Unraveling the Metabolic Regulatory Mechanisms Driving Lactate Runaway in CHO Cell Cultures Using a Multi-Omics Approach

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September 26th, 2025

CASSS 2025 Multi-Omics Session

Cell Engineering & Analytical Sciences
Therapeutics Development & Supply

Johnson&Johnson

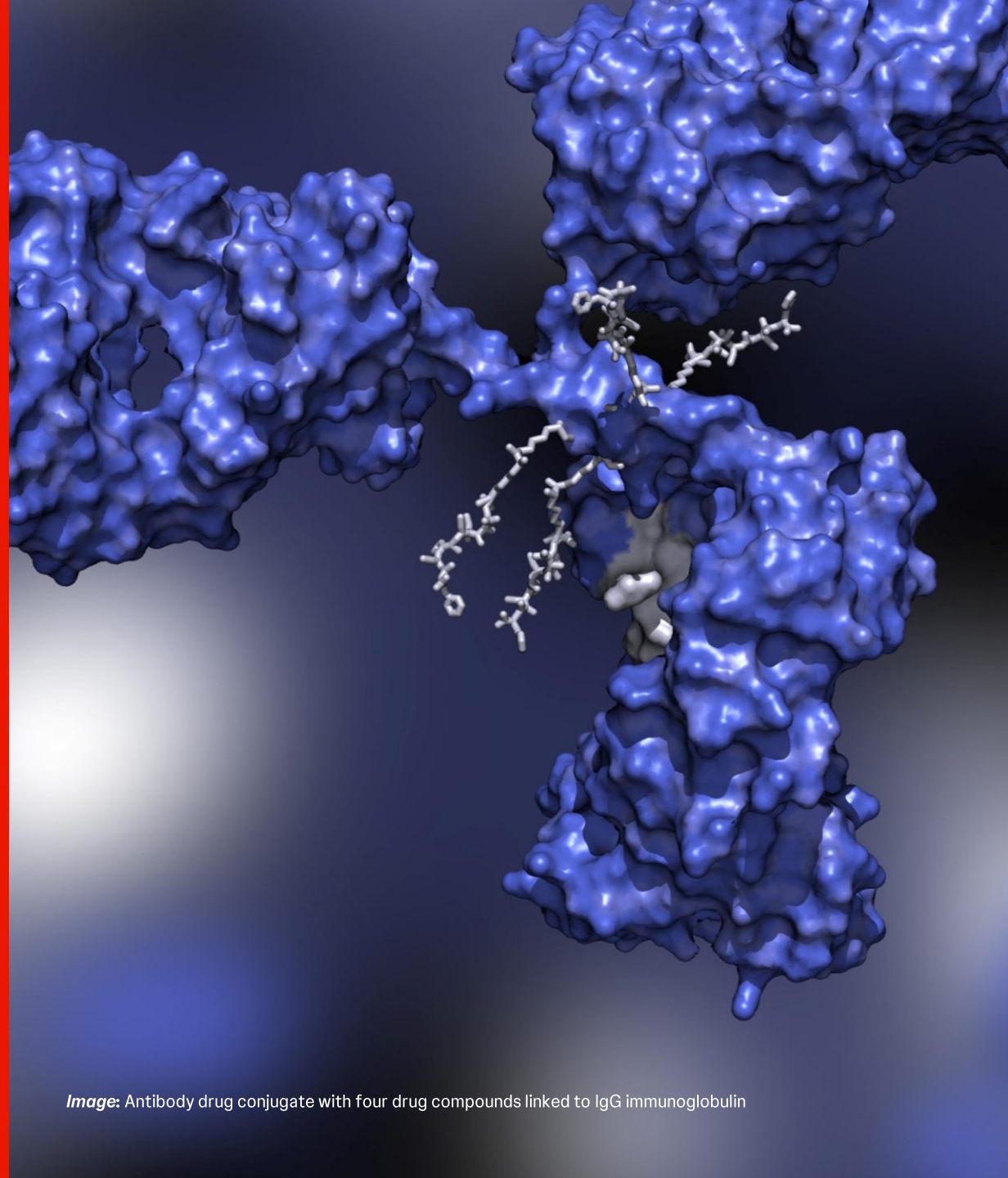
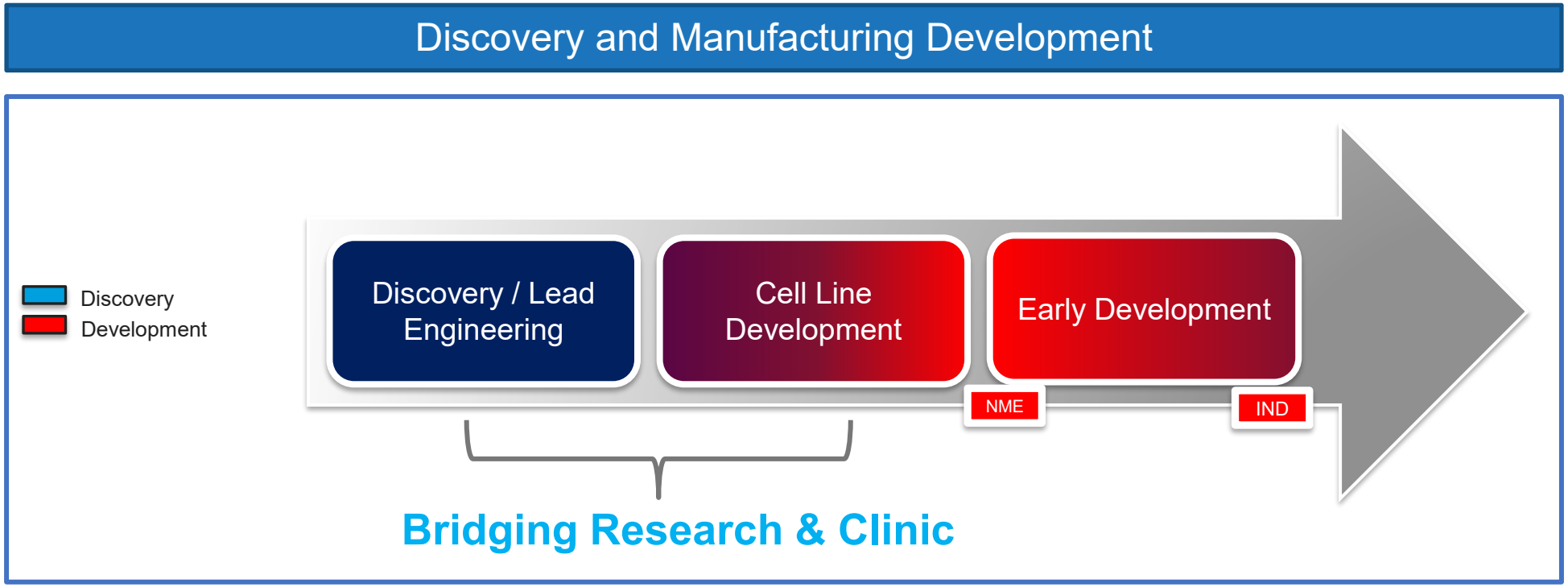


Image: Antibody drug conjugate with four drug compounds linked to IgG immunoglobulin

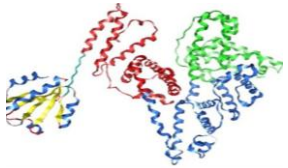
Outline

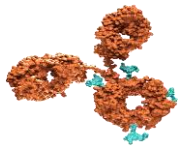
1. CEAS Group @ J&J Innovative Medicine
2. Cell Engineering Efforts
3. Implementation of Multi-Omics
4. Case Study: Using Multi-Omics to Uncover Strategies to Address Lactate Runaway
5. Conclusions
6. Acknowledgments

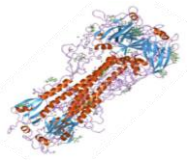
Cell Engineering & Analytical Sciences (CEAS)

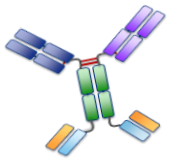


Deliver the manufacturing cell line for lead candidates.


Alternative Scaffolds

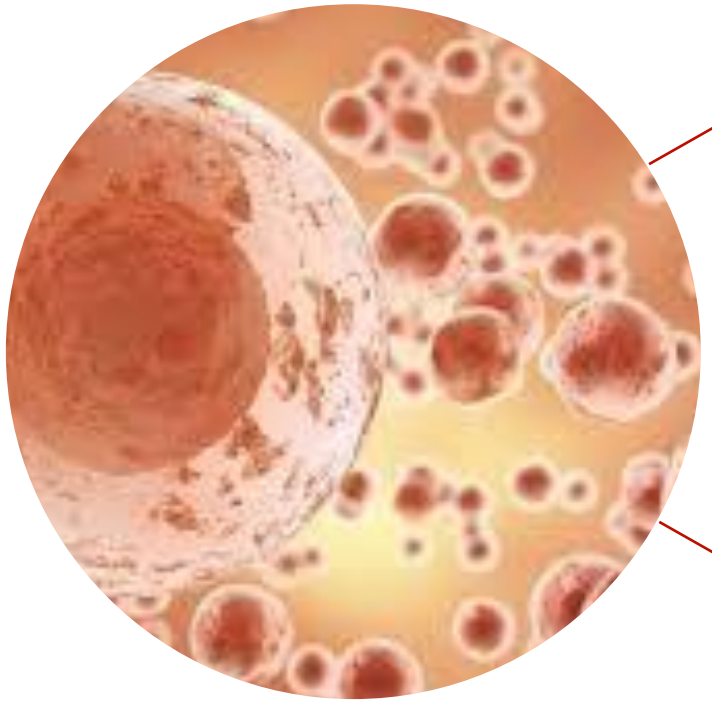

Antibody Drug Conjugates


Vaccines


Multi Specifics

Increasing # of new protein modalities

Cell Engineering Efforts



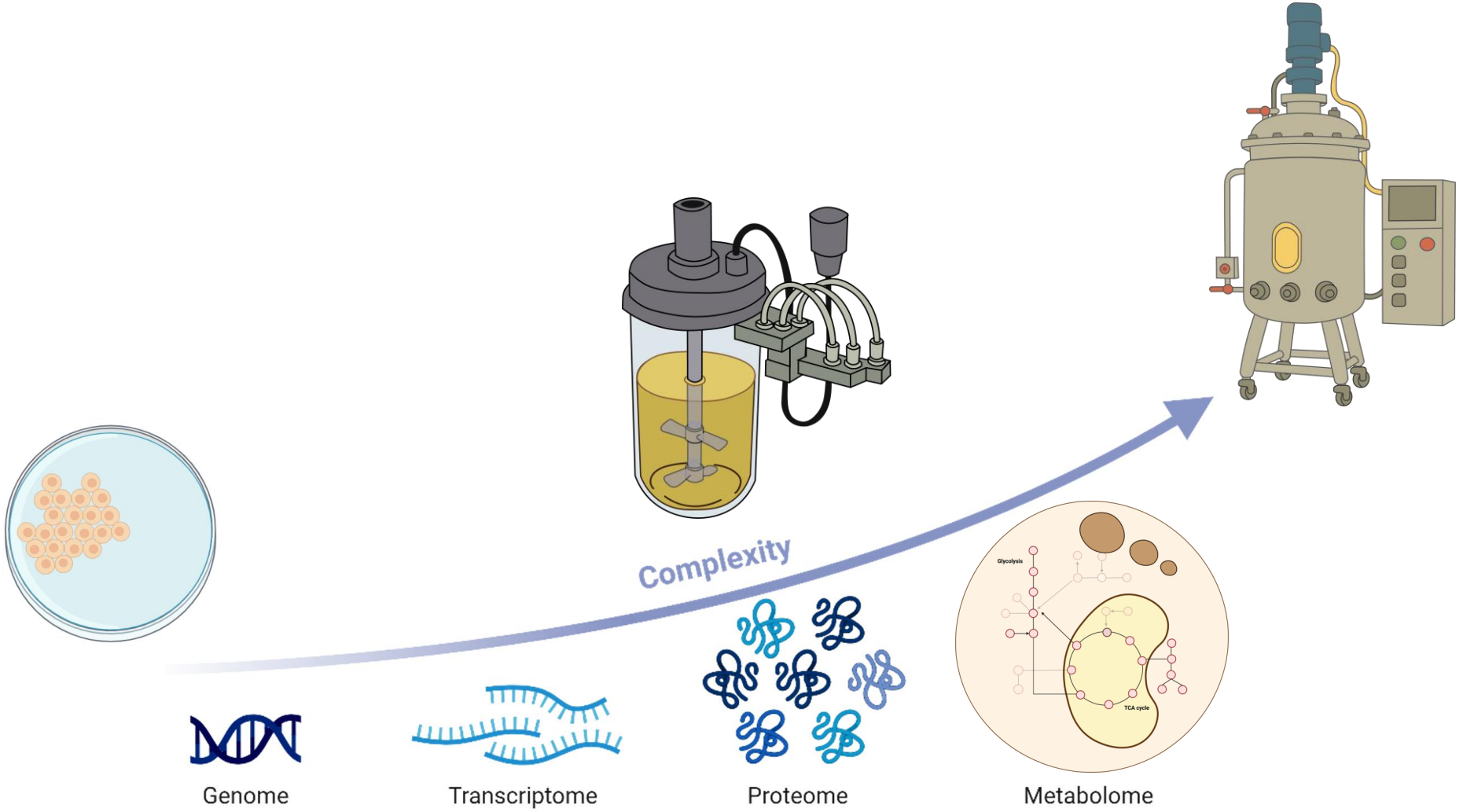
Cell Factory

- Improve PQ
- Control Lactate

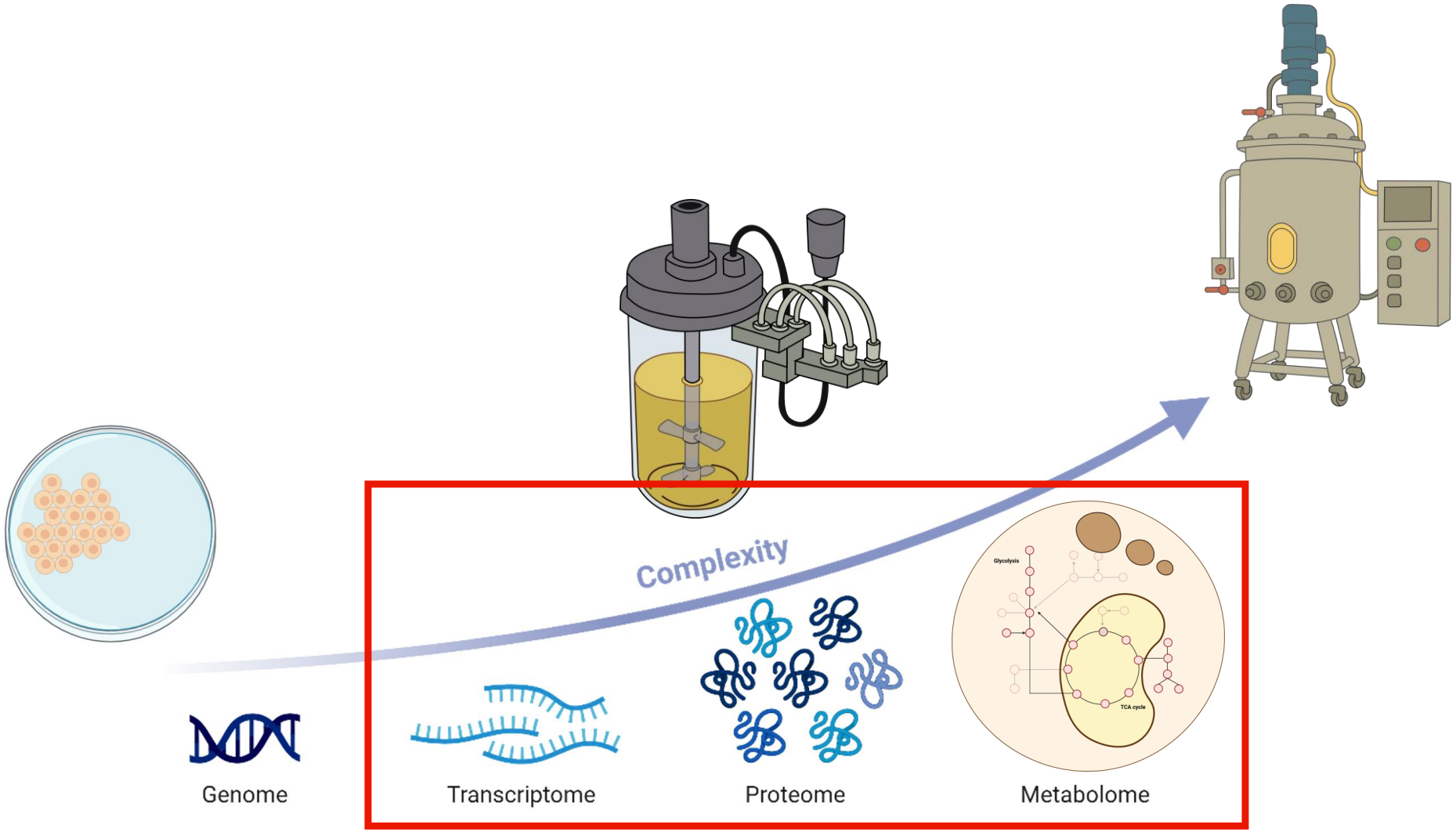
Titer

- ↑ titer
- Improve qP [pg/cell/day]

Implementation of Multi-Omics



Implementation of Multi-Omics



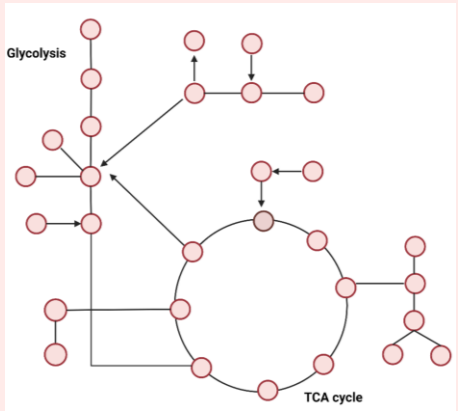
Explore Use of Genome Scale Models

Genome scale metabolic modeling (GEM) is a systems biology tool that models and predicts the complexities of a biological system.

GEM:

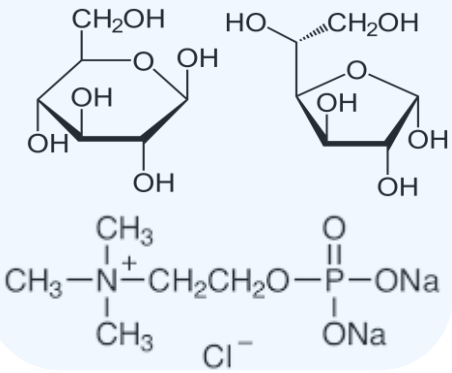
1. Reconstructs and simulates an organism's entire metabolic network.
2. Integrates all omics data
3. Maps known metabolic reactions.
4. Uses techniques like flux balance analysis to predict fluxes and phenotypes.

GEM

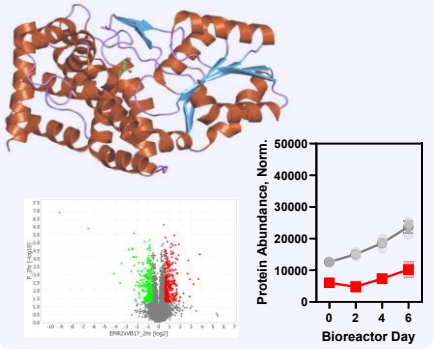


A → B
B → C
C + D → E
F → E
F → G
H → F
...

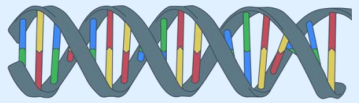
Metabolomics

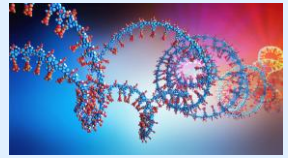


Proteomics

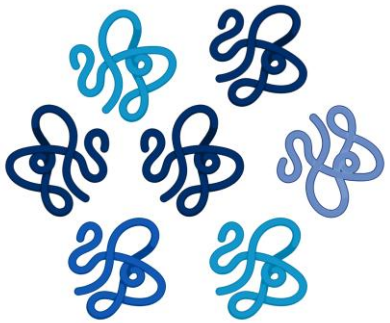


Transcriptomics





Integrating Proteomics + Metabolomics with Data Science



Proteomics

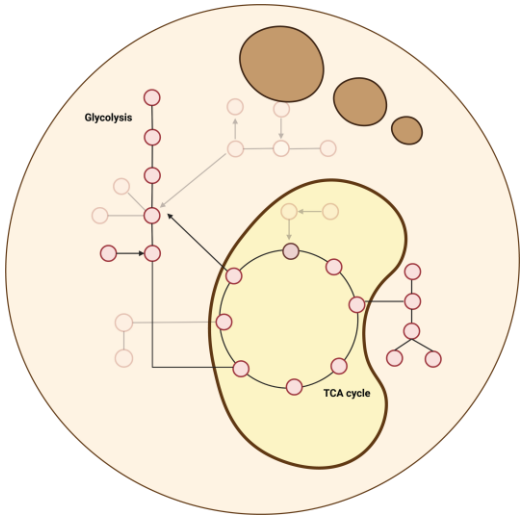
- Insight into functional proteins
- Analysis of PTM

- Complex interpretation
- Resource-intensive

Metabolomics

- Direct reflection of biological state
- Wide range of compounds

- Detection limits
- Stability issues



Data Science

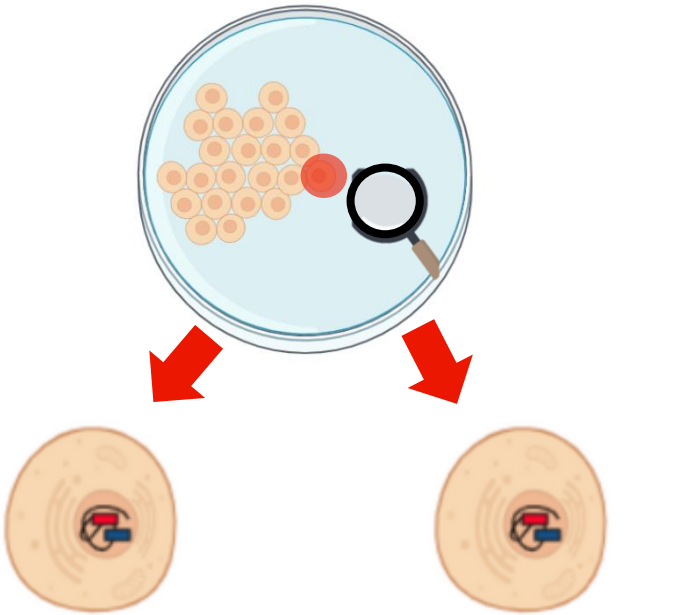
- Customize the GEM with omics experimental data.
- Flux estimation
- Identify pathways that have a direct impact on a variable of interest

Case Study: Using Multi-Omics to Uncover Strategies to Address Lactate Runaway

Johnson&Johnson
Innovative Medicine

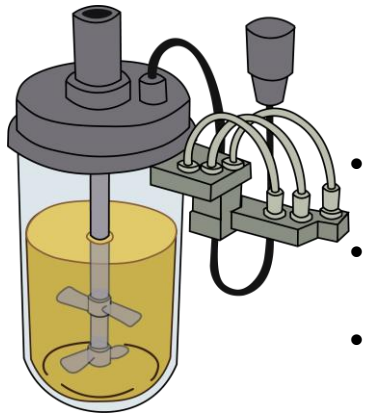
Experimental Set Up

TFX Pool Expressing a Biotherapeutic



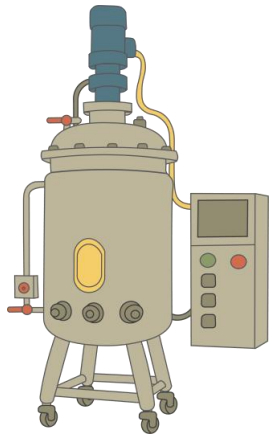
High Lactate Phenotype (HL)

Low Lactate Phenotype (LL)



Ambr250

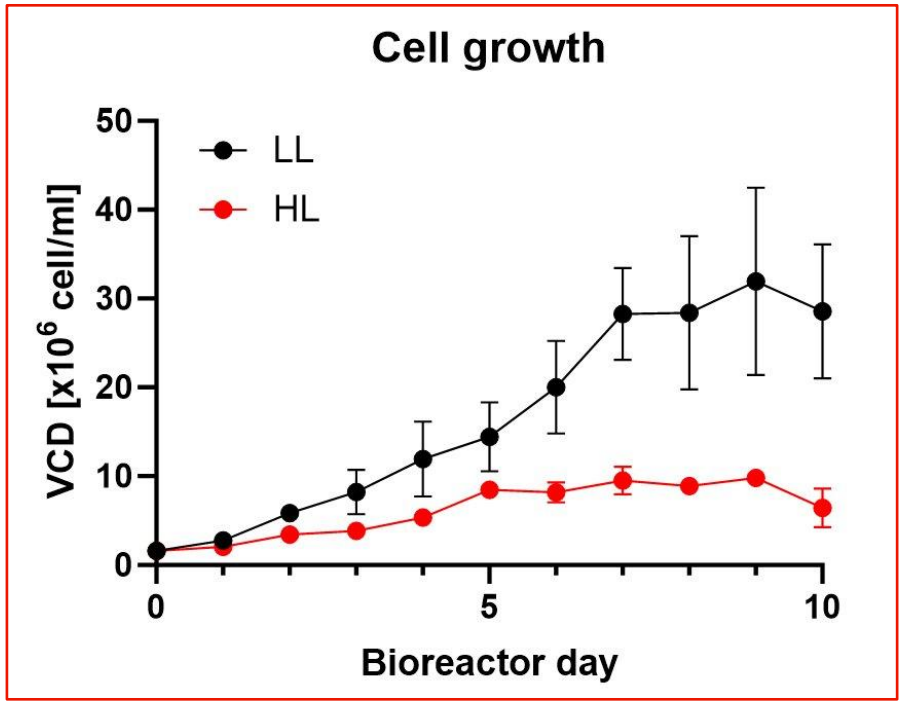
- 1 High lactate condition
- 2 Low lactate conditions
- 1 TFX Pool
- Duplicate bioreactors

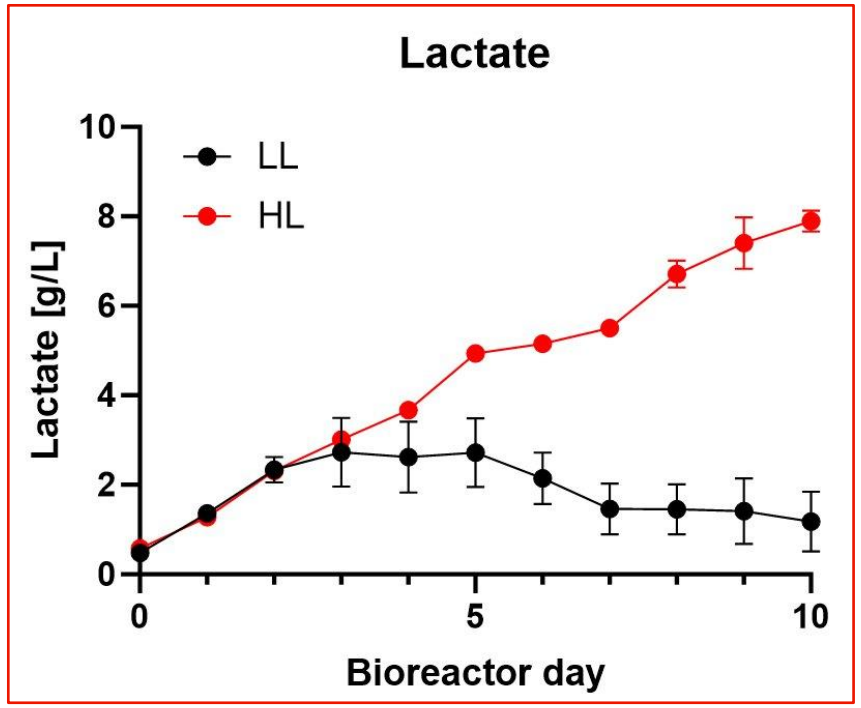


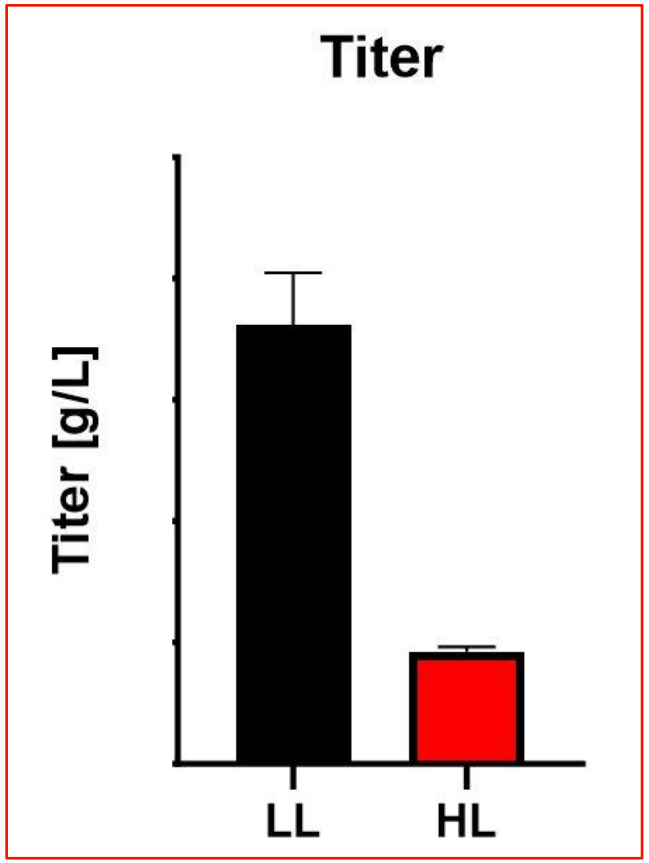
10L

- 1 High lactate condition
- 2 Low lactate conditions
- Duplicate bioreactors for HL and no replicate for LL.

Growth Conditions in 10 L Bioreactors

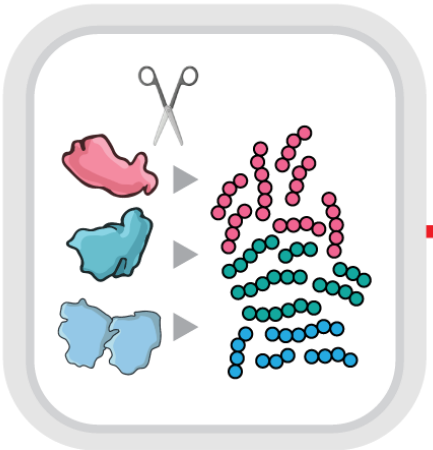




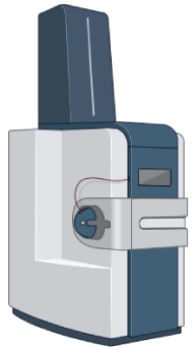


HL Phenotype have significant lower growth leading to much smaller titer.

Proteomics + Metabolomics Workflows



Sample Preparation



LC-MS/MS

In Silico nnAA

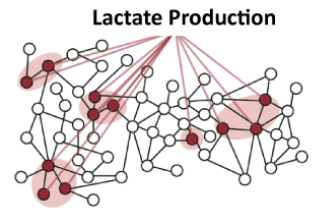
Raw Data Search

Clustering

Data Process

Ingenuity Pathway Analysis

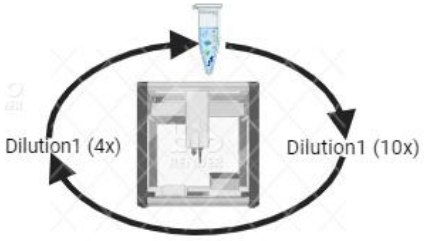
Data Interpretation

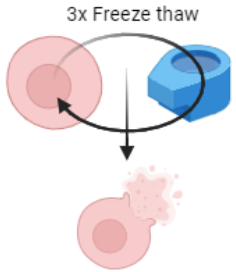


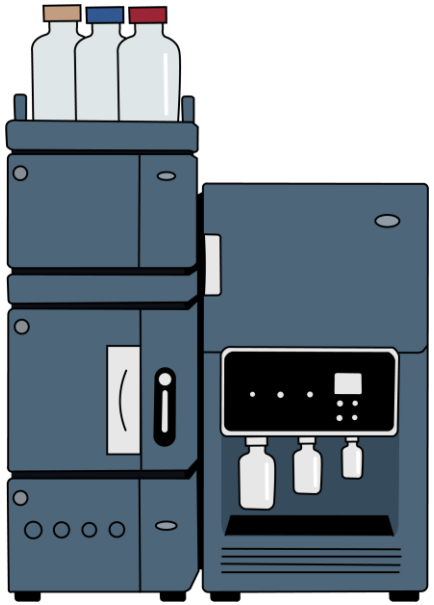
Biological Processes Specific to HL

Supernatant

Cell Pellets





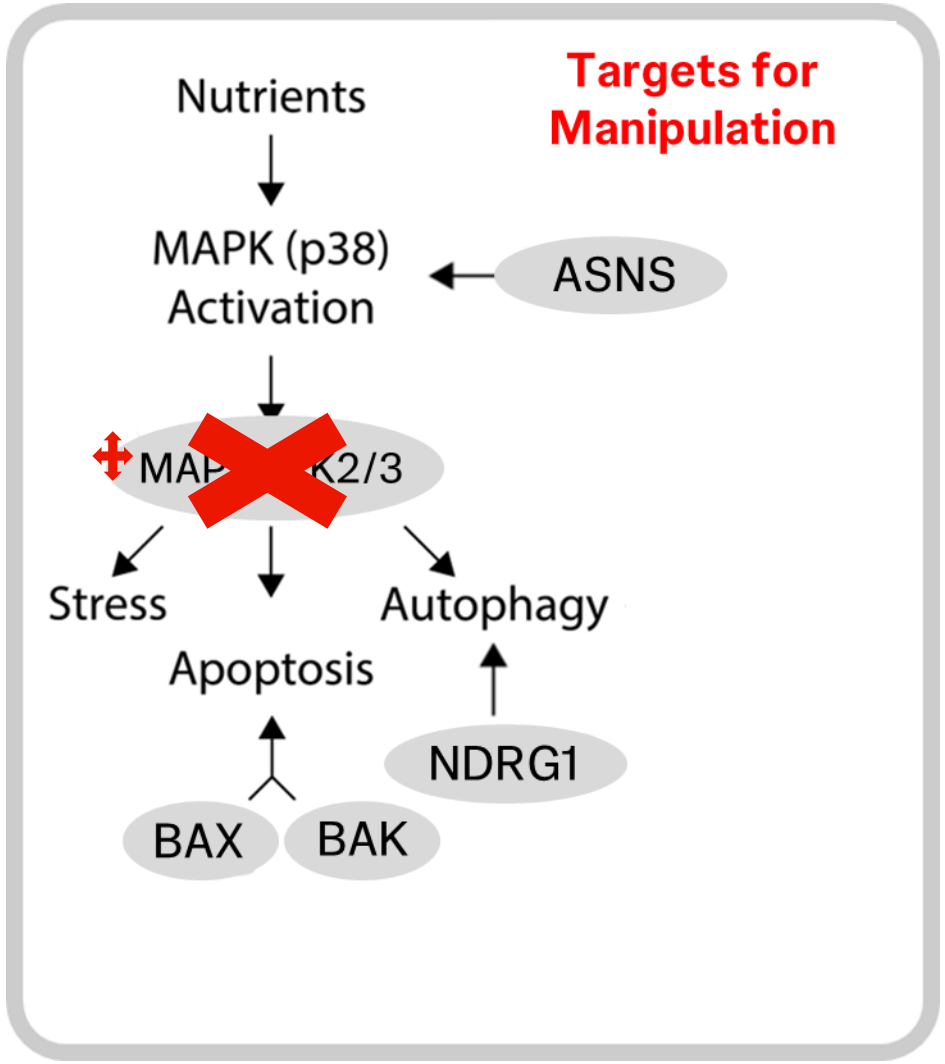


Semi-Targeted Metabolomics

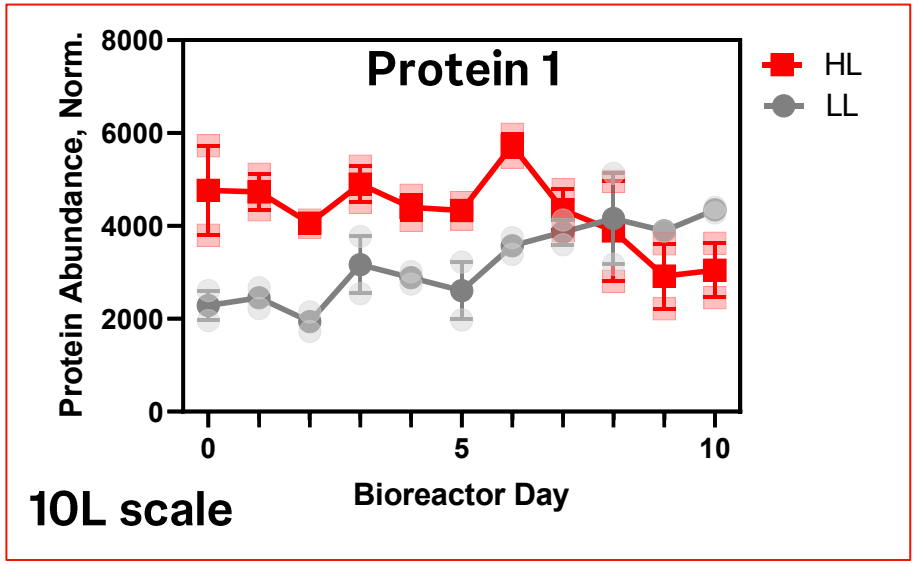
- Waters BioAccord
- MS1 Full scan
- Spent media analysis
- Amino acid analysis on pellets

Proteomics Data: Target 1 (MAPKAPK2)

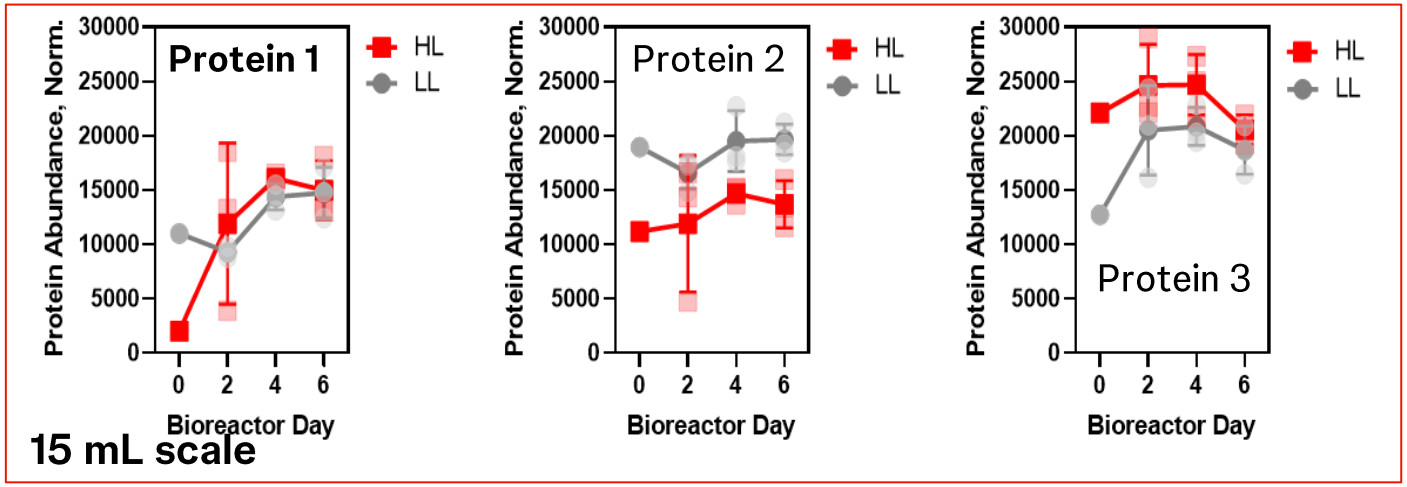
Autophagy, Apoptosis Model



Protein abundance over Bioreactor Days

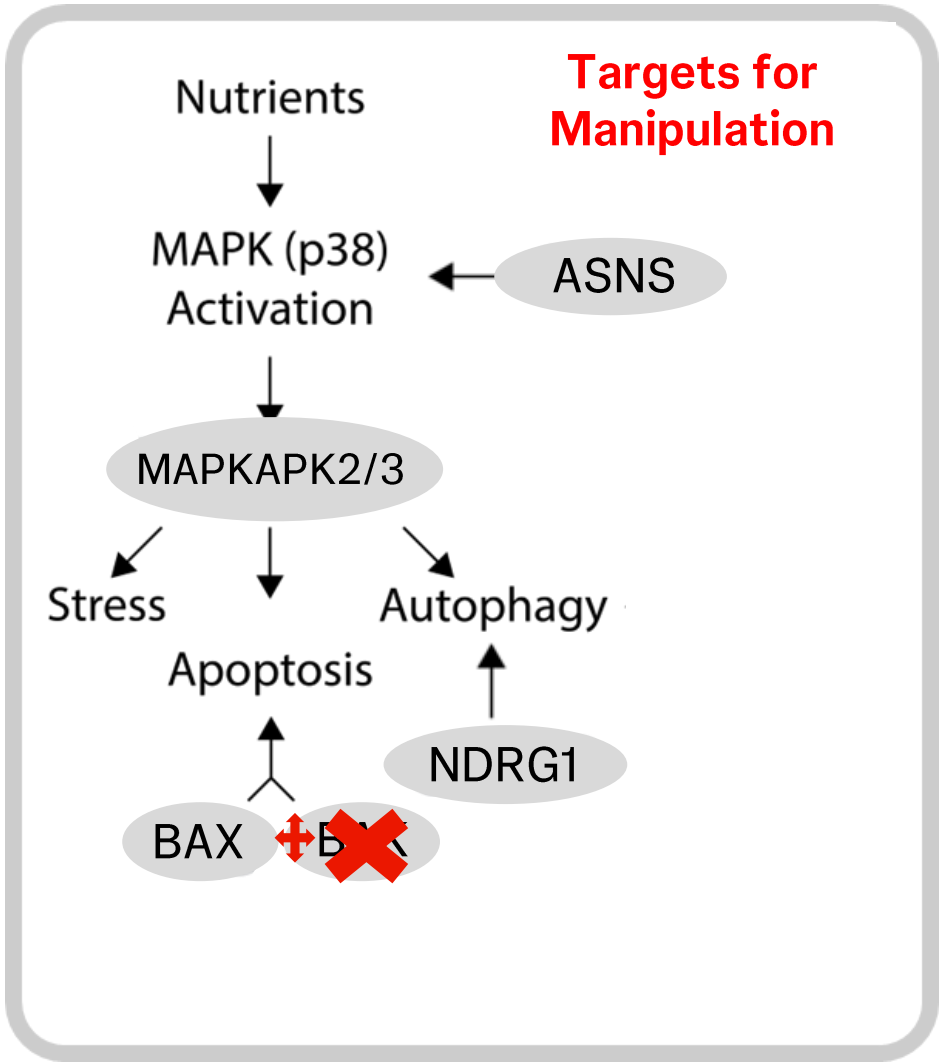


Protein abundance over Bioreactor Days

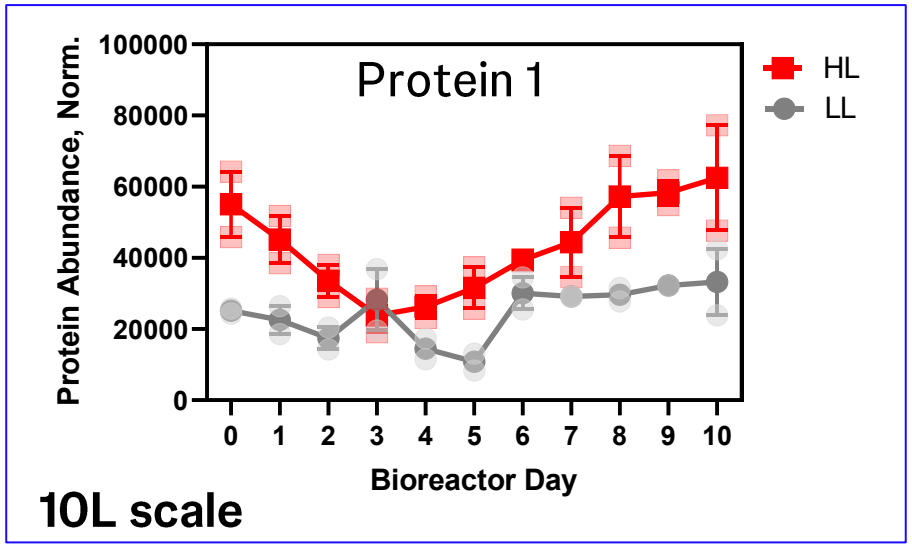


Proteomics Data: Target 2 (BAK)

Autophagy, Apoptosis Model

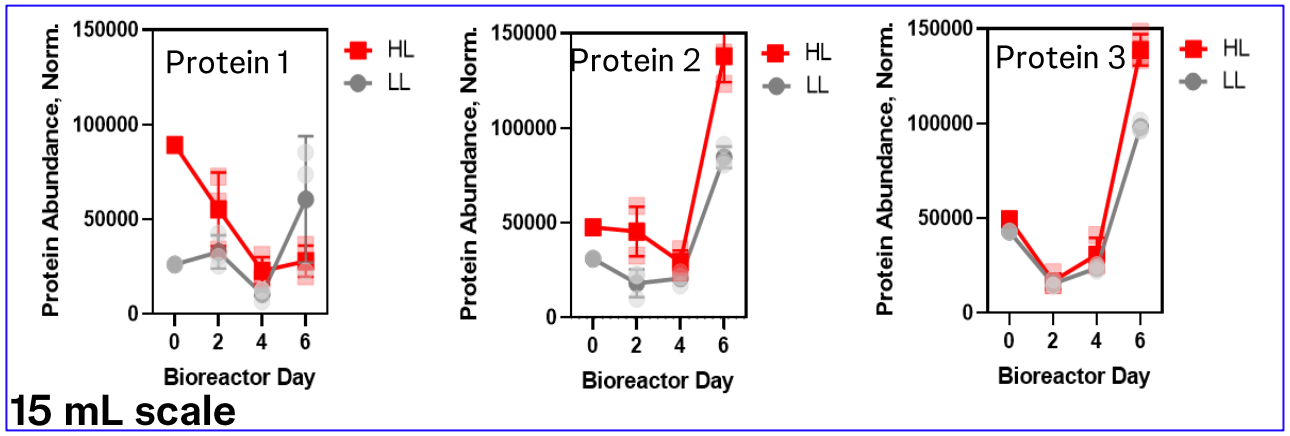


Protein abundance over Bioreactor Days



10L scale

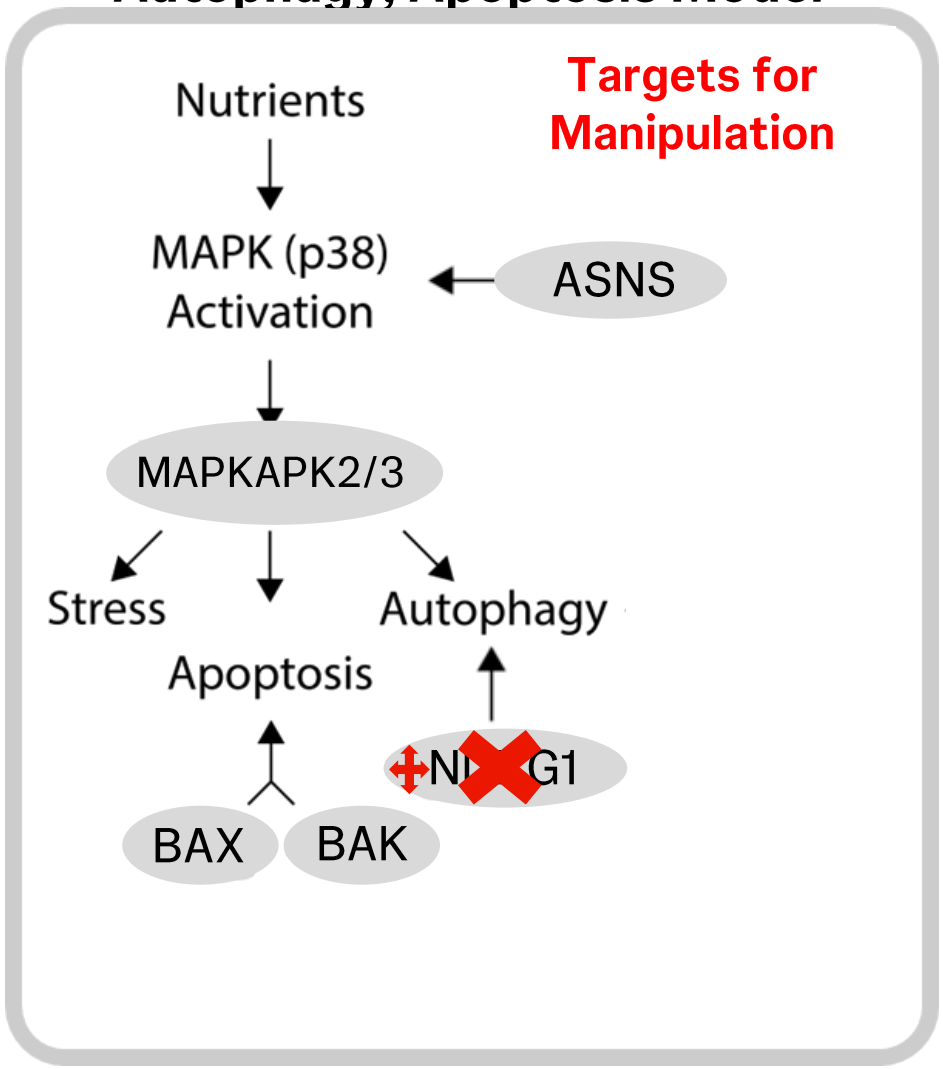
Protein abundance over Bioreactor Days



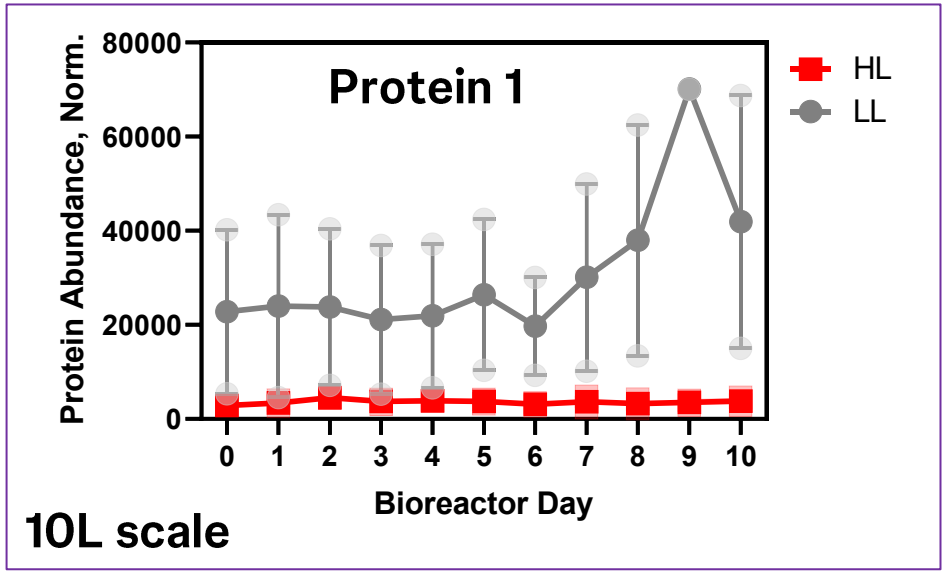
15 mL scale

Proteomics Data: Target 3 (NDRG1)

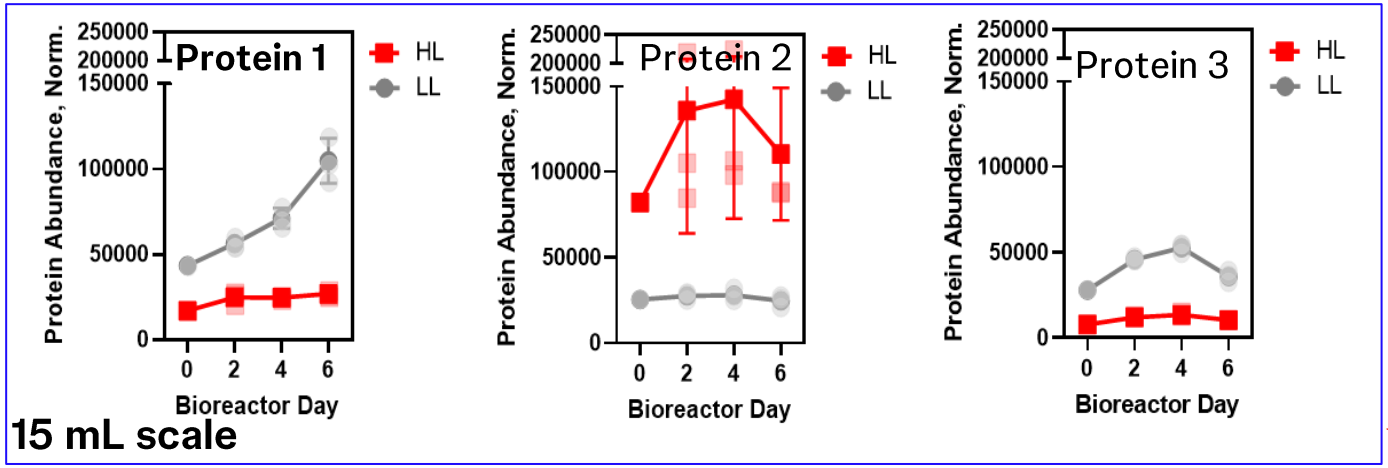
Autophagy, Apoptosis Model



Protein abundance over Bioreactor Days

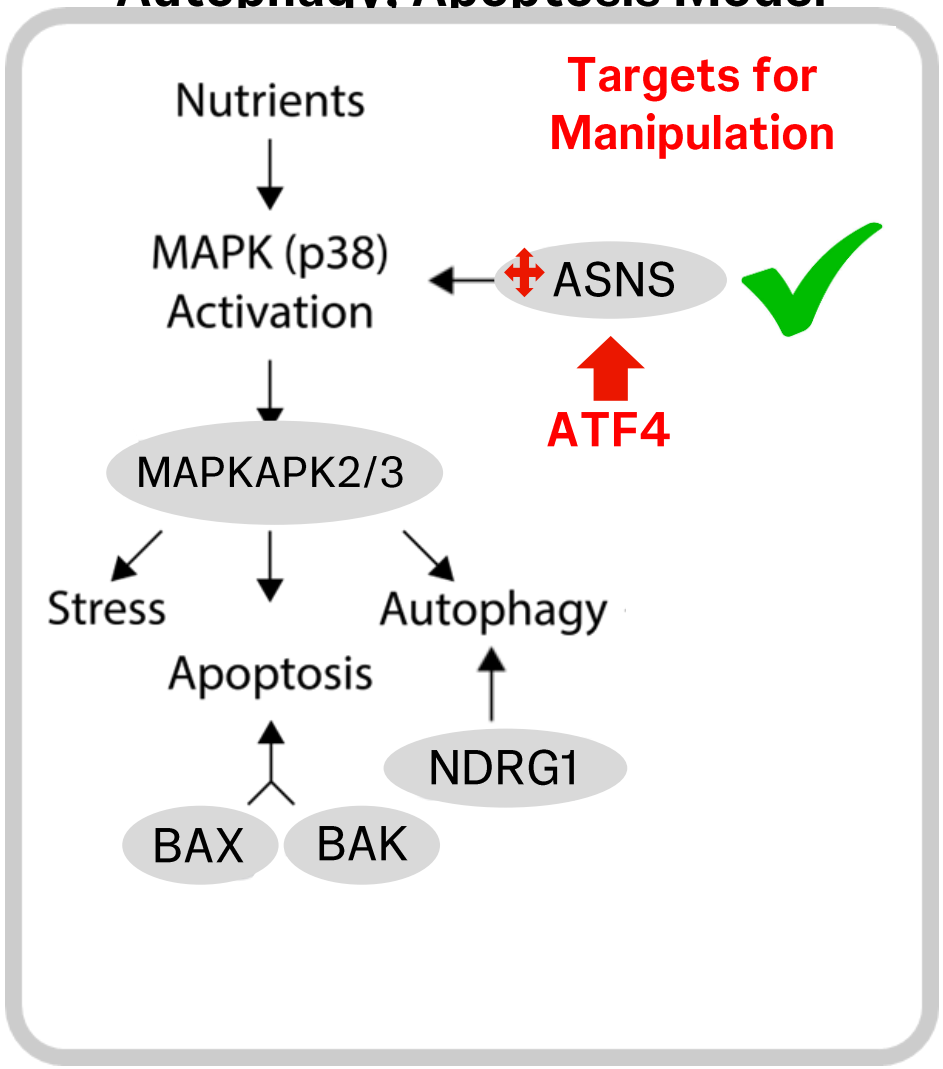


Protein abundance over Bioreactor Days

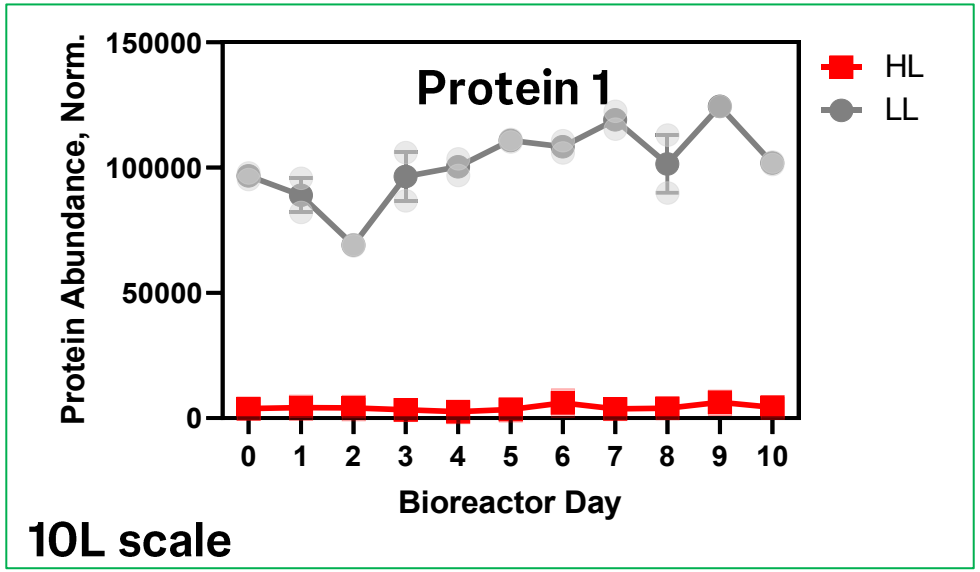


Proteomics Data: Target 4 (ASNS)

Autophagy, Apoptosis Model

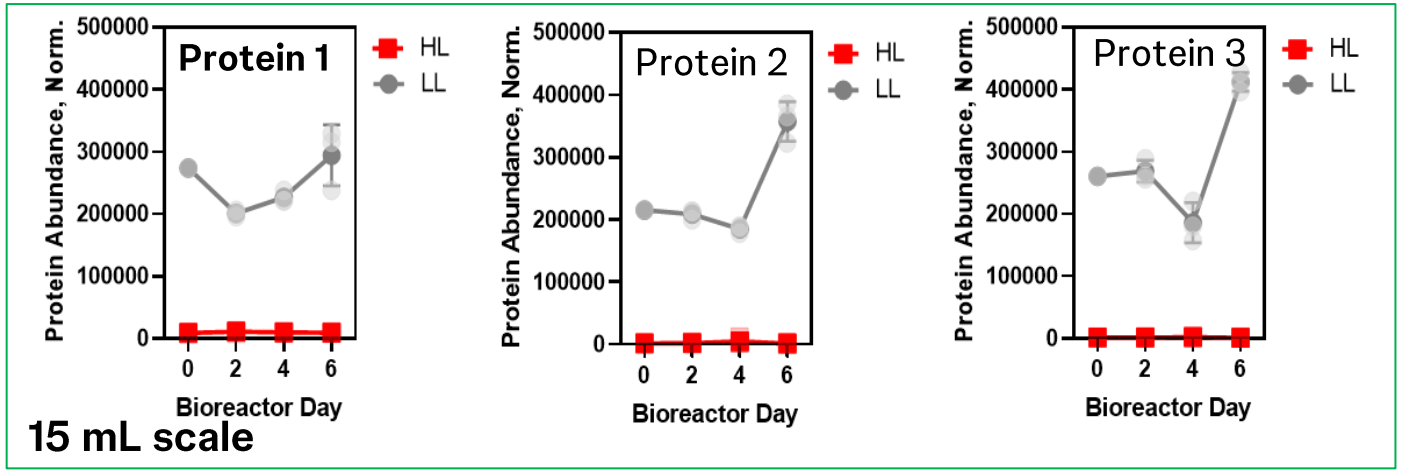


Protein abundance over Bioreactor Days



10L scale

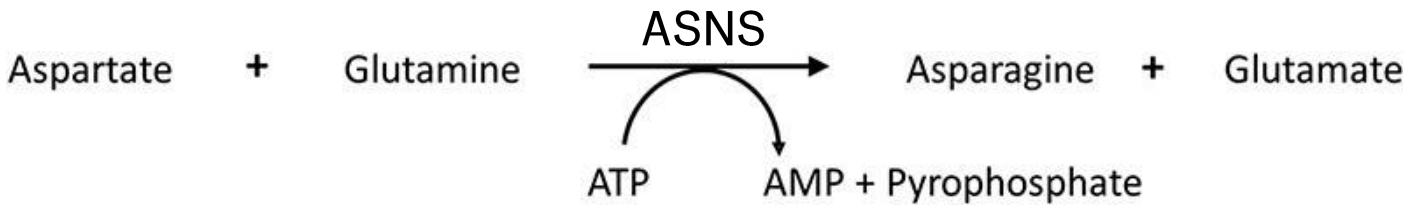
Protein abundance over Bioreactor Days



15 mL scale

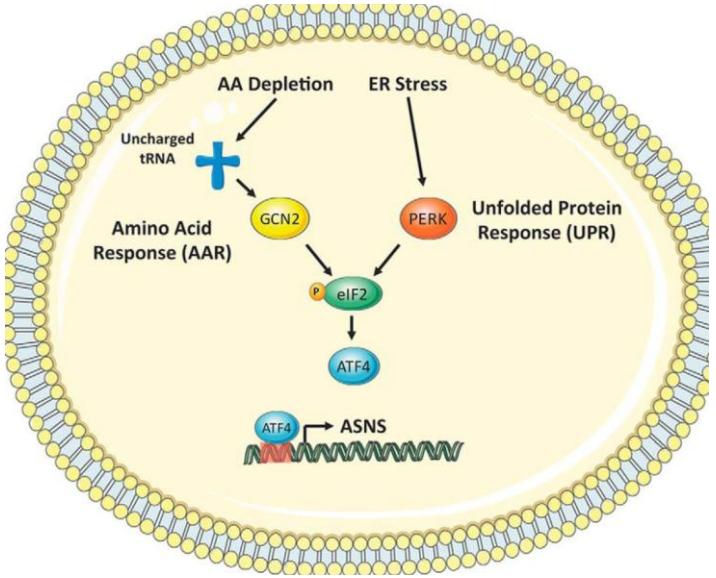
Asparagine Synthetase (ASNS) Enzyme

1. Housekeeping enzyme involved in biosynthesis of asparagine from aspartate and glutamine. [1]



2. Many research that suggest that ASNS gene is activated by cells amino acid response pathway due to AA deprivation. [2]

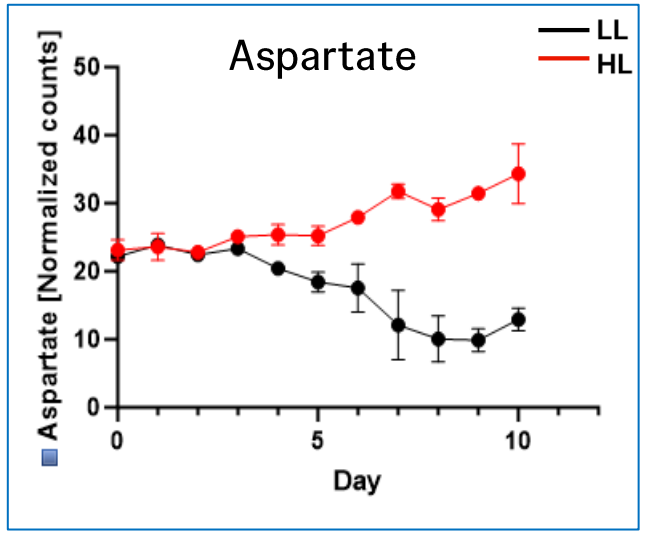
3. ASNS (common ATF4 responsive target gene) transcriptional activation can be from either AA limitation or ER stress. [3]

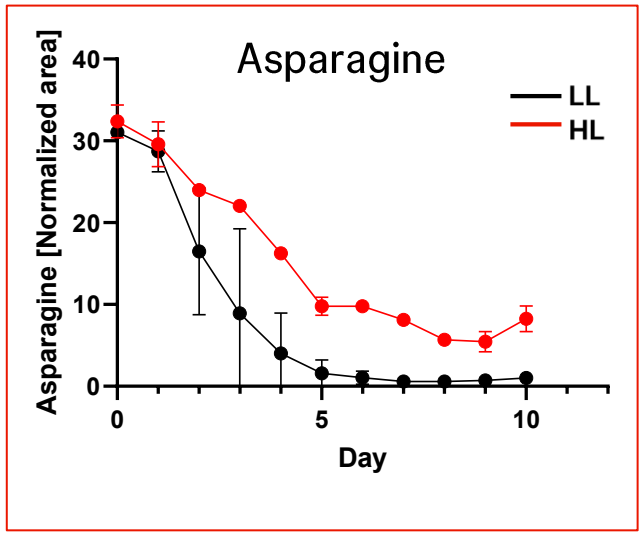


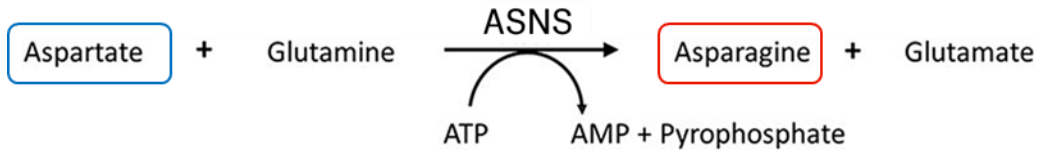
1. Andrulis et al., 1987
2. Gjymishka et al., 2009
3. Balasubramanian et al., 2013

Metabolomics Spent Media Data for 10L Bioreactor

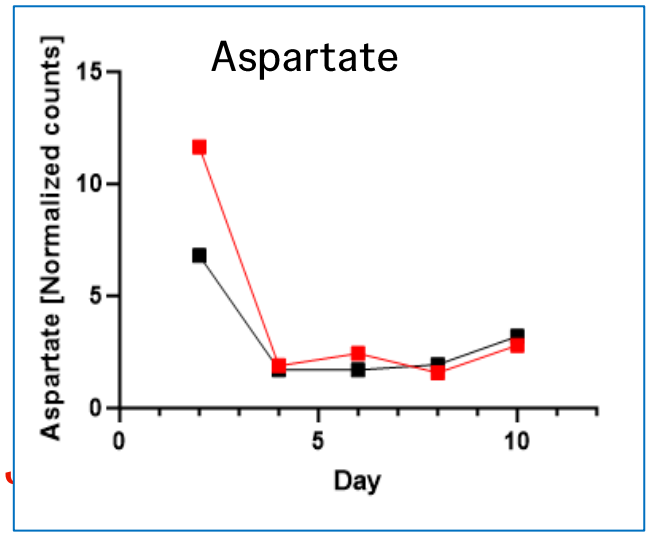
Extracellular

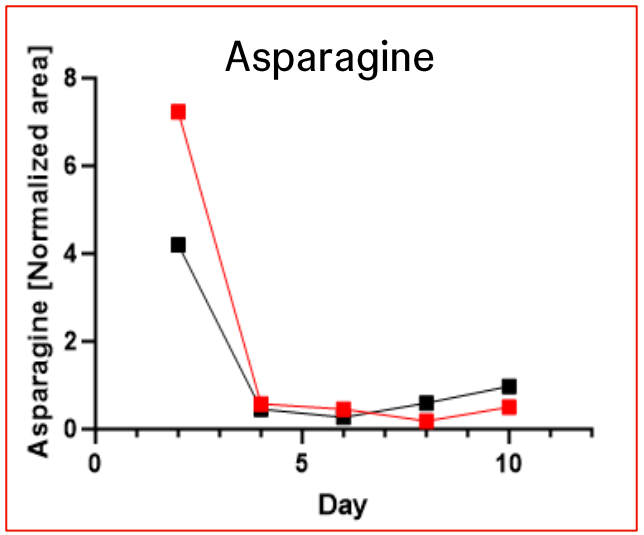






Intracellular





Conclusions:

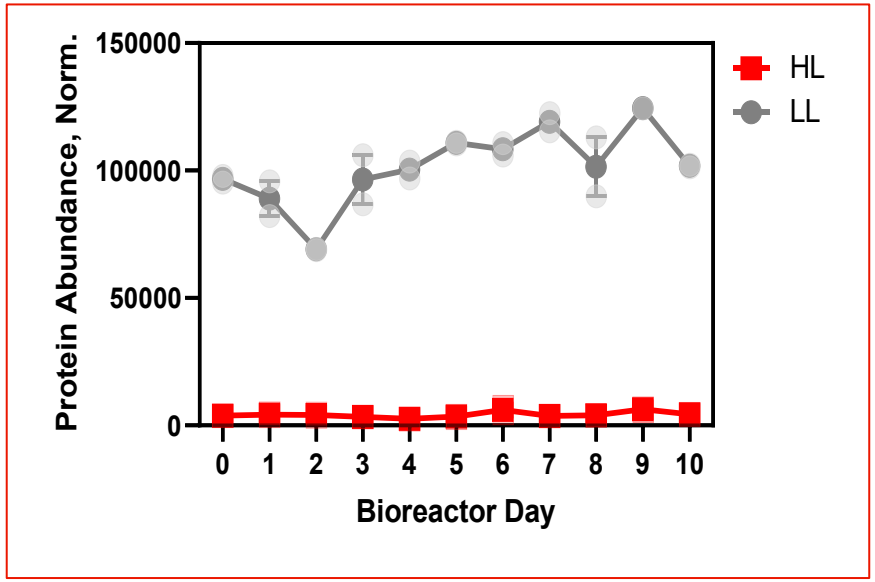
1. In LL clones, Asn levels deplete, matching Asp consumption.
2. Aspartate is converted to Asn during depletion, reducing Asp levels.
3. No significant difference in intracellular Asn levels; they remain stable due to cellular homeostasis.
4. Media concentration changes better reflect substrate availability and utilization.

Patent Pending

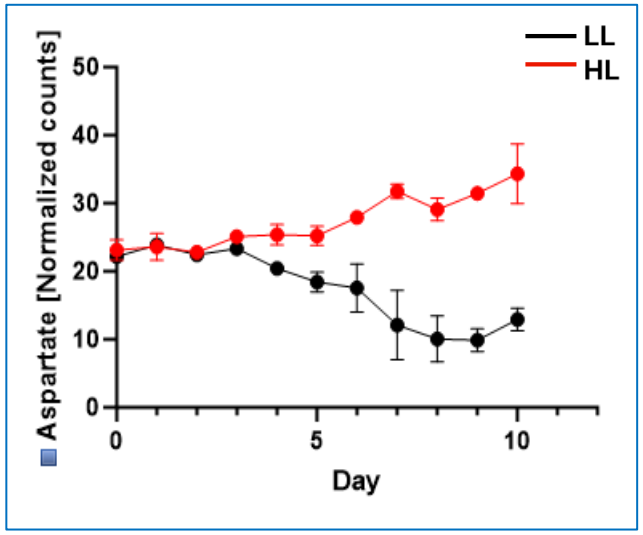
Integrated Multi-omics: Proteomics & Metabolomics Synergy

10L scale

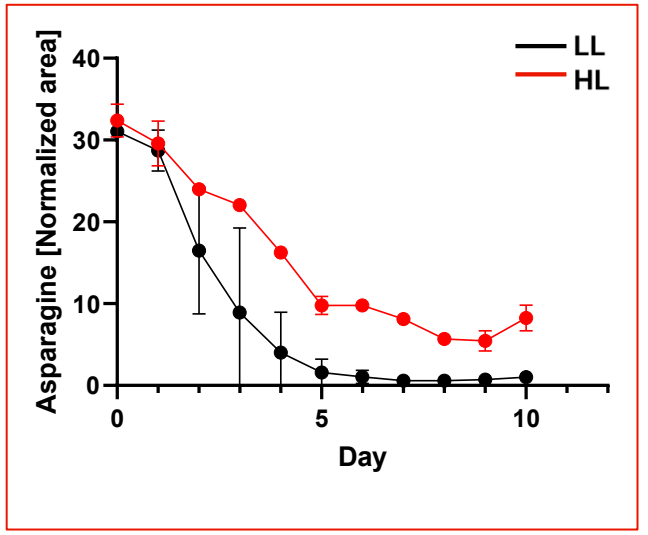
Protein abundance over Bioreactor Days

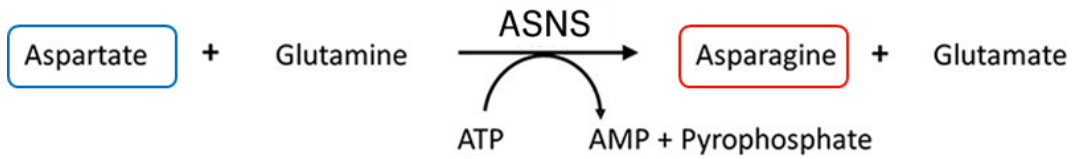


Aspartate Normalized Levels



Asparagine Normalized Levels

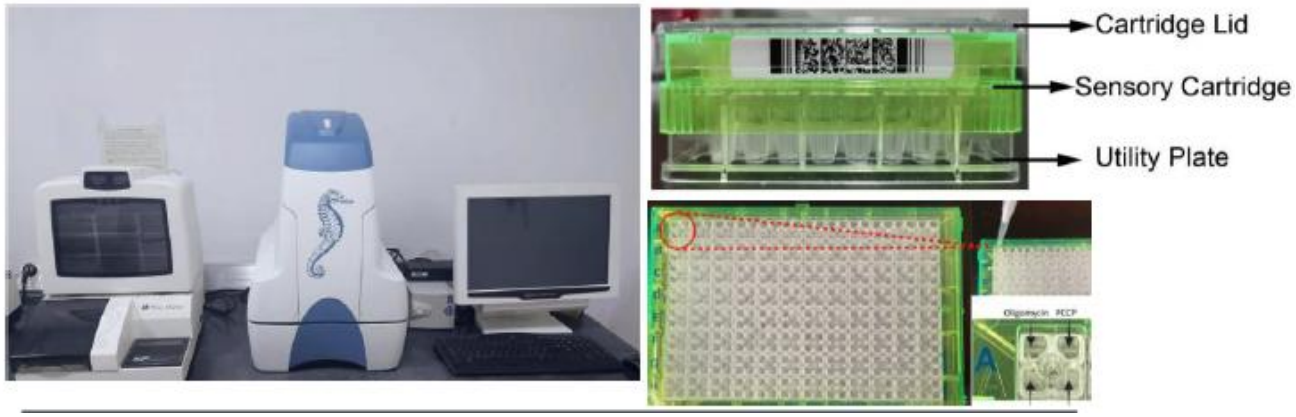




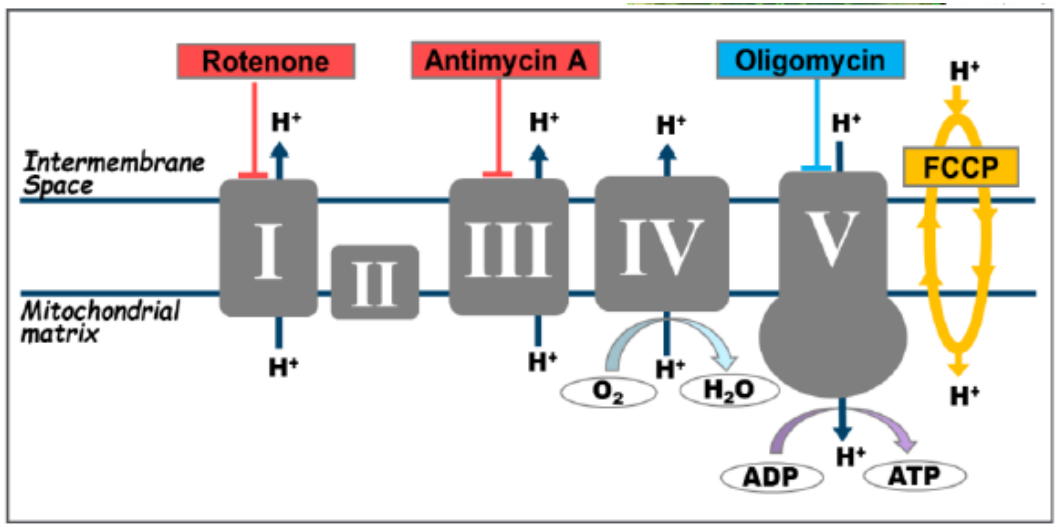
Patent Pending

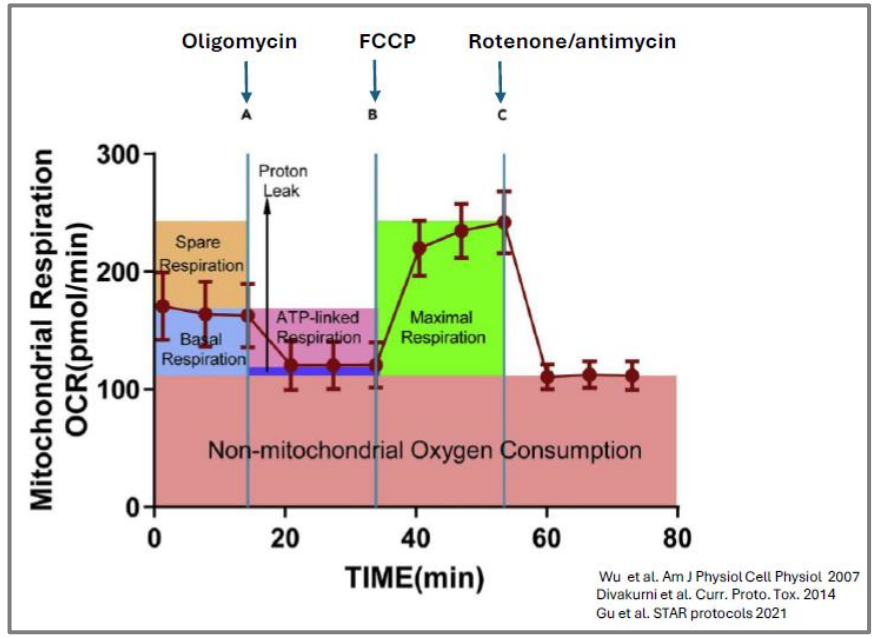
Introduction to Seahorse Assay

1. The Seahorse assay is used to measure cellular metabolism in real-time.
2. It evaluates key metabolic functions: mitochondrial respiration and glycolysis by detecting changes in oxygen consumption rate (OCR).
3. Provides insights into cellular energy production, metabolic flexibility, and mitochondrial health.

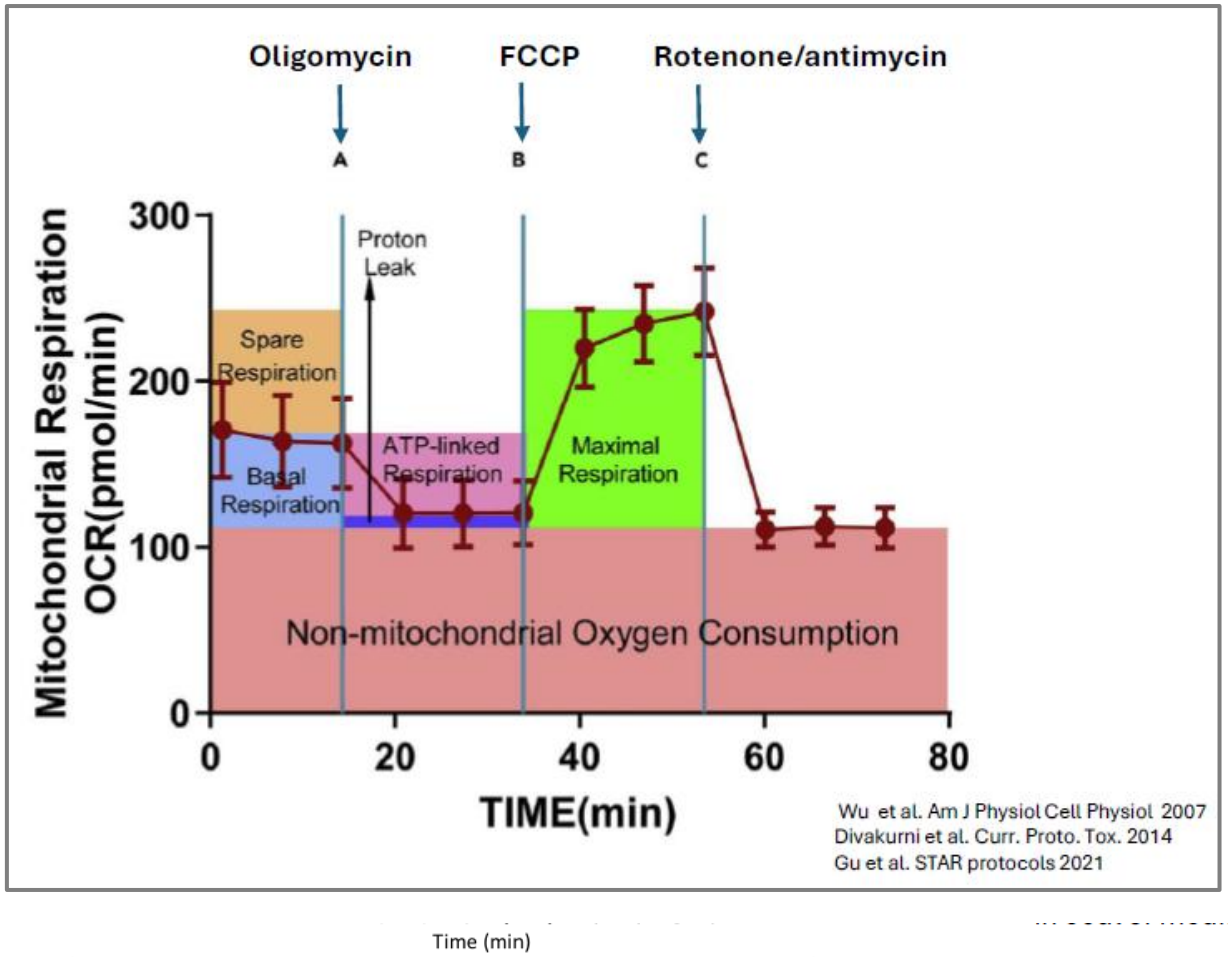


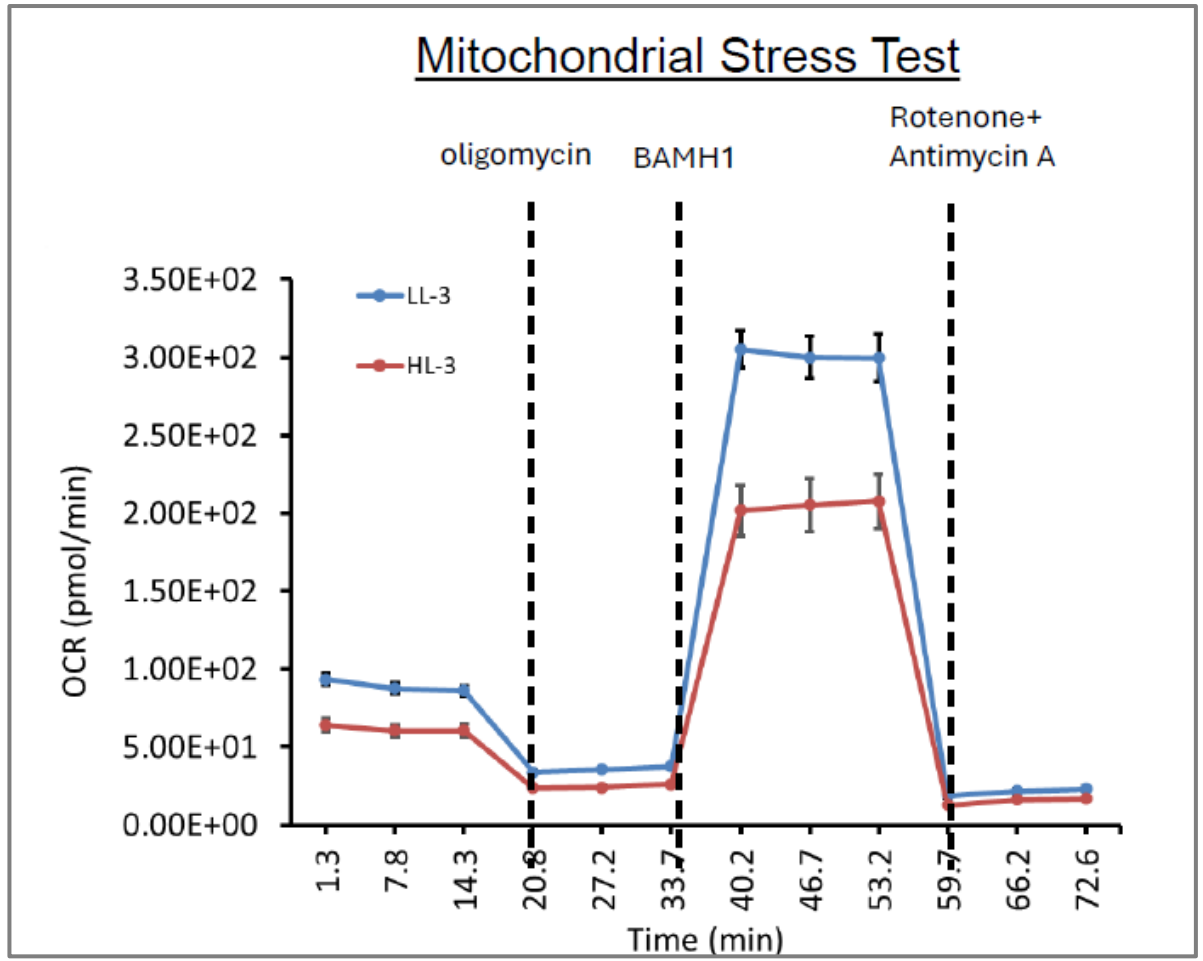
Agilent Seahorse XF





Introduction to Seahorse Assay



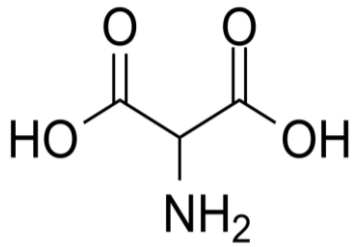


Time (min)

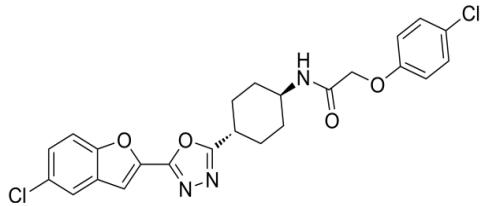
- **Basal respiration**: Oxygen consumption used to meet cellular ATP demand and engage Kreb's cycle is much reduced in high lactate clone . Shows energetic demand of the cell under baseline conditions.
  - LL clone at three different cell densities shows higher OCR compared to HL clones

ote: OCR=oxygen consumption rate

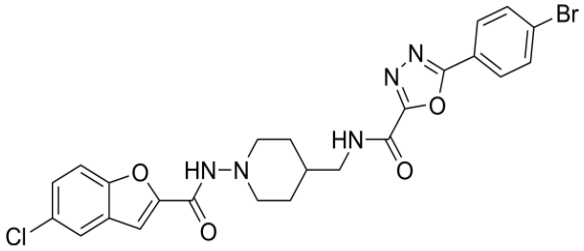
ASNS inhibition reduces OCR with ATF4-in2 in LL Phenotype



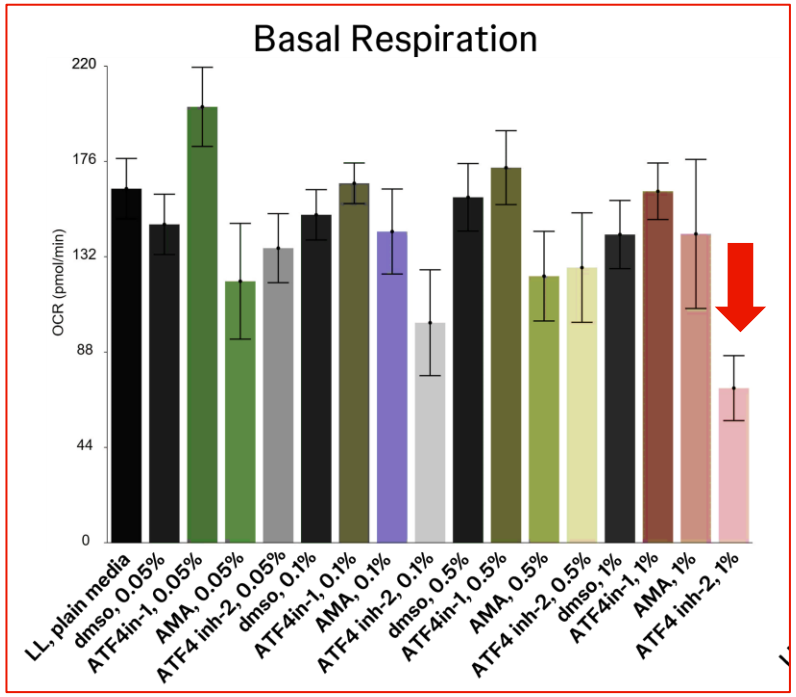
Amino-malonic acid (direct ASNS inhibitor)

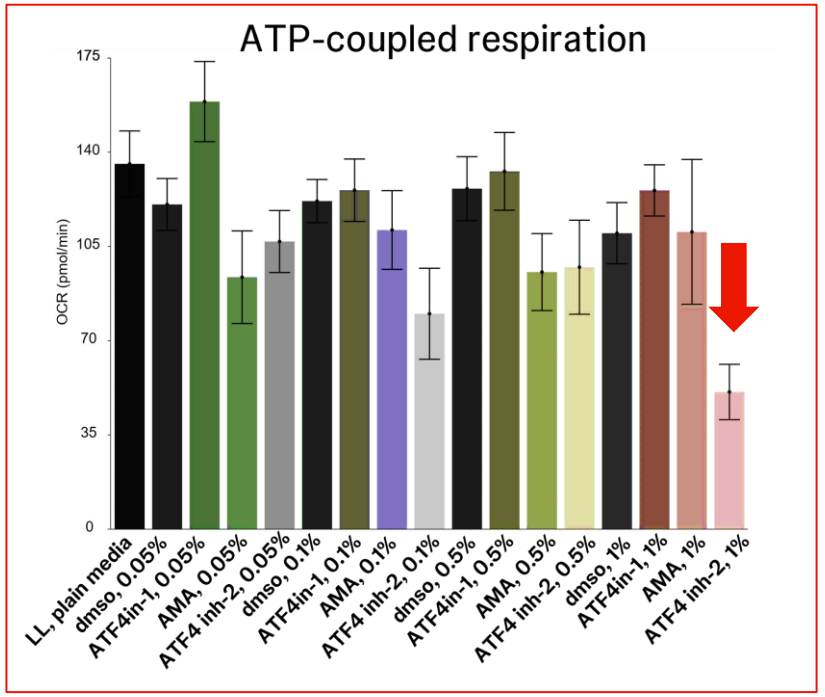


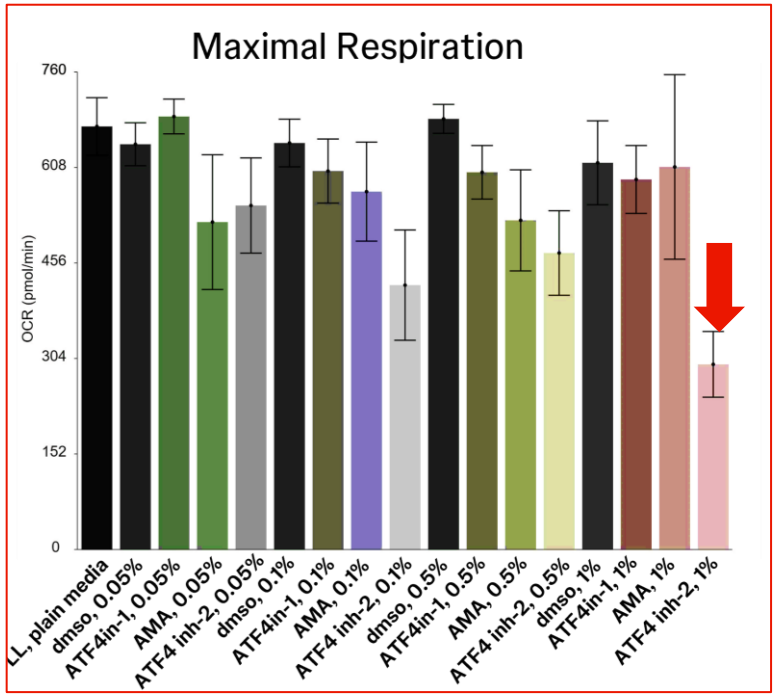
ATF4-158201 (ATF4 in, eIF4B activator)



ATF4-in-2 (specific ATF4 inhibitor) Inhibitor 1







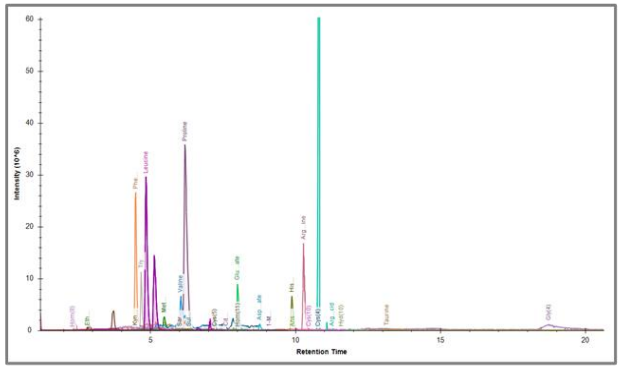
J&J

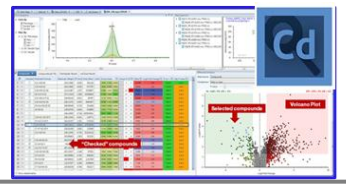
Patent Pending

Path Forward

Support Metabolomics Research Projects

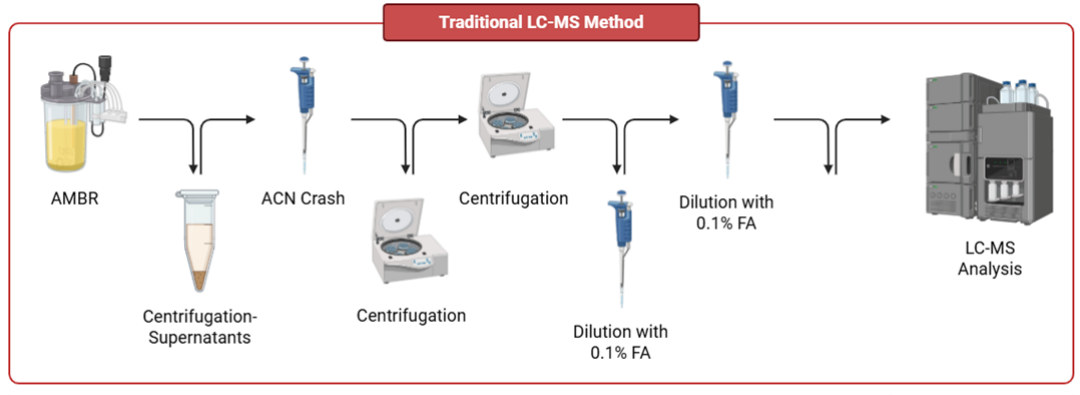
Current Untargeted Method | Current Targeted Method

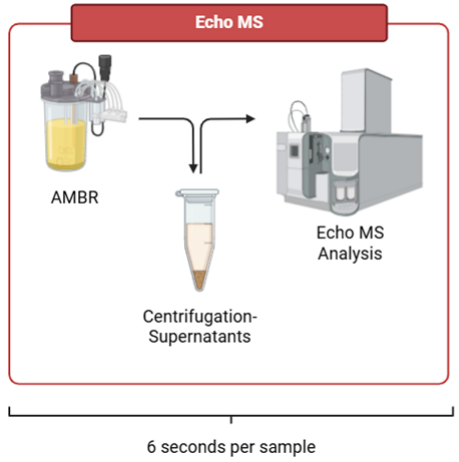




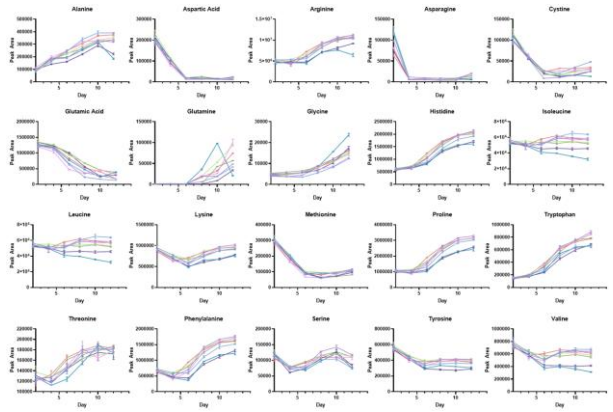
Skyline

Support Cell Line Development Projects





6 second/sample amino acid quantitation in crude supernatants
783 injections in 75 minutes
Automation friendly formats



Patent Pending

Conclusions

Cell Engineering Efforts @ CEAS

✓ Leveraging cell engineering to address titer and PQ improvements.

✓ Using multi-omics to streamline analysis and support engineering efforts.

✓ Integrating genome-scale modeling with multi-omics data for tailored, data-driven insights.

Showcasing an Application of Multi-omics to Get Insight on Lactate Runaway

✓ Identified a model to study lactate runaway using high and low lactate phenotypes.

✓ Collected proteomics and metabolomics data to identify overexpression targets.

✓ Validated omics findings with additional methods like 15mL scale and Seahorse assay.

❑ Implemented a Metabolomics & ECHO MS Packages to Our Group

- ✓ The untargeted metabolomics package supports all research projects.
- ✓ ECHO MS is used for relative amino acid profiling across CLS projects.

Acknowledgments

CEAS Team

Partha Chowdhury

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Bo Zhai

Abby Chiang

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Mona Goli

Thank You for Your Attention!