Utilizing Machine Learning and Mechanistic Understanding to Appreciate the Impact of pH, DO, and pCO_2 on Upstream AAV Yield and Product Quality

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SESSION: AAV critical quality attributes

DO: Dissolved Oxygen
pCO_2: Dissolved CO_2

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- I am a full-time employee at Solid Biosciences

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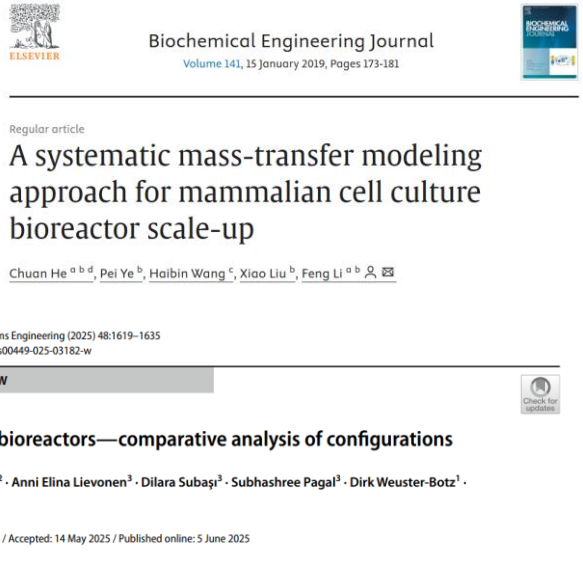
Why should you care about pH, DO, and pCO_2? They tend to vary as you scale-up your bioreactor, impacting PQ!

Google Scholar: >250K publications on the impact of scale-up upon product quality. Established literature is mAb centric. Literature considering gene therapy is limited.



ELSEVIER

Biochemical Engineering Journal

Volume 141, 15 January 2019, Pages 173-181

Regular article

A systematic mass-transfer modeling approach for mammalian cell culture bioreactor scale-up

Chuan He [a b d], Pei Ye [b], Haibin Wang [c], Xiao Liu [b], Feng Li [a b]

Bioprocess and Biosystems Engineering (2025) 48:1619–1635
https://doi.org/10.1007/s00449-025-03182-w

CRITICAL REVIEW

Scale-down bioreactors—comparative analysis of configurations

Prasika Arulrajah[1,2] · Anni Elina Lievonen[3] · Dilara Subaşı[3] · Subhashree Pagal[3] · Dirk Weuster-Botz[1] · Anna-Lena Heins[2]

Received: 19 March 2025 / Accepted: 14 May 2025 / Published online: 5 June 2025
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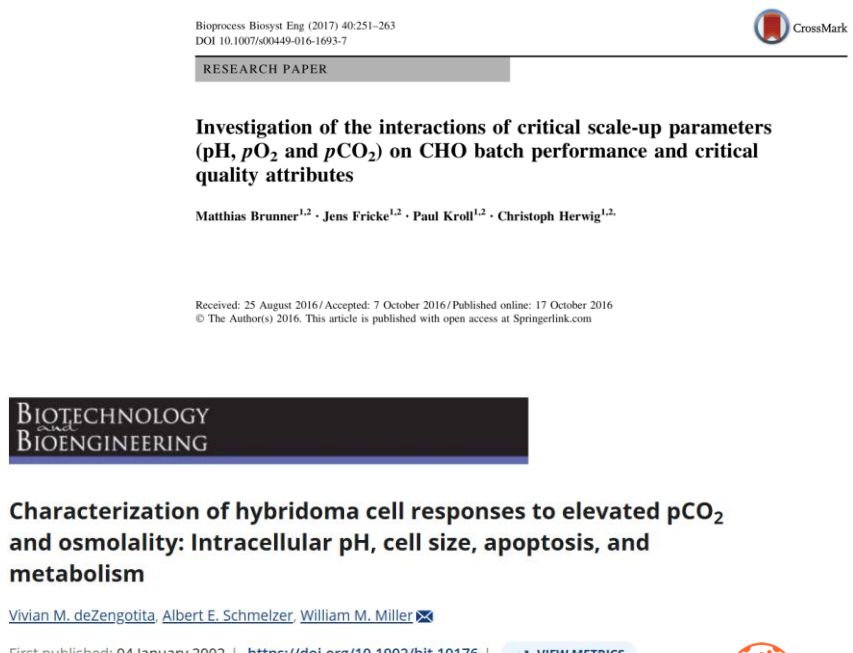

Bioprocess Biosyst Eng (2017) 40:251–263
DOI 10.1007/s00449-016-1693-7

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RESEARCH PAPER

Investigation of the interactions of critical scale-up parameters (pH, pO_2 and pCO_2) on CHO batch performance and critical quality attributes

Matthias Brunner[1,2] · Jens Fricke[1,2] · Paul Kroll[1,2] · Christoph Herwig[1,2,]

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BIOTECHNOLOGY AND BIOENGINEERING

Characterization of hybridoma cell responses to elevated pCO_2 and osmolality: Intracellular pH, cell size, apoptosis, and metabolism

Vivian M. deZengotita, Albert E. Schmelzer, William M. Miller

First published: 04 January 2002 | https://doi.org/10.1002/bit.10176 | VIEW METRICS

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So pH, DO, pCO2 matter...what to do about it? We executed 24 related batches with goal to:

- Evaluate process sensitivity by directly or indirectly manipulating pH, pCO_2, or DO profile.
- Determine impact to batch performance at harvest via 3 assays.
  - ddPCR yield
  - Multiplex ddPCR: Genomic Integrity
    - 2 primers used simultaneously to verify that the genome is complete
  - Full Capsid Ratio: ddPCR/Capsid ELISA
    - High-throughput method that overestimates on an absolute basis, but predictive on relative basis

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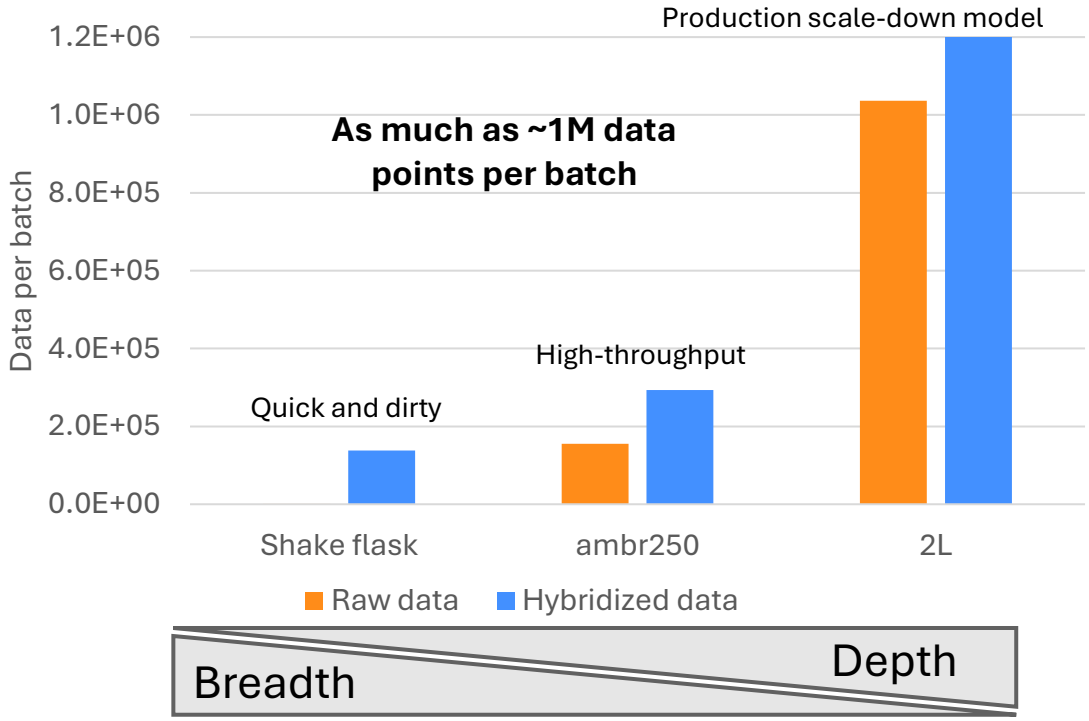
Why is Process Development uniquely poised to leverage ML?

Our experiments are rich with data! Below are the 3 small-scale platforms we use with relative advantages and disadvantages.



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Product quality predictions are complex. What can machine learning offer?

Predictive Modelling

- Predict outputs such as GOI ddPCR titer, full ratio (GOI ddPCR/Capsid ELISA), and multiplex ddPCR genomic integrity
- Enable “what if” questions to be answered without the need for added experiments.

If you do predictive modeling, don’t waste this opportunity to assess batch normalcy (using same dataset)!

Batch Normalcy Detection

- Determine normal batch to batch variability
- Determine whether or not a given batch is performing normally

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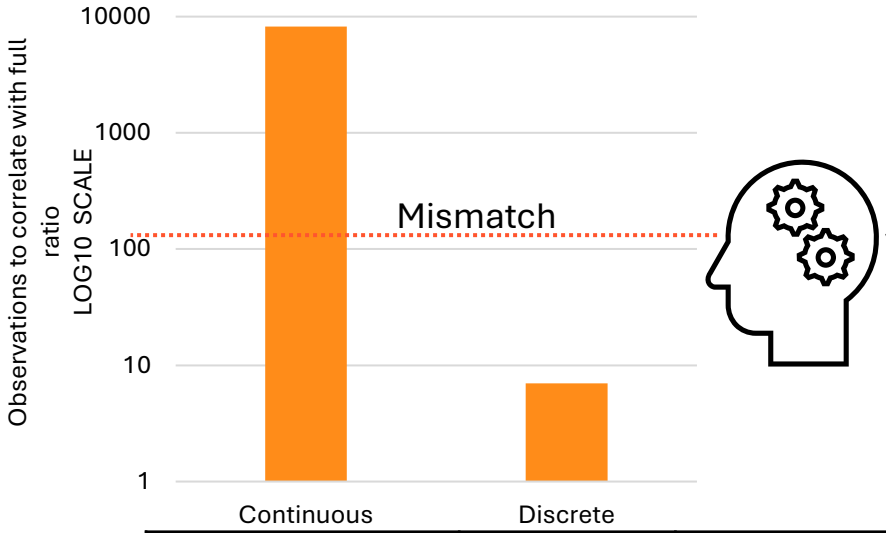
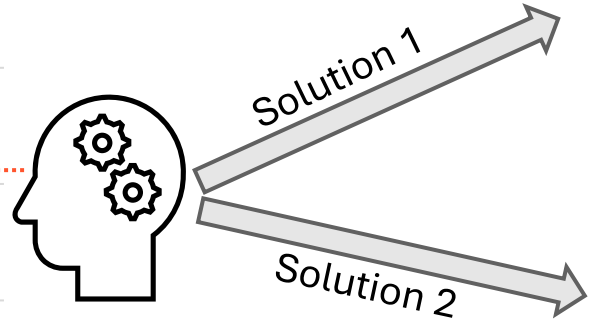
Why is it a challenge to apply ML to process development? What shortcomings are common when applied?

The challenge: Sample Frequency divergence



Oxygen Flowrate	[Glucose]	**Example of Measurement**
Oxygen uptake rate (OUR)	Glucose consumption	**Relevant cell-specific flux**

Note: Each observation is an individual recorded data point provided to the model.



Common shortcomings:

Predict outcome with separate models (even though data sets inherently related)

Predict outcome with unbalanced model with ~1000x the continuous data for a given variable as compared to discrete

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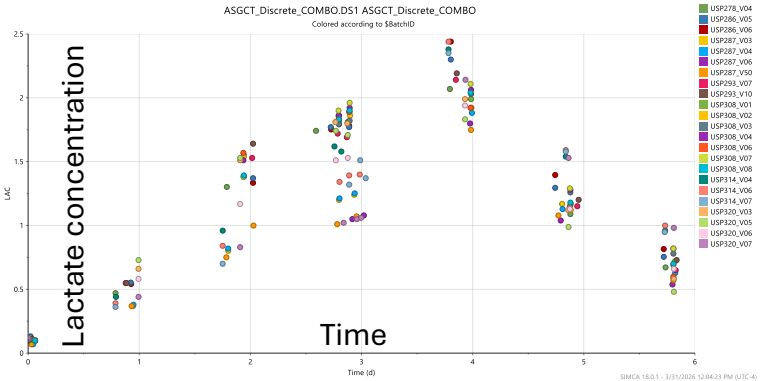
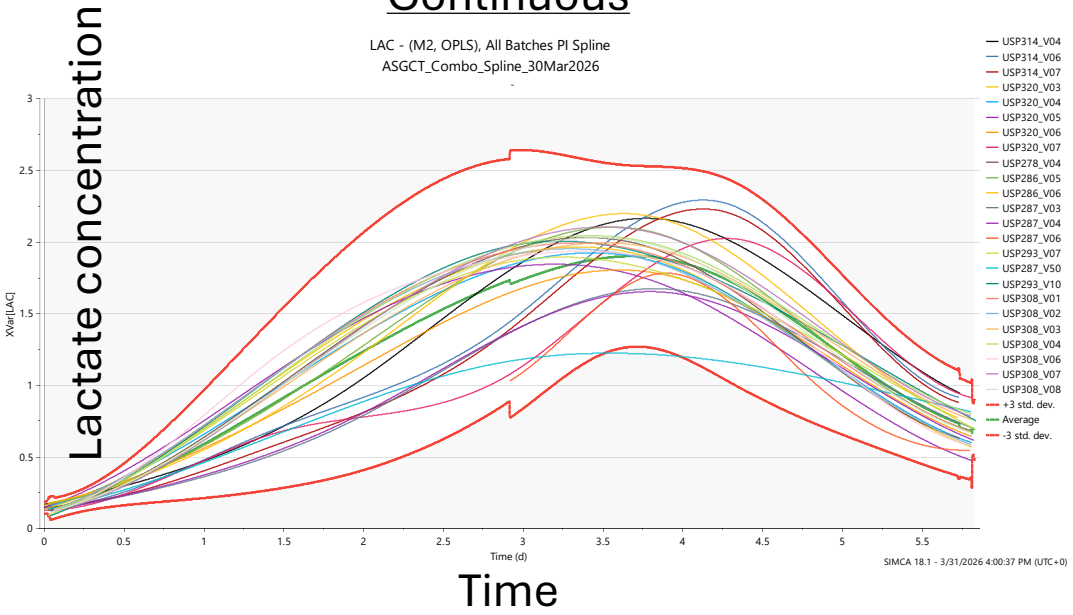
Solution: Hybridize your datasets using metabolic flux analysis

Leverage entire data set, not a subset

Discrete



Continuous



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Multivariate predictions by 3 ML models

Level playing field: Same dataset for all models. 24 batches x 180K variables.

1) Hybridized dataset (discrete->continuous)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
pCO_2	pO_2	Viable Cell Density (VCD)	Viability	Glutamax	ASN	ASP	GLC	GLU	K	LAC	LDH	Na	NH_3	Pyruvate	Osmolality

2) Online dataset (continuous by nature)

17	18	19	20	21	22
DO	pH	Temperature	CO_2 Flow Rate	Base Flowrate	O_2 Flowrate

3) Batch performance

23	24	25
ddPCR titer	Capsid Full Ratio	Genomic Integrity

24 batches insufficient

ML Model	Orthogonal Partial Least Squares (OPLS)	Random Forest (RF)	Bayesian Neural Networks (BNN)

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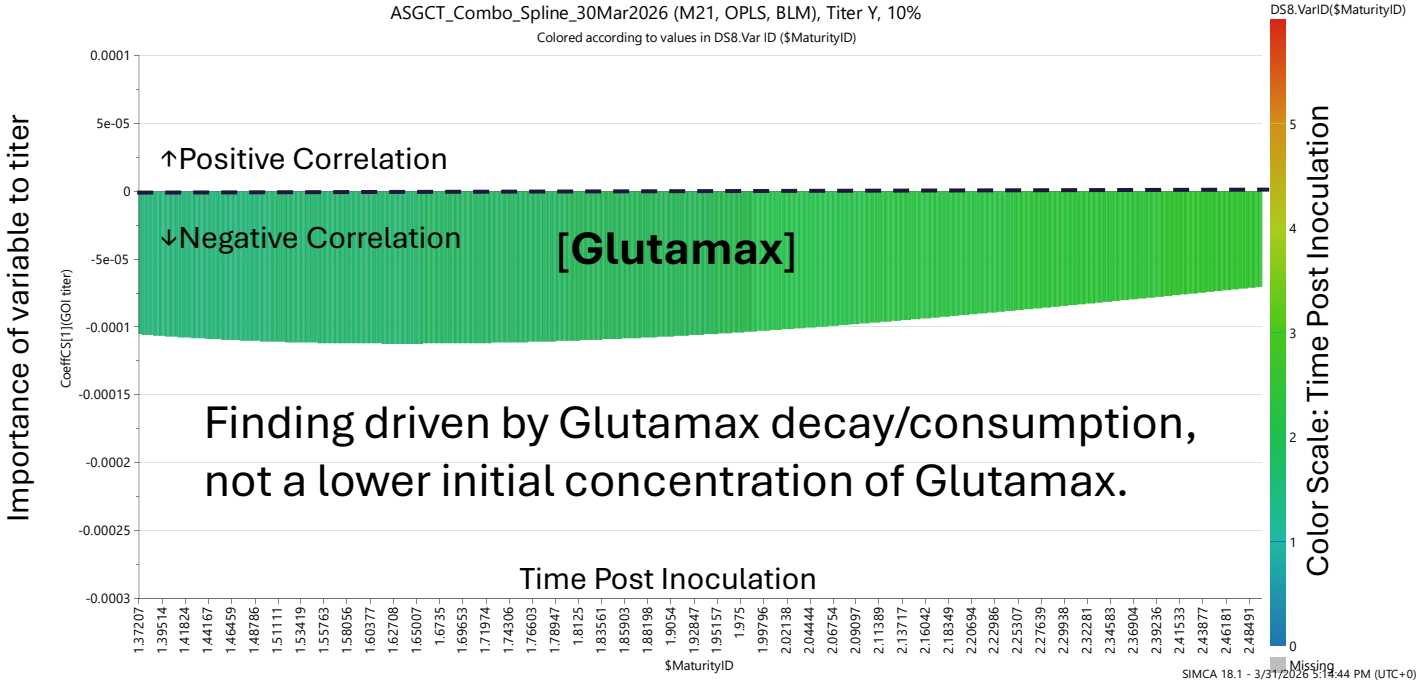
Out of ~180K variables, what are the top 1% of ddPCR harvest titer correlates? When do they matter?

- [**Glutamax**] most relevant in 36 hours prior to Transfection. Lower the glutamax, higher the titer.
- Second most significant in Random Forest (RF), most significant in Orthogonal Partial Least Squares (OPLS).



Note:
Glutamax is a dimer of Gln and Ala

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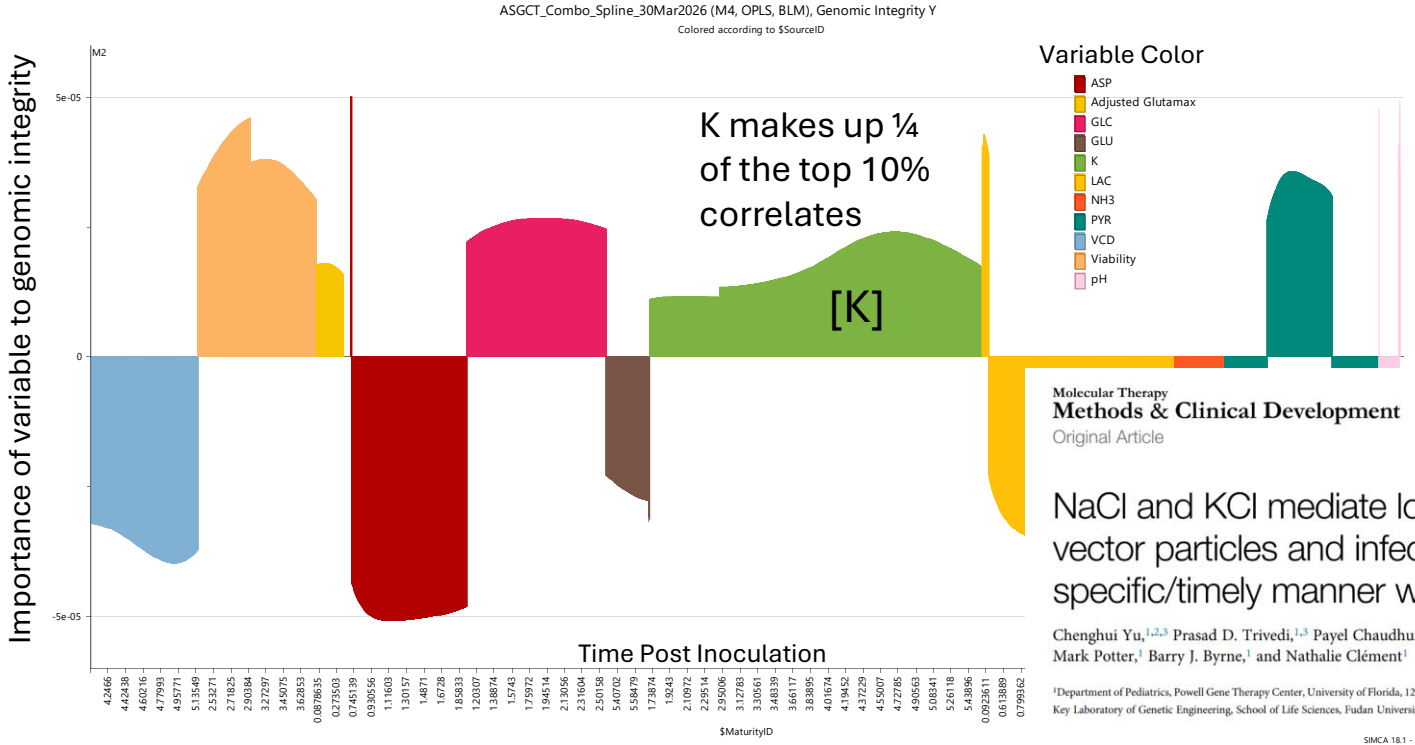
What are the top 10% of genomic integrity correlates? When do they matter?

[Potassium] ([K]) was a significant factor in all 3 ML models. [K] correlated with genomic integrity most strongly 1-2 days post transfection (period of peak ddPCR productivity). Higher [K], higher genomic integrity.



Genomic integrity drives protein expression

Molecular Therapy
Methods & Clinical Development
Original Article

NaCl and KCl mediate log increase in AAV vector particles and infectious titers in a specific/timely manner with the HSV platform

Chenghui Yu,[1,2,3] Prasad D. Trivedi,[1,3] Payel Chaudhuri,[1] Radhika Bhake,[1] Evan J. Johnson,[1] Tina Caton,[1] Mark Potter,[1] Barry J. Byrne,[1] and Nathalie Clément[1]

[1]Department of Pediatrics, Powell Gene Therapy Center, University of Florida, 1200 Newell Drive, Academic Research Building, RG-187, Gainesville, FL 32610, USA; [2]State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai 200438, China

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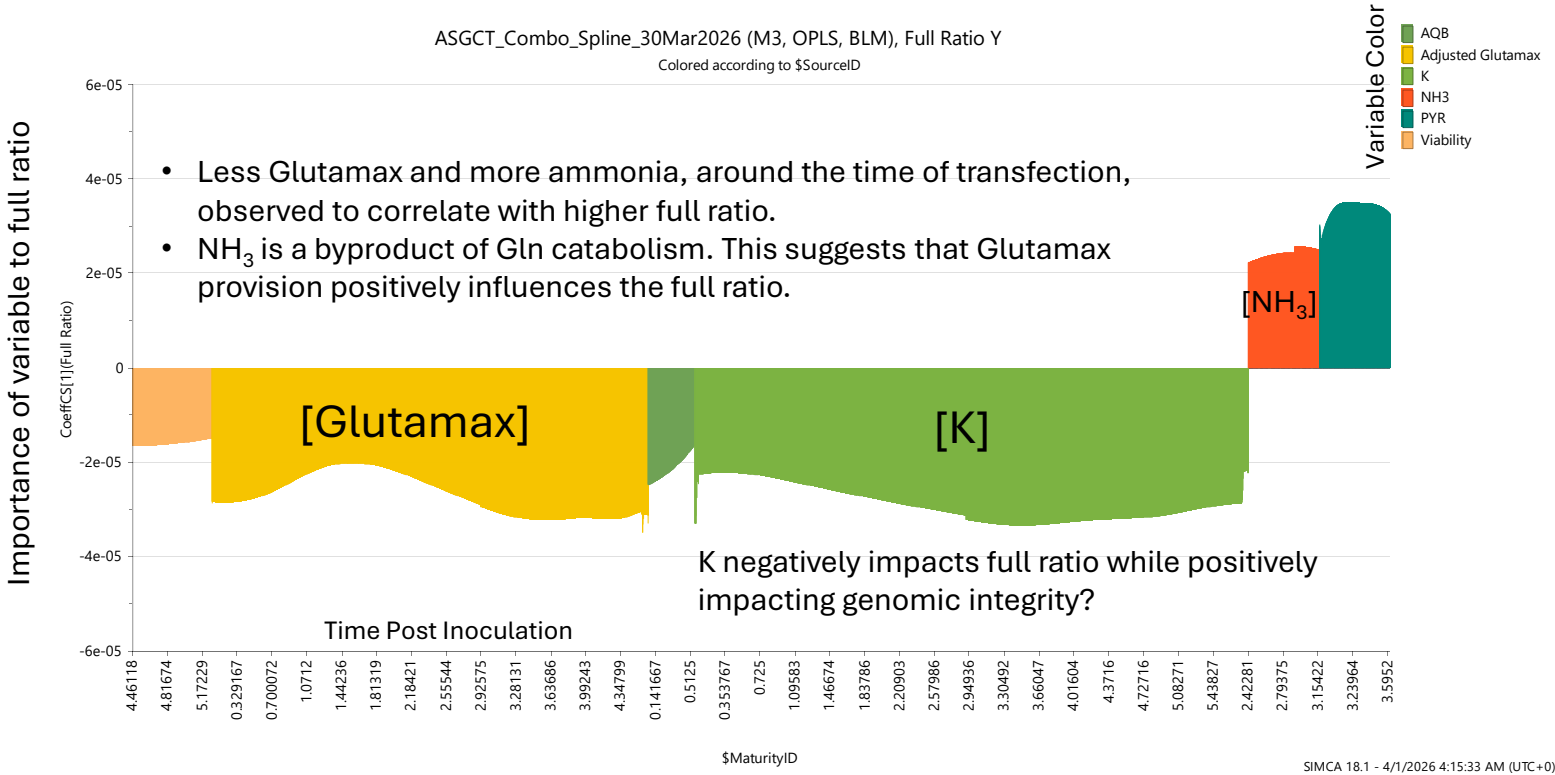
What are the top 10% of capsid full ratio correlates? When do they matter?

When examining top 10% of correlates in Orthogonal Partial Least Squares, Glutamax and Potassium (K) make up 70% of it. Lower [K] or [Glutamax], higher capsid full ratio. Likewise, Random Forest highlighted the importance of these variables.



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Conclusions and recommendations for applying ML

- When applying ML, context matters! pH, DO, pCO2 might not correlate with PQ directly, but they directly drove downstream metabolism which did correlate!
- We applied 3 ML approaches in parallel. Corroborative data is invaluable and tangible with ML.
- ML offers correlations, not causations. Logical deduction and systematic understanding remain crucial to successfully leveraging ML.
- Higher glutamax decay/consumption positively corresponded with yield and full ratio. Conversely, higher ammonia did not negatively correlate with yield or full ratio. At some point, ammonia will become toxic, but that limit was not observed at 2-3mM. Additional glutamax provision has the potential to improve full ratio and titer and merits future exploration.
- Potassium positively correlated with genomic integrity but negatively correlated with full ratio? Within similar window of time? Corroborative assessment needed (e.g. AUC) given that ddPCR to Capsid ELISA tends to overestimate full ratios. If conflict can be addressed, given the reported correlation of genomic integrity to potency within literature, and observed correlation of potassium to genomic integrity, future exploration is merited.

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Thank you!

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