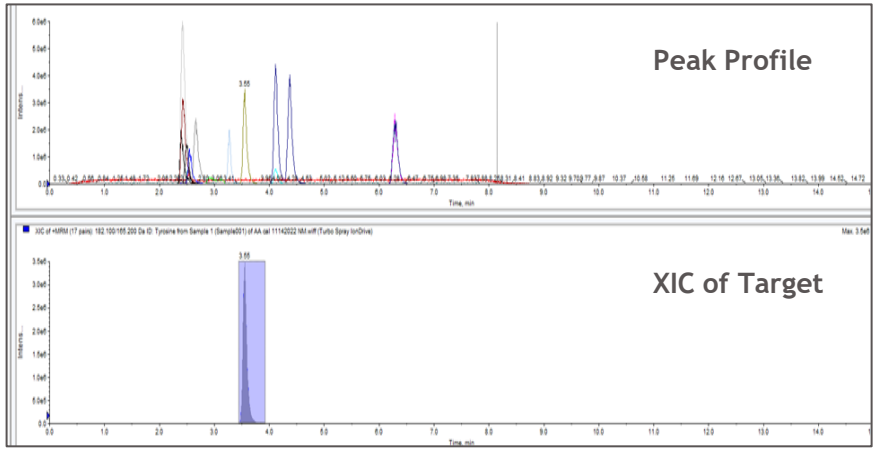
Targeted MRM Principle



MRM Transitions

Q1	Q3	RT	ID
76.100	30.000		glycine
90.100	44.000		Alanine
106.100	60.000		Serine
116.100	70.000		Proline
118.100	55.000		Valine
120.100	103.200		Threonine
132.100	86.000		Lucine Isoluc
132.100	69.000		Isoleu 2
134.100	74.000		aspartic acid
147.100	84.000		Lysine
148.100	84.000		Glutamic acid
150.200	104.000		methionine
156.100	110.000		histidine
166.100	103.000		phenylalanine
175.200	70.000		Arginine
182.100	165.200		Tyrosine
241.100	152.100		Cystine

TIC, XIC, Peak area, Calibration and Quantification



Targeted MRM Method Development: Amino Acid

LC

Gradient Table:

Time	Flow Rate	%A	%B	Curve
Initial	0.150	99.0	1.0	Initial
1.00	0.150	95.0	5.0	6
2.00	0.150	90.0	10.0	6
6.00	0.150	75.0	25.0	6
7.00	0.150	65.0	35.0	6
8.00	0.150	50.0	50.0	6
10.00	0.150	25.0	75.0	6
12.00	0.150	1.0	99.0	6
13.00	0.150	99.0	1.0	6
15.00	0.150	99.0	1.0	6

QQQ- transition- +ve MS

Q1	Q3	RT	ID
76.100	30.000		glycine
90.100	44.000		Alanine
106.100	60.000		Serine
116.100	70.000		Proline
118.100	55.000		Valine
120.100	103.200		Threonine
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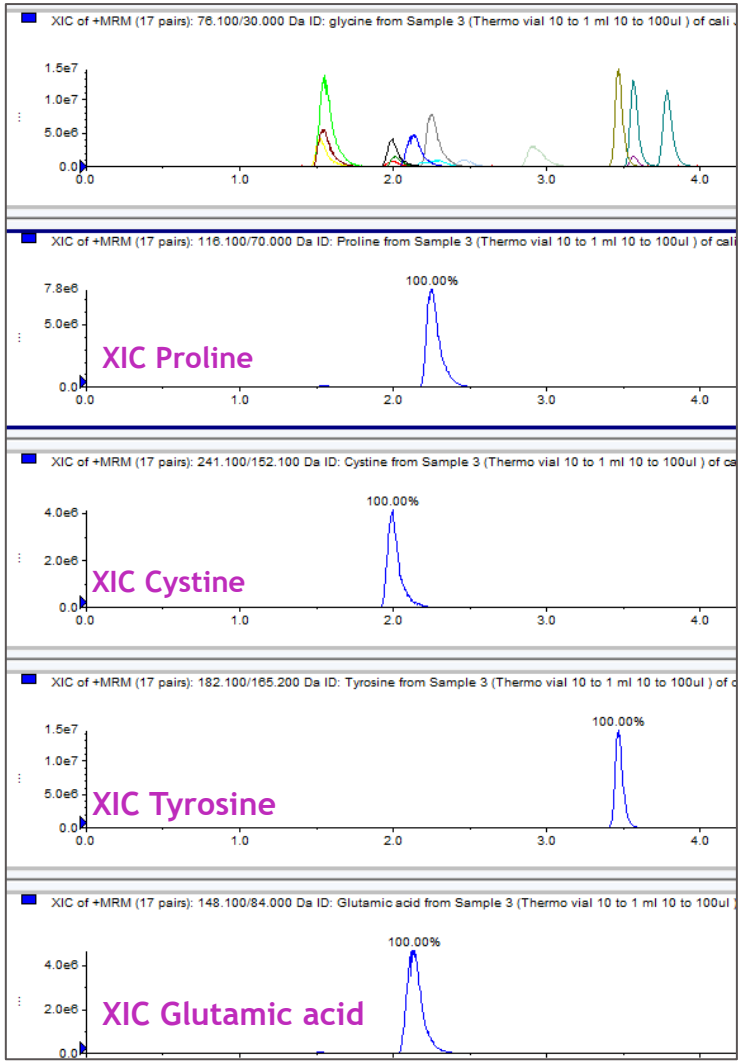
148.100	84.000		Glutamic acid
150.200	104.000		methionine
156.100	110.000		histidine
166.100	103.000		phenylalanine
175.200	70.000		Arginine
182.100	165.200		Tyrosine
241.100	152.100		Cystine

- 15 min Run Time, Waters UPLC/Sciex 6500 QQQ
- Reverse Phase Chromatography
- Quantitation: External Linear Calibration
- Analyst Software
- CHO -Cell culture media diluted and analyzed directly

E.g: LCMS amino acid profile



TCA Cyle Metabolite Analysis: Method

LC

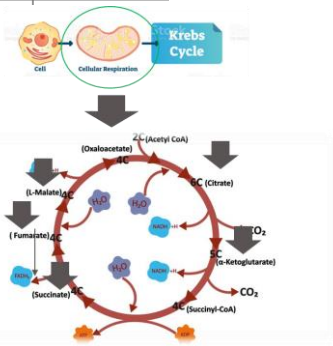
Gradient Table:

Time	Flow Rate	%A	%B	Curve
Initial	0.200	90.0	10.0	Initial
1.00	0.200	90.0	10.0	6
6.00	0.200	10.0	90.0	6
7.00	0.200	90.0	10.0	6
10.00	0.200	90.0	10.0	6

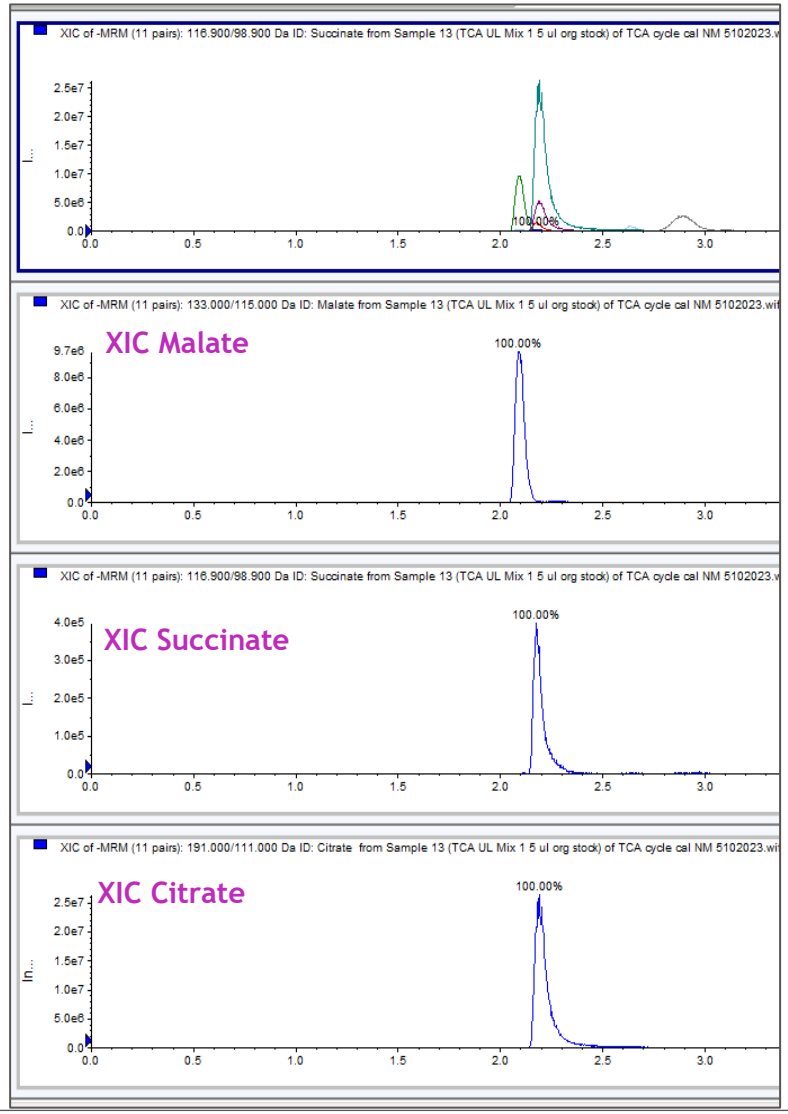
QQQ- transition- -ve MS

	Q1 Mass (Da)	Q3 Mass (Da)	Dwell Time (msec)	ID
1	116.900	98.900	5.0	Succinate
2	117.000	73.000	5.0	Succinate -b
3	133.000	115.000	5.0	Malate
4	145.000	101.000	5.0	Alpha ketp glutar
5	115.000	71.000	5.0	Fumarate
6	131.000	87.000	5.0	Oxaloacetate
7	191.000	111.000	5.0	Citrate
8	191.000	87.000	5.0	Citrate b
9	117.900	98.900	5.0	suc iso
10	87.000	43.000	5.0	Pyruvate
11	87.000	32.000	5.0	pyruvate b

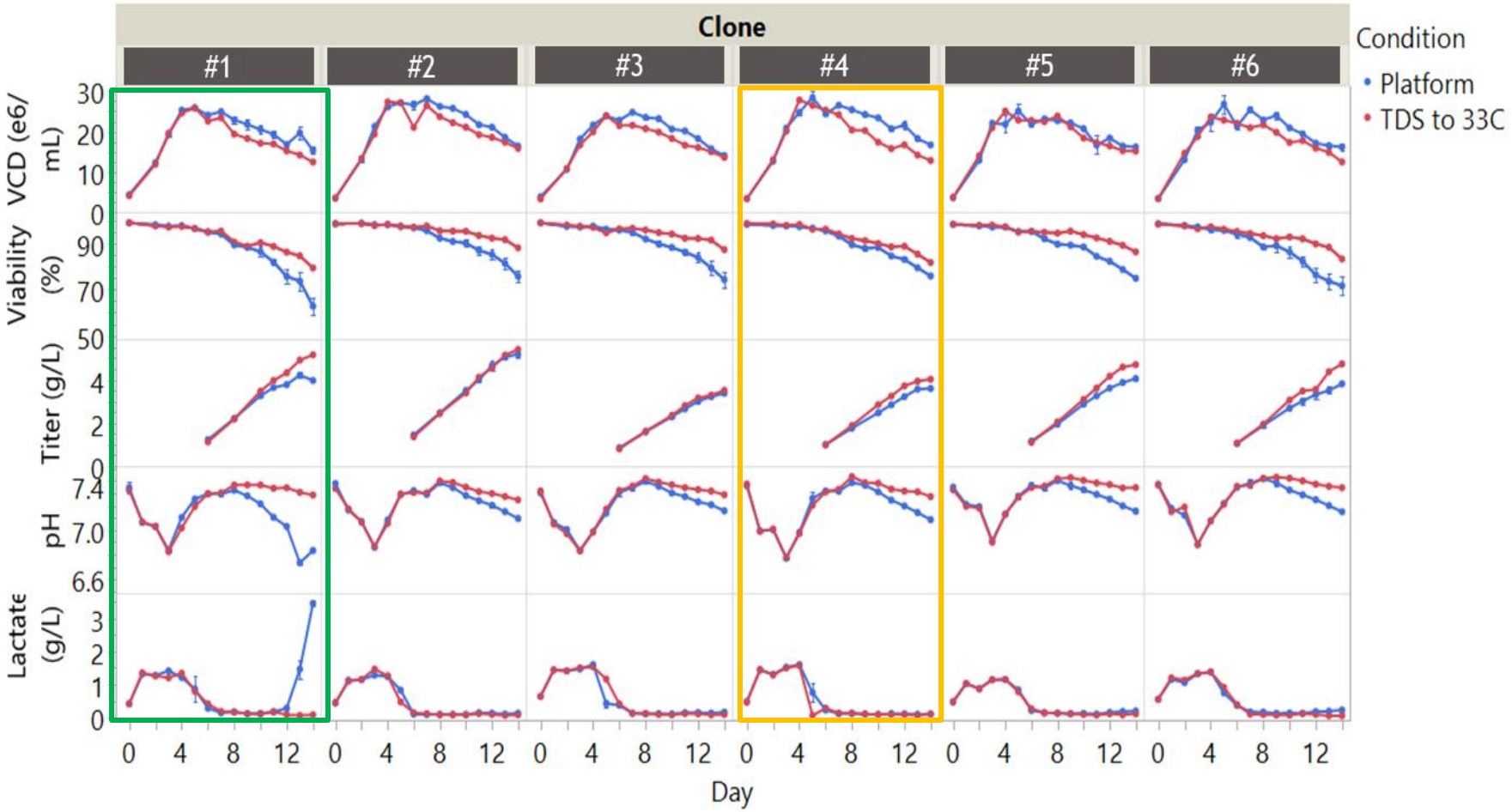
- 10 min Run time, **Waters UPLC/Sciex 6500 QQQ**
- Reverse Phase
- Quantitation: External Linear Calibration
- Analyst Software
- Cell pellet: Freeze and Thaw, Acidified Methanol Extraction



E.g: LCMS TCA Cycle Metabolite Profile

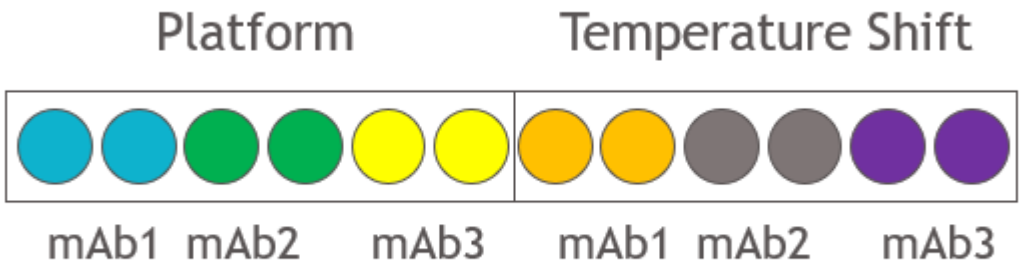


Case Study: Background: Historical Process Performance and Details



- **Issues**
  - pH drift followed by steep decline
  - Lactate runaway in the stationary phase
  - Titer stops increasing
- **Options**
  - Choose a different clone
    - Not possible due to stability and PQ
  - Find a control strategy for manufacturing
    - Attempted control strategy didn't work during scale-up

Case Study: Experimental Design



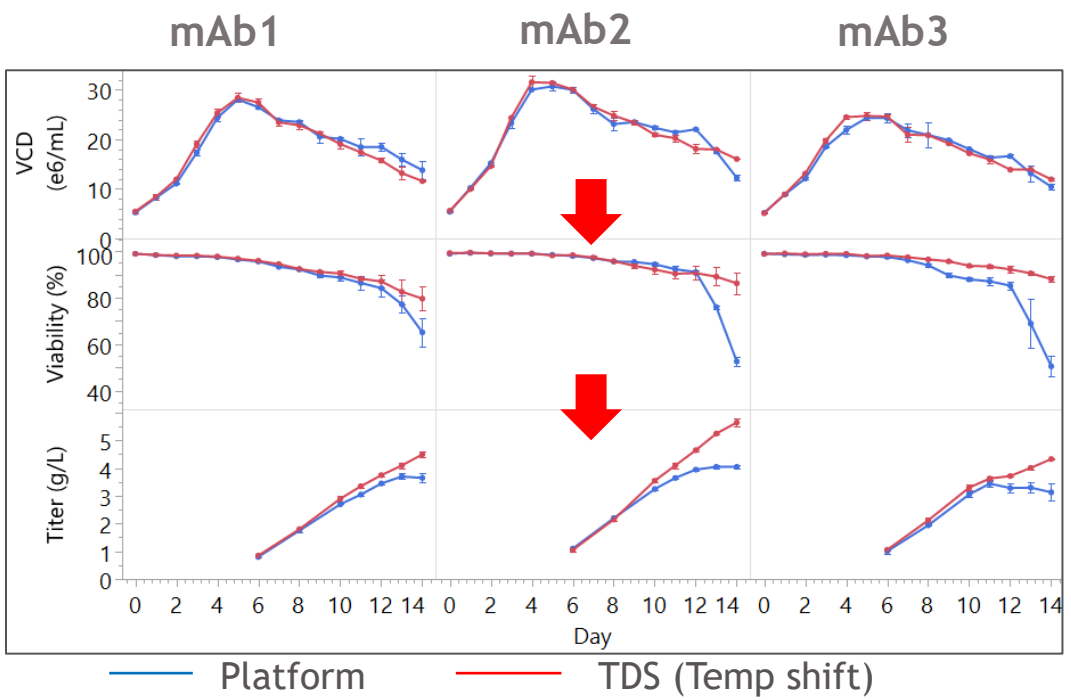
Process

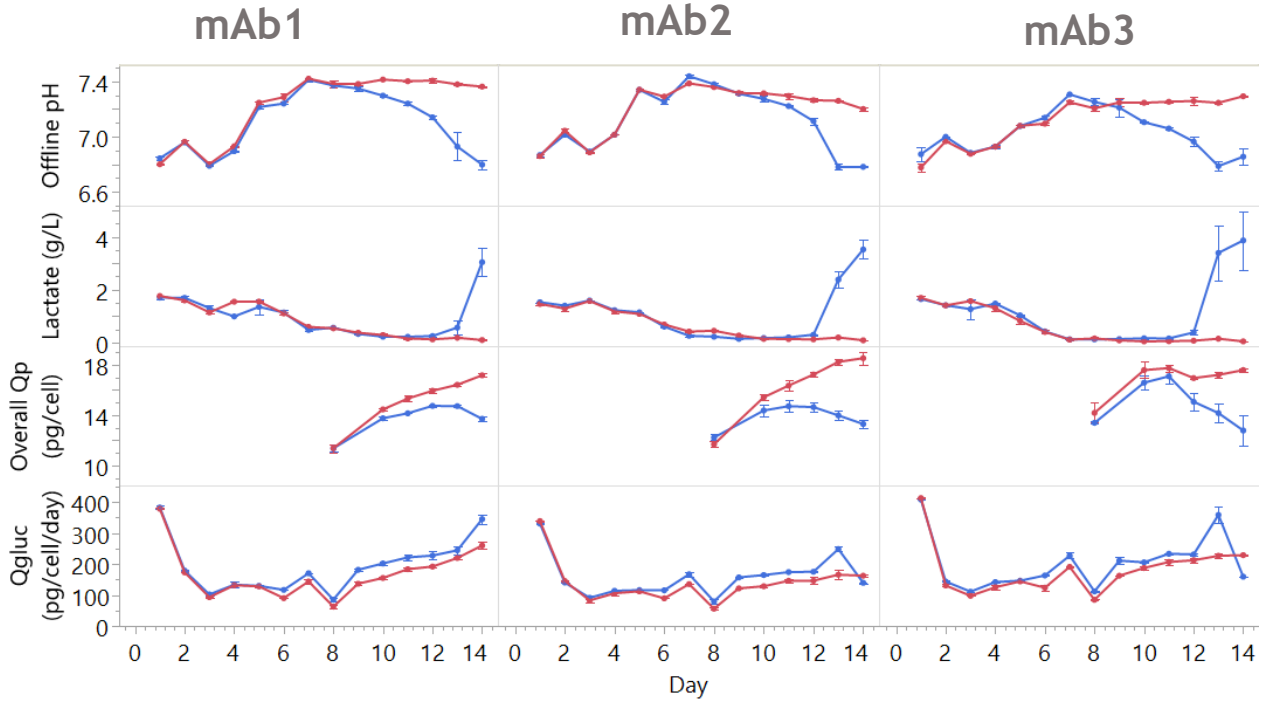
- Reactors – 12x ambr250
- Conditions – FIH Platform vs Temperature Downshift
- Programs – Bispecific mAb1, mAb2, mAb3
- Replicates – 2x of each program
- Sampling: Pellets and supernatant:0, 3, 6, 9, 12, 14 Days

LCMS Target Metabolomics

- Intracellular TCA cycle metabolite Sample preparation: Freeze and Thaw and acidified methanol extraction
- Extracellular amino acid: Dilution

Results: Case Study: Cell Culture Performance

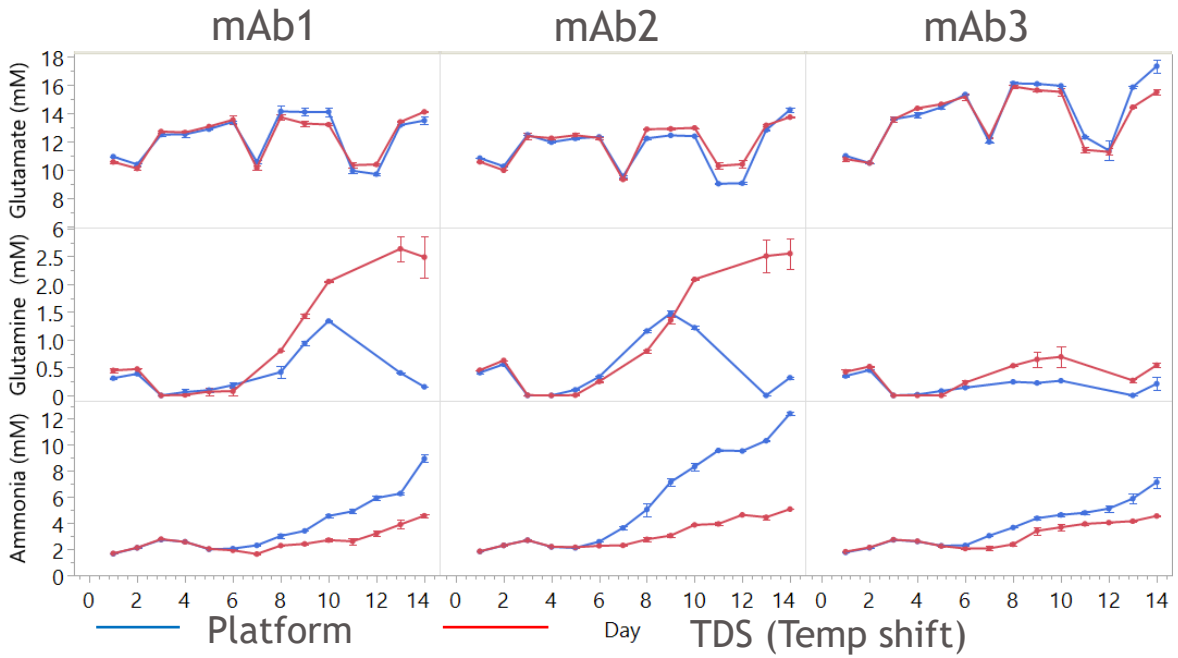


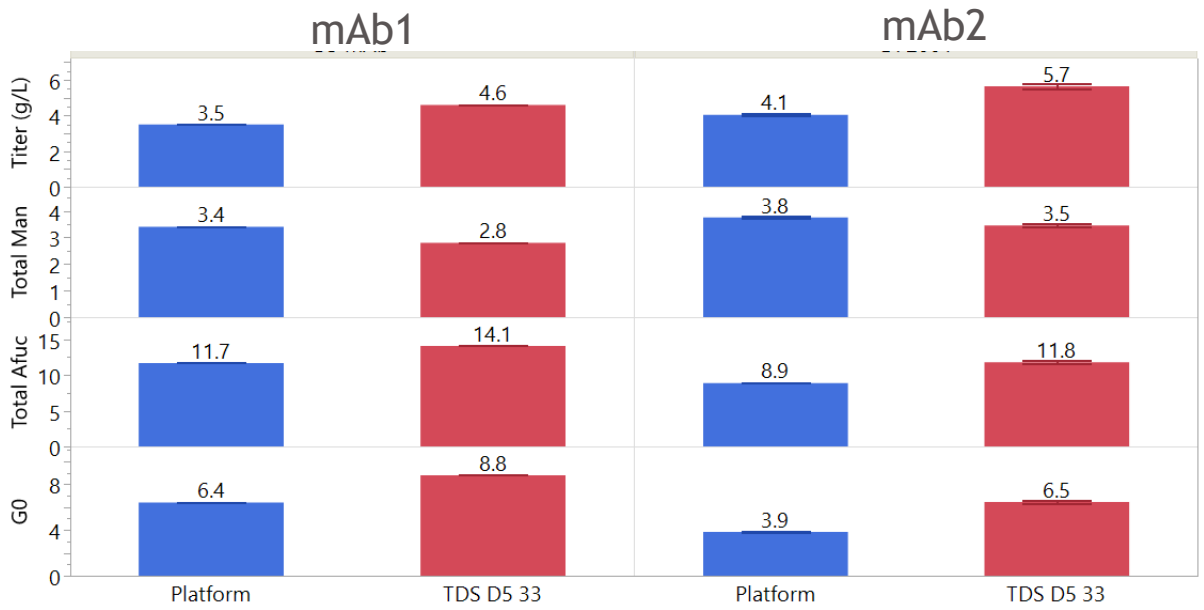


- Distinct difference in viability
  - Magnitude is mAb specific
- Titer flattens out

- pH slowly decreases
- Lactate increases D12-D13
- Qp decreases

Results: Case Study: Other Metabolites & Product Quality

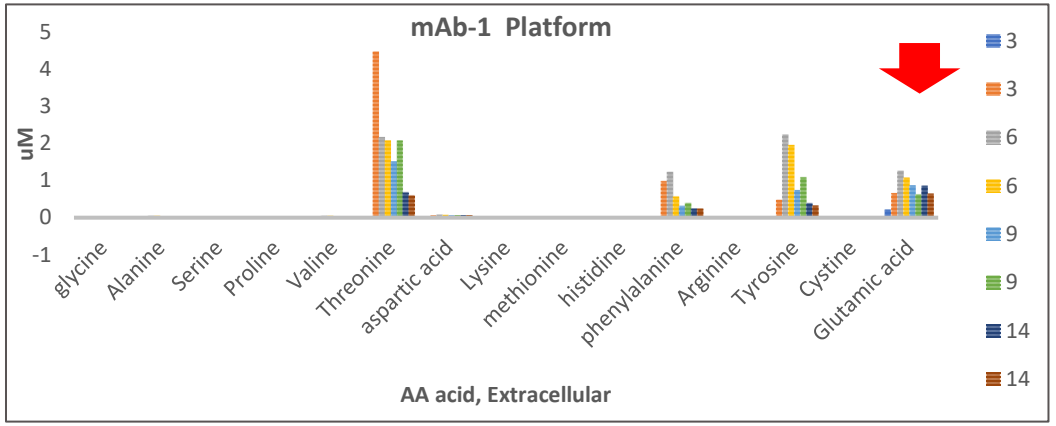


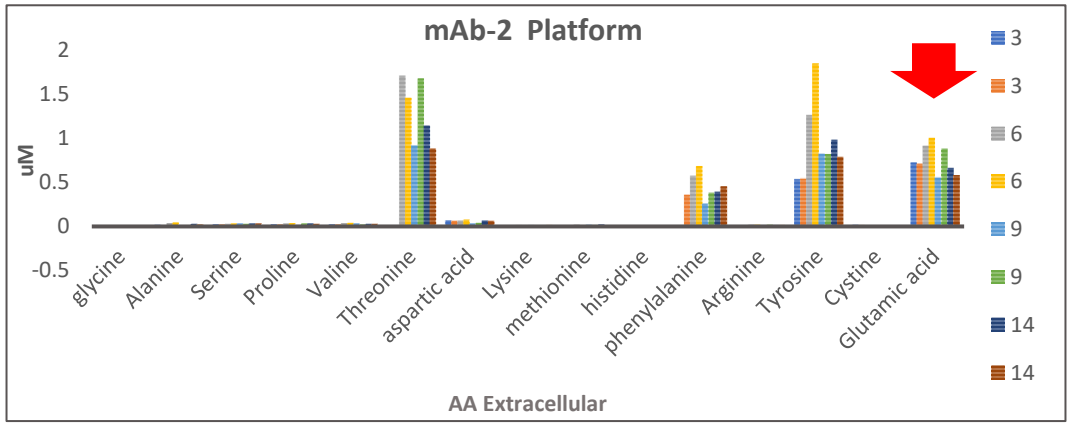


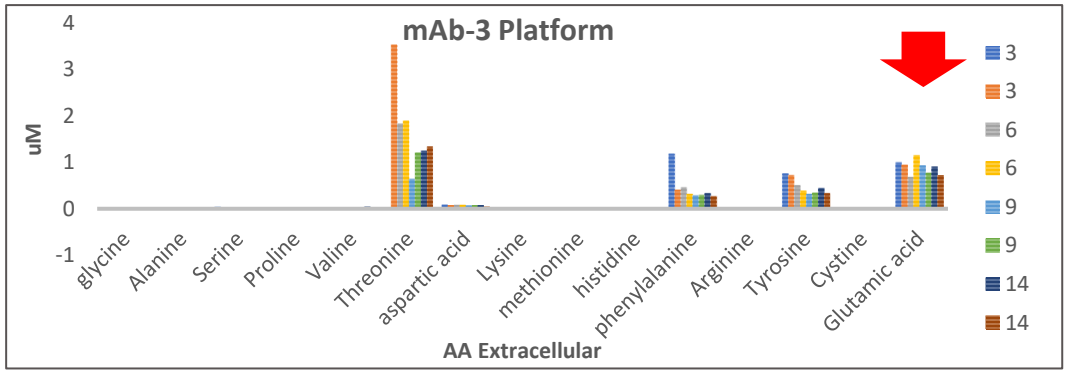
- Glutamate is high for both conditions
- Increased gln consumption for platform
- Higher ammonia production for platform

- Introducing a temperature shift was effective
  - Impacted product quality

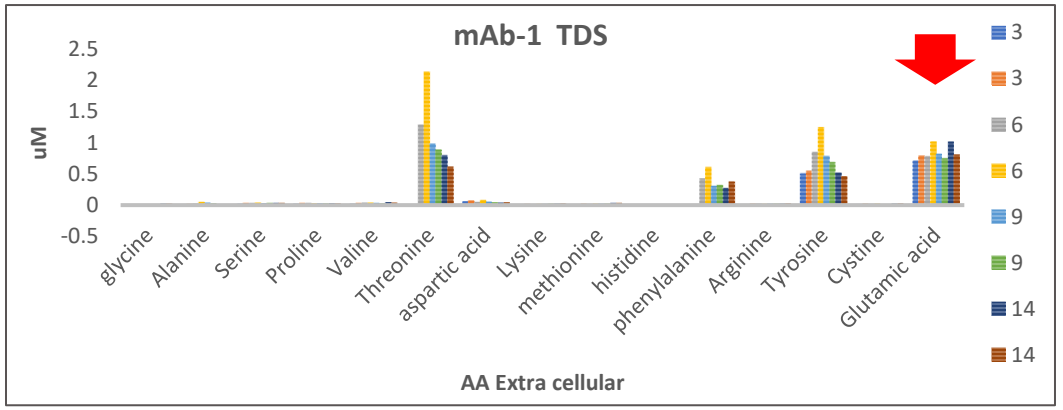
Targeted Metabolomics: Extracellular AA analysis- Process Day 3-14

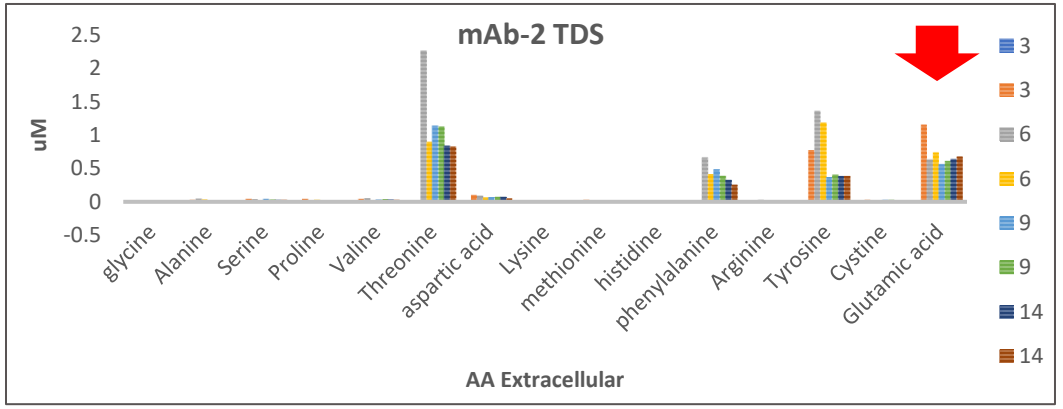


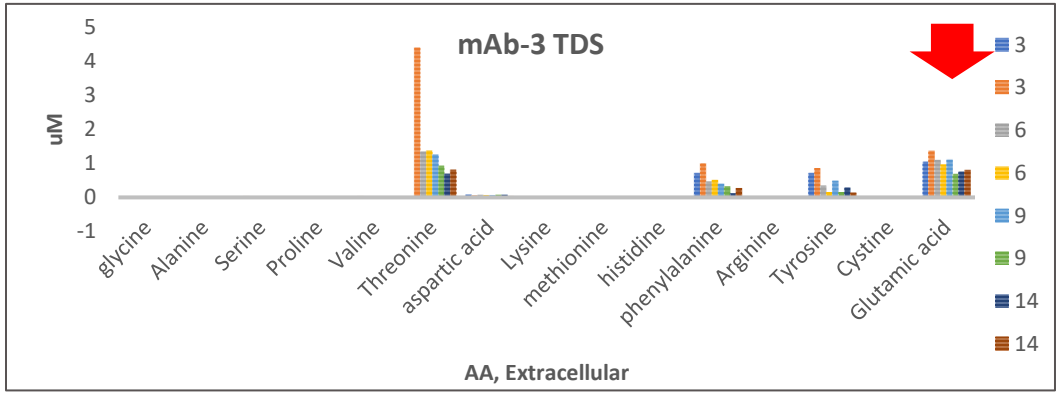




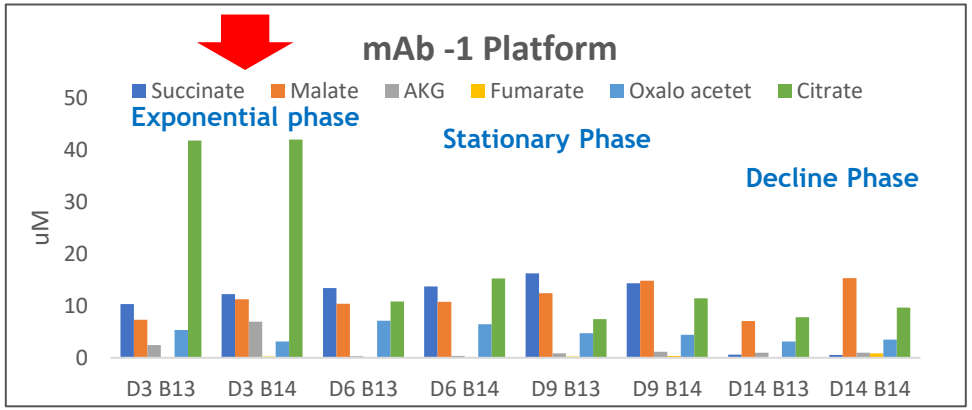
- All amino acid present throughout the cell culture
- Lucine, Phenyl Alanine, Threonine, Tyrosine, Glutamate present and high throughout the cell culture

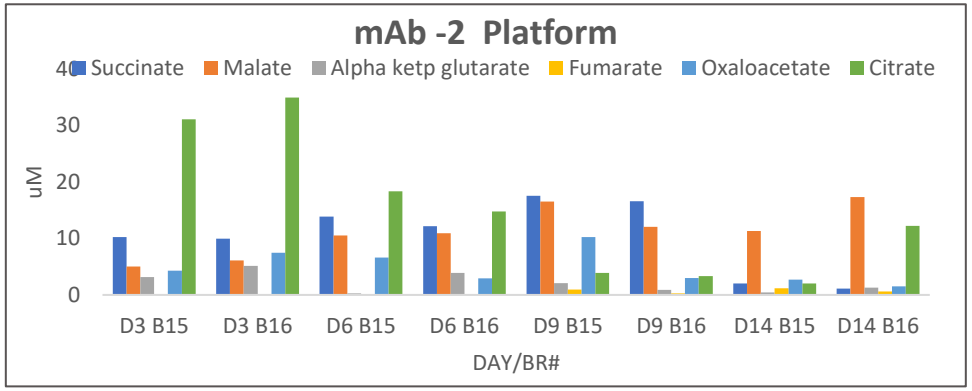


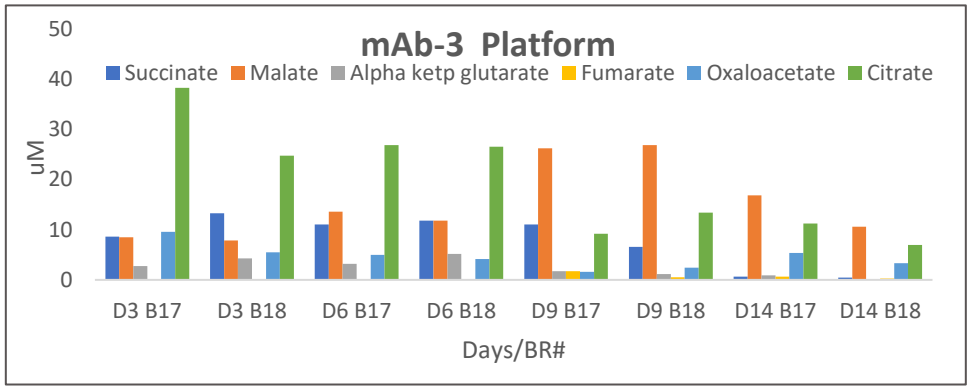




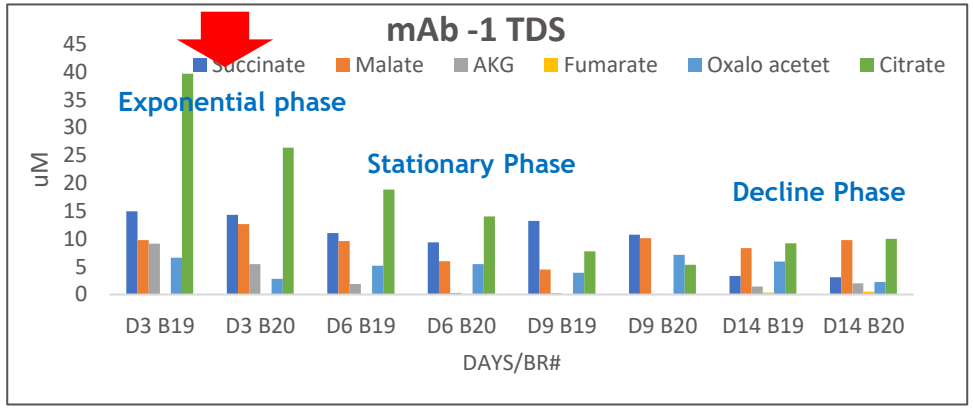
Targeted Metabolomics: TCA intracellular metabolite

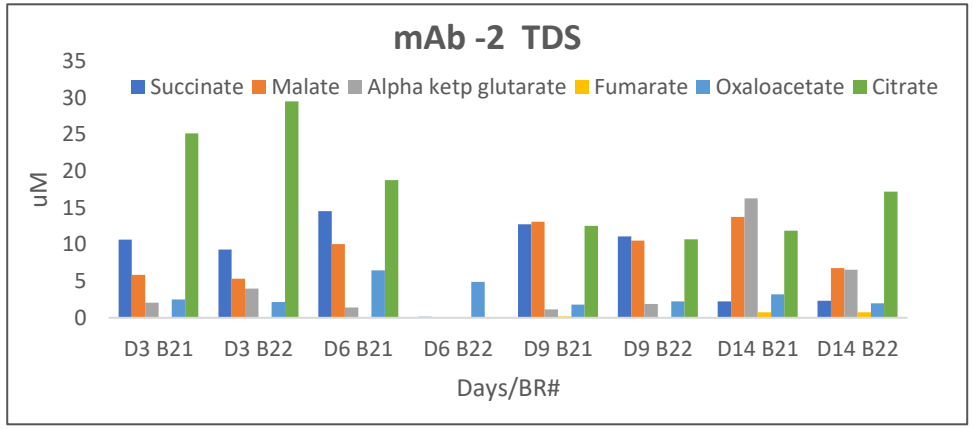


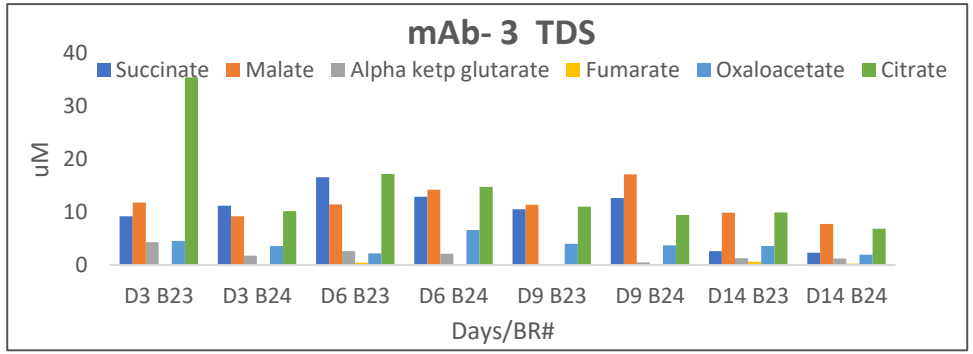




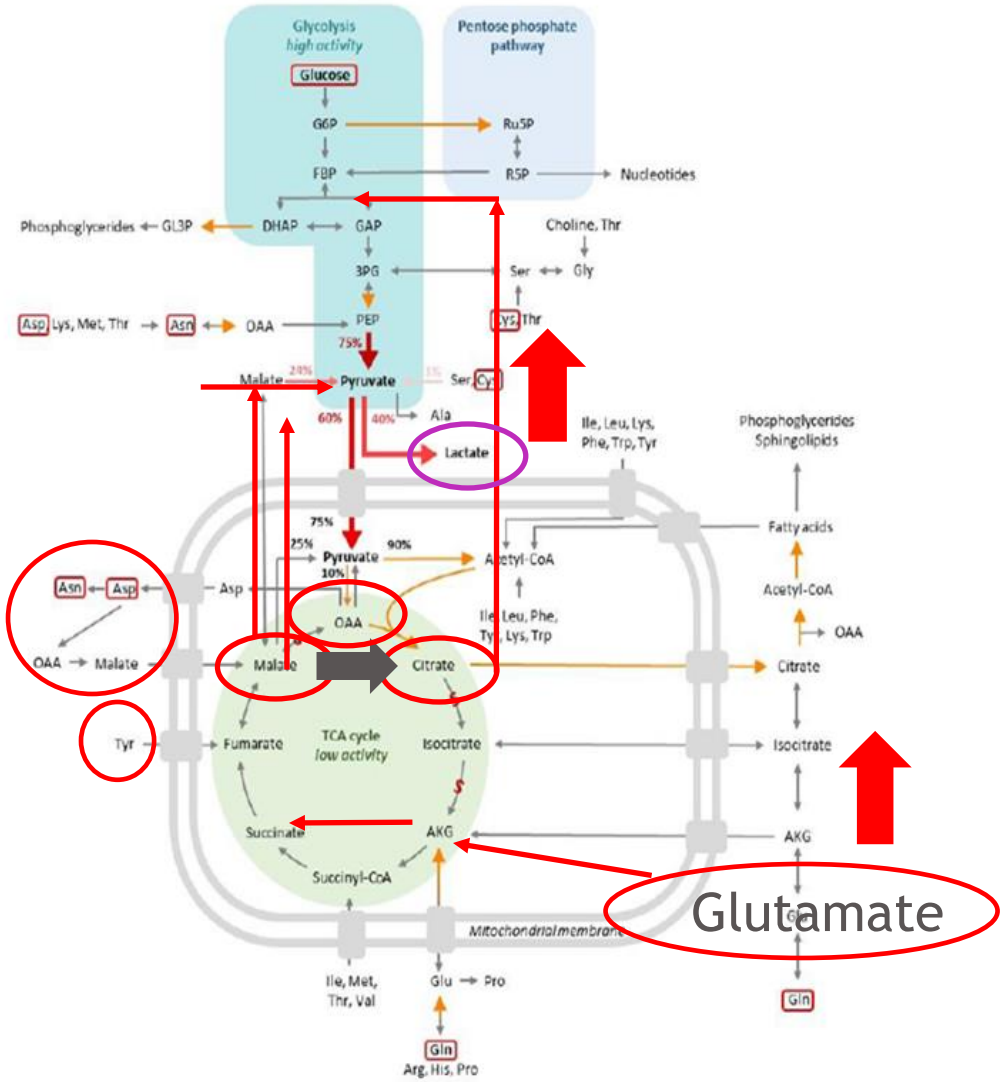
- Quantified intracellular TCA metabolites
- **Citrate, succinate, Malate, Oxaloacetate** present and relatively high at exponential phase
- Early TCA overflow?







Targeted Metabolomics: Key Observations



Coulet, Mathilde, et al. "Metabolic profiling of CHO cells during the production of biotherapeutics." *Cells* 11.12 (2022): 1929.

- Elevated TCA Cycle metabolite observed during exponential phase
  - High citrate levels were observed and can inhibit glycolysis
  - Citrate inhibition of PFK occurs when there are high intracellular levels of citrate.
- Lactate can be produced from glycolysis or TCA
  - Malate can convert to pyruvate or lactate
  - Glutamate and other AA can enter TCA at any phase and trigger malate and lactate accumulation

- Lactate accumulation may be due to both glycolysis/TCA overflow
- Over feeding of amino acid early? No lactate reutilization?

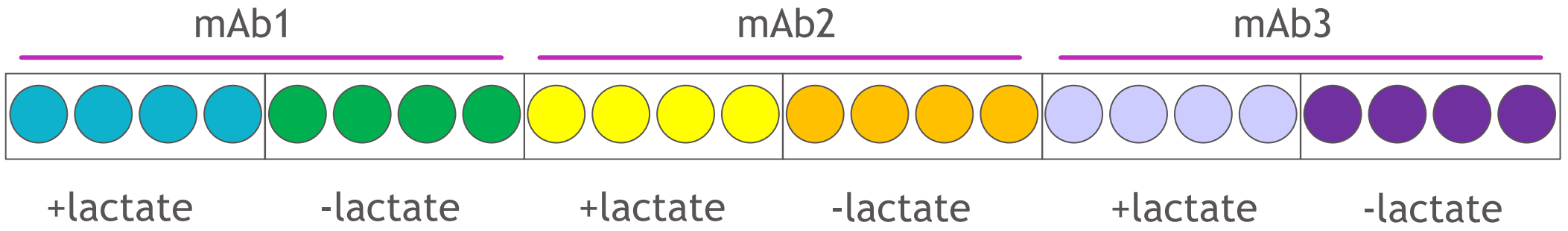
Conclusions:

- **Implementation of a right and relatively high-throughput " targeted omics" tool provided key insight of CHO Cell Bioprocess and Metabolism for multiple mAb molecules**

- **Exponential Phase:**
  - Potential TCA overflow indicated by high citrate, malate and OAA
    - Inhibits glycolysis via PFK
  - All essential and nonessential amino acids are present, higher Glutamate
    - Alternative higher carbon or nitrogen sources can contribute lactate overflow and its reduced reutilization at any growth phase

- **Stationary Phase:**
  - Glucose consumption initially decreases, but starts increasing
    - Needed to generate ATP, but not going towards protein production
  - Metabolism shifts from lactate consumption to lactate production and accumulation
    - Indicates pyruvate not entering TCA
  - Succinate drop may indicate ETC issues
  - Rise in malate may result in lactate production and can related to overfeeding of glutamate

Future Work: Experimental Design- Mitochondrial Focus



- Targeted Metabolomics (TCA, glycolysis, PPP, ROS)
- Targeted proteomics –Intra and extracellular
- GSH and NAD assays
- Mitochondrial Membrane Potential

Thank you

Bristol Myers Squibb™

Acknowledgements

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DevEx Team


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