



Comparison of cell culture product quality at development and manufacturing scales

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Outline

- Background and Motivation
 - Process outlines
 - Definition of protein quality
- Review of small scale vs large scale processes
 - Seed train
 - Production bioreactor
- Comparison of improved model to large scale data
- Summary and conclusions

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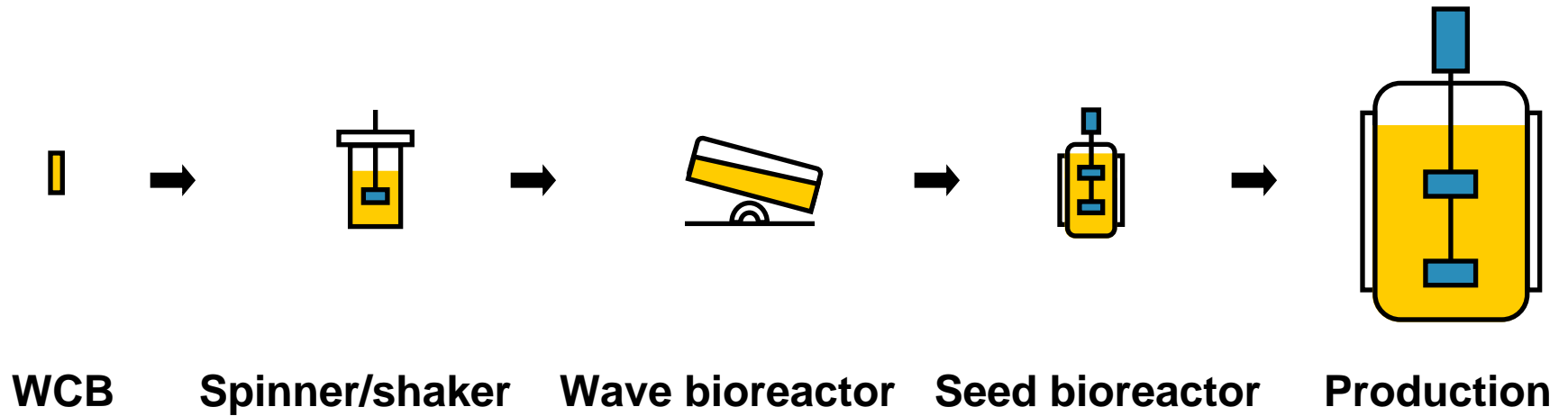
- Discover and develop new medicines for immune-mediate and inflammatory diseases
- Develop biological-based medicines for other therapeutic areas
- Manufacture biologics for clinical and commercial use



Motivation

- Scheduled tech transfer and scale-up activities for late-stage projects
- Review of scale-down model to support scale-up activities
 - Platform projects using similar scale down model
 - Growth, productivity, protein quality
- Use understanding gained to ensure successful transfer and scale up with minimal issues

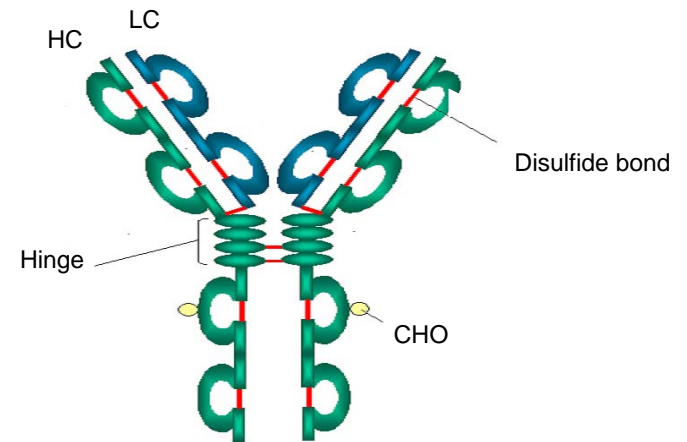
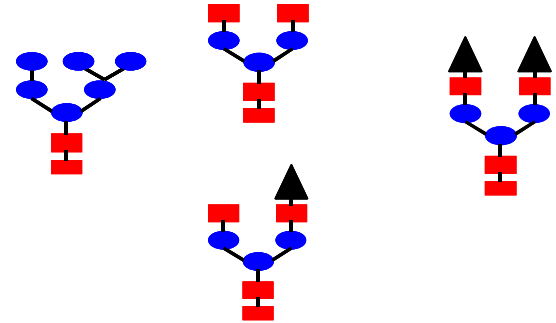
Process Outlines



- Project A: fed-batch
- Project B: batch

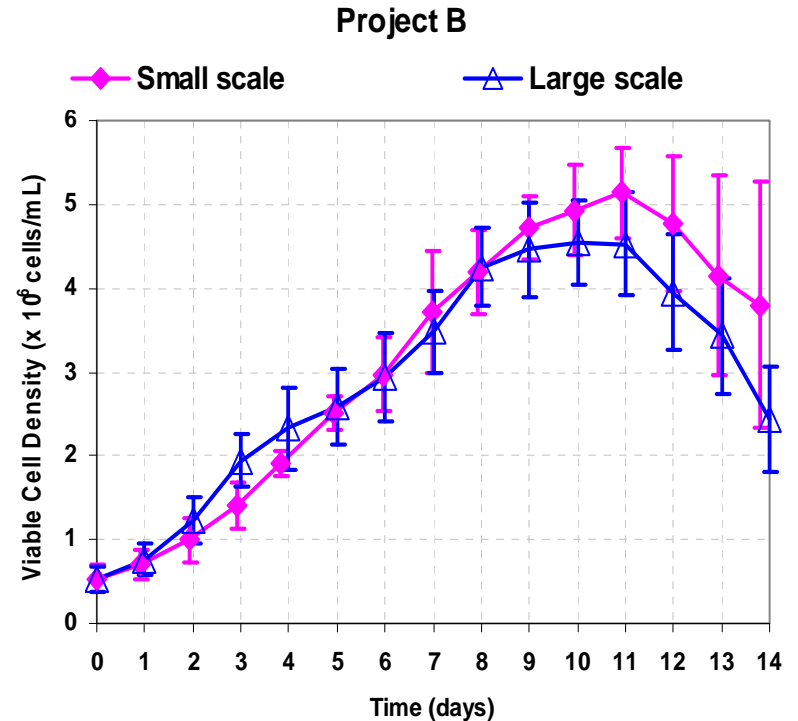
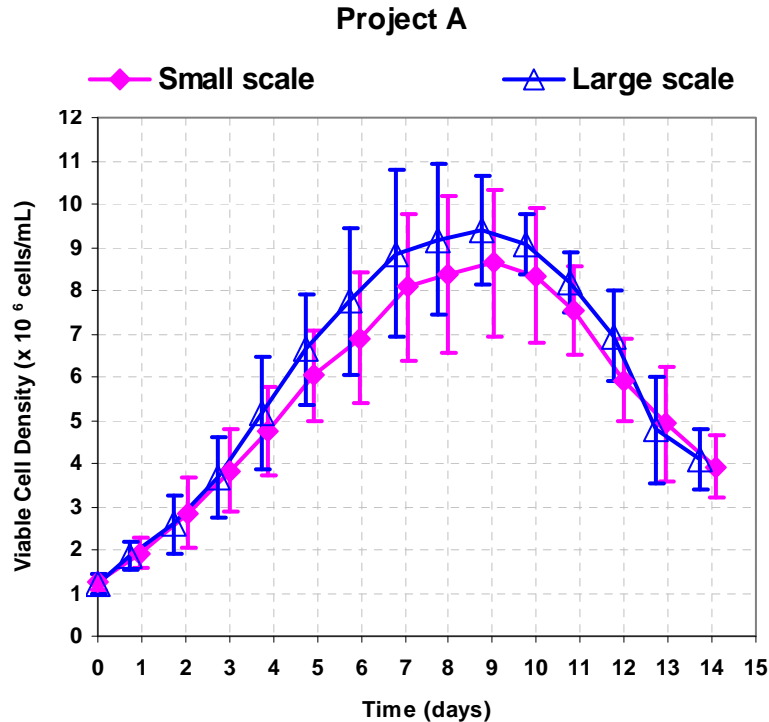
Protein quality indicators

- Oligosaccharide analysis
 - Distribution of glycoforms
- Charge heterogeneity
 - Ion-exchange separation of charged variants
- Size exclusion chromatography
 - Monomer vs aggregate



Initial model review – Cell density

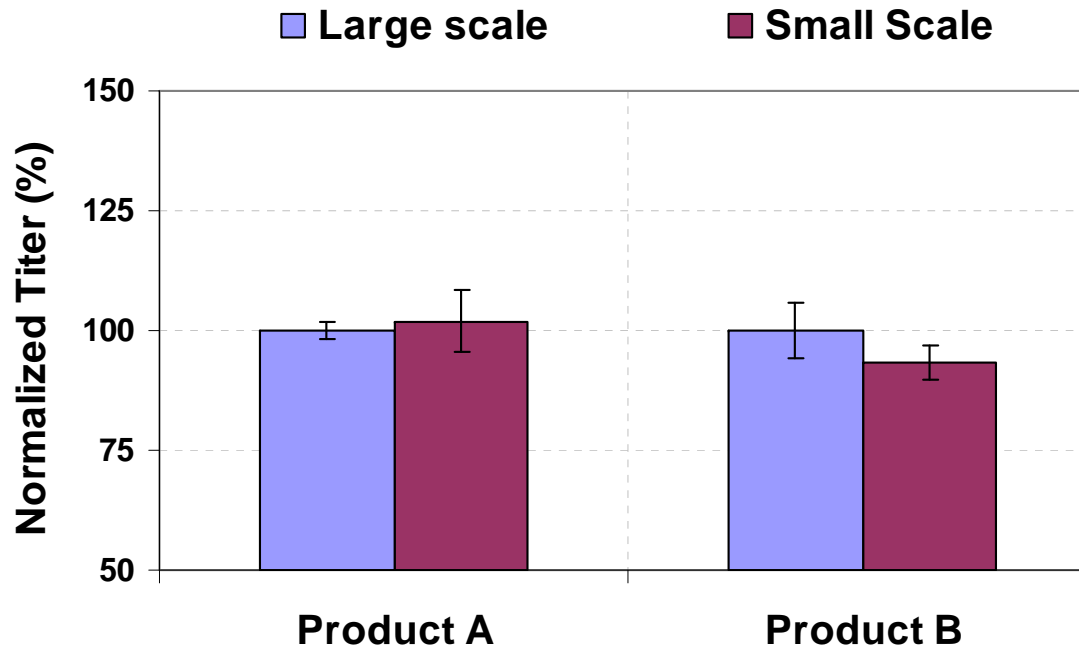
- Comparison of small scale (3L) vs large scale (6000L)



Comparable growth observed for both projects

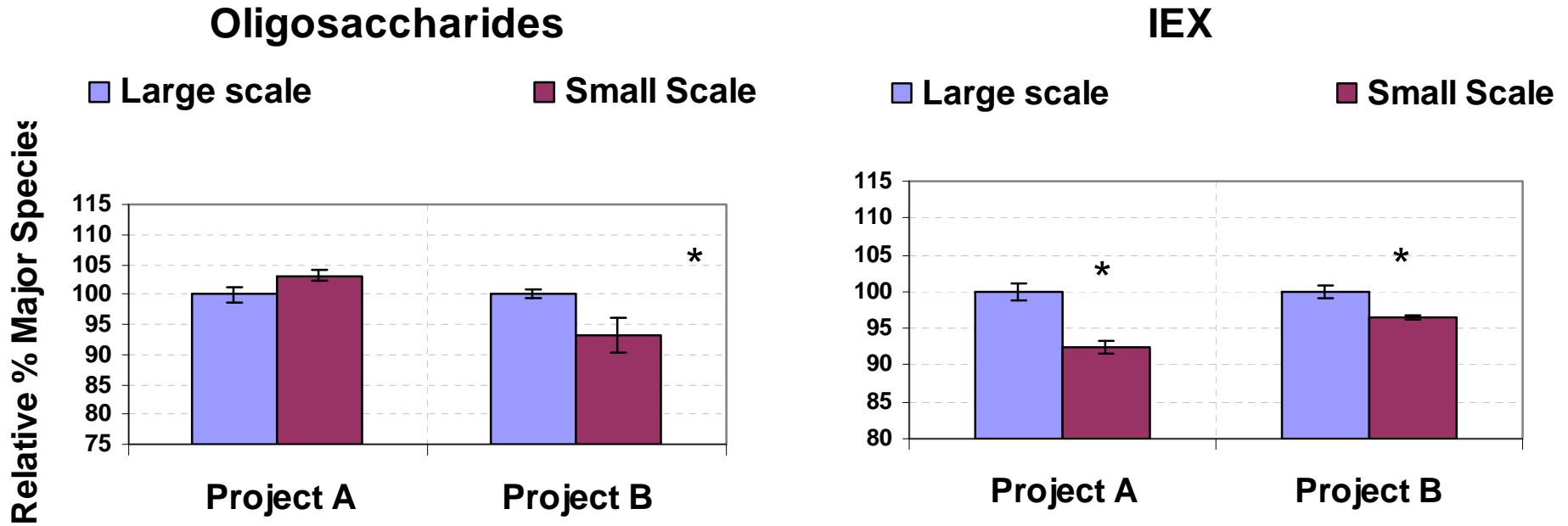
Initial model review – Productivity

- Comparison of small scale (3L) vs large scale (6000L)



Comparable productivity observed for both projects

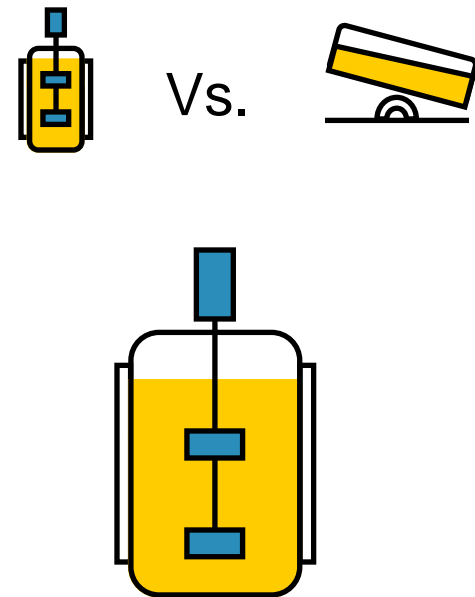
Initial model review – major protein quality species



Differences observed between small and large scale processes
Project to project variability

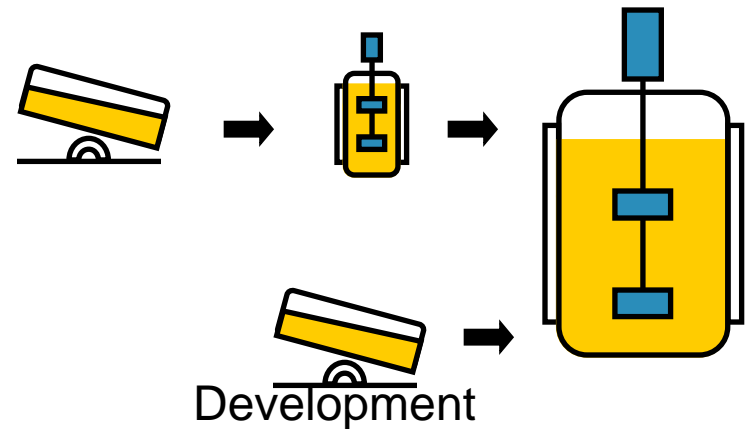
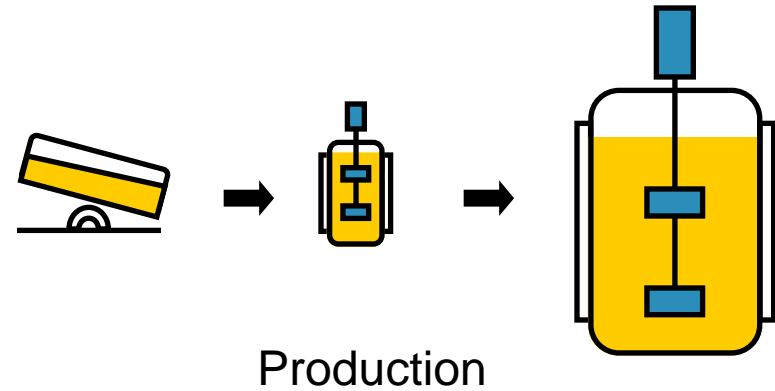
Scale down model review – potential areas for study

- Analytical methods
 - Methods have no impact on measured PQ
- Seed train
 - Source of production reactor inoculum
- Production bioreactor
 - Operating parameters
 - Mass transfer and mixing



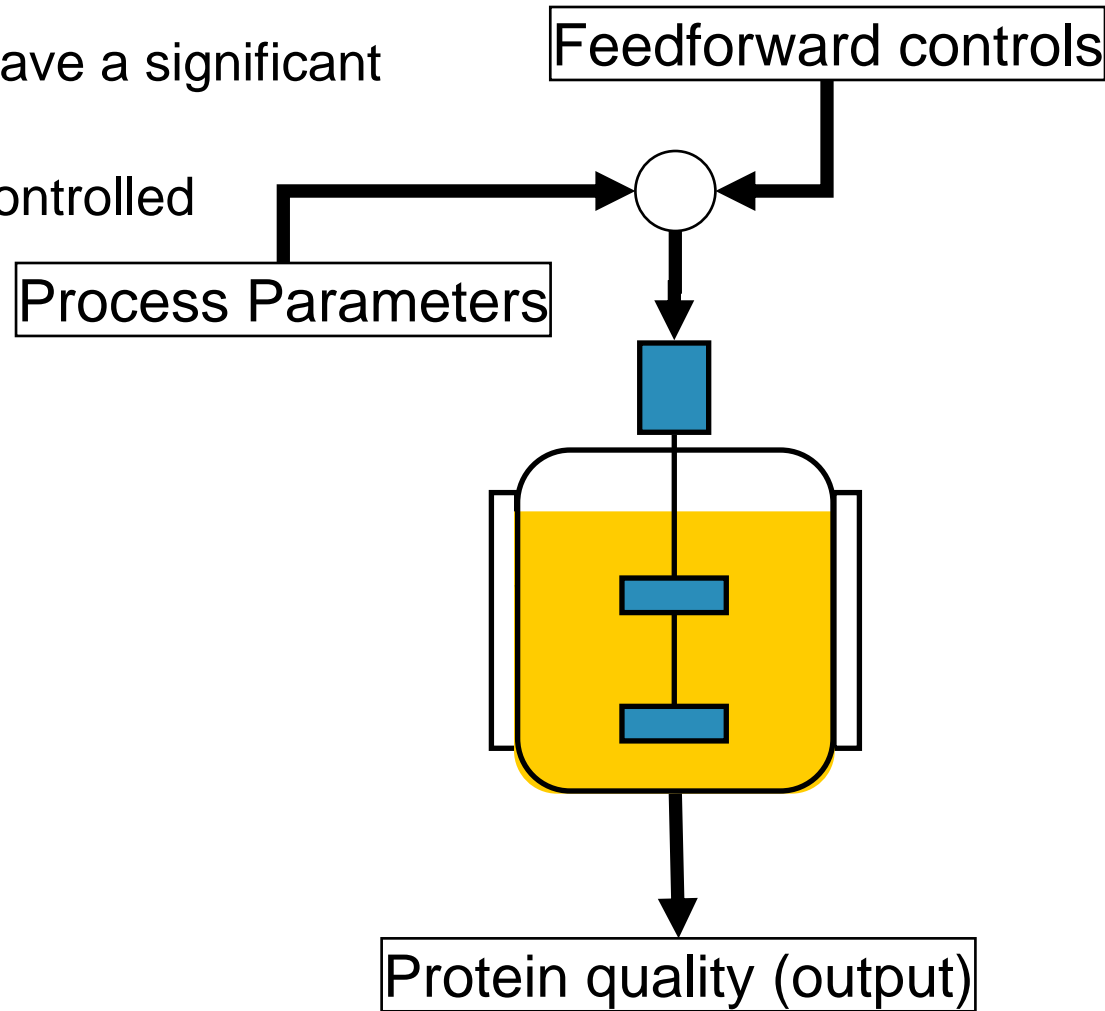
Differences in cell source

- Production uses defined process
- Development has flexible approach
 - Wave bioreactor seed trains less well-controlled as compared to seed bioreactors
- No impact of cell source on protein quality observed from historical data
 - Satellite runs used to eliminate seed train as source of variation
 - Planned further experiments to explore impact of seed train



Production bioreactor methodology

- Cell culture conditions can have a significant impact on protein quality
- Protein quality is indirectly controlled
 - Feed-forward strategy
- Operating parameters
 - Scaled appropriately
- Mass transfer and mixing
 - Scale agitation by P/V
 - Gas flow cascade to match



Comparison of initial production bioreactor model

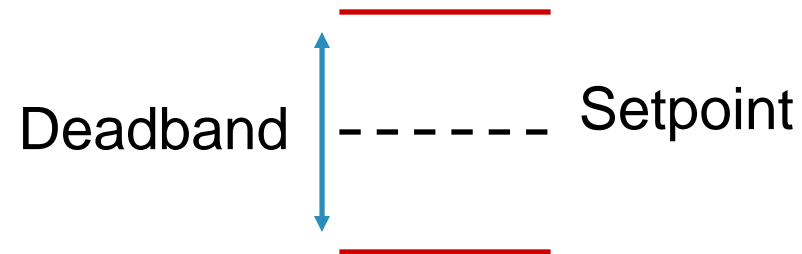
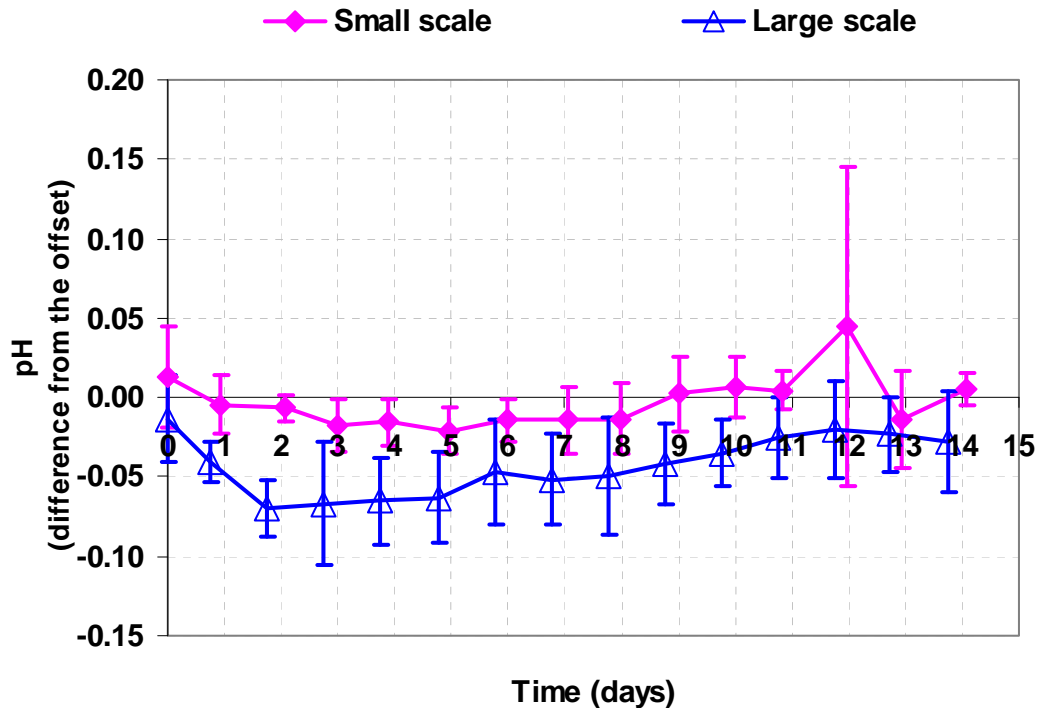
Online Parameters	3 L vs 6000 L
Temperature	Green
pH	Yellow
Dissolved oxygen	Green
Mass transfer	Green
Gas flows	Yellow
Base Consumption	Yellow
Offline Parameters	3 L vs 6000 L
Growth and viability	Green
Glucose	Green
Osmolality	Yellow
pO ₂ , pCO ₂	Red

Review of initial production bioreactor model

Online Parameter	3L vs 6000L
Temperature	Grey
pH	Yellow
Dissolved oxygen	Grey
Mass transfer	Grey
Gas flows	Grey
Base Consumption	Grey
Offline Parameters	3L vs 6000L
Growth and viability	Grey
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Osmolality	Yellow
pO ₂ , pCO ₂	Red

Control of pH

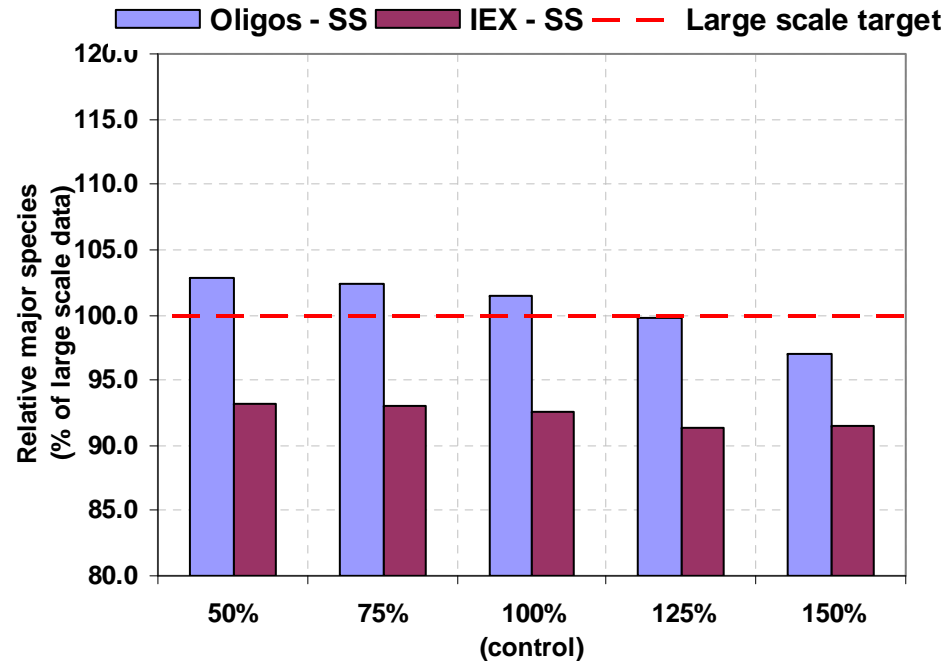
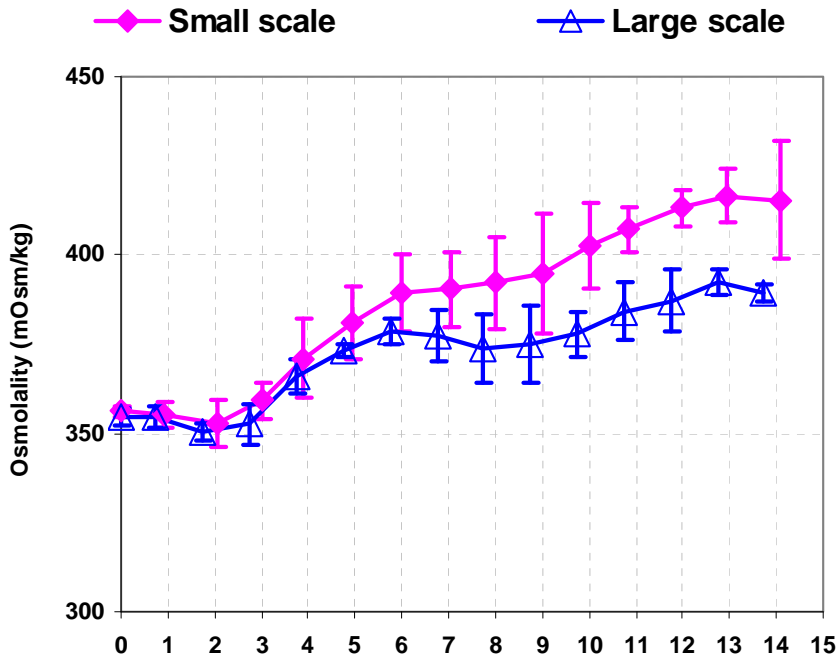
- Previous history identified pH as key parameter for product quality



- Adjust small scale controllers to match large scale control

Osmolality during fed-batch process

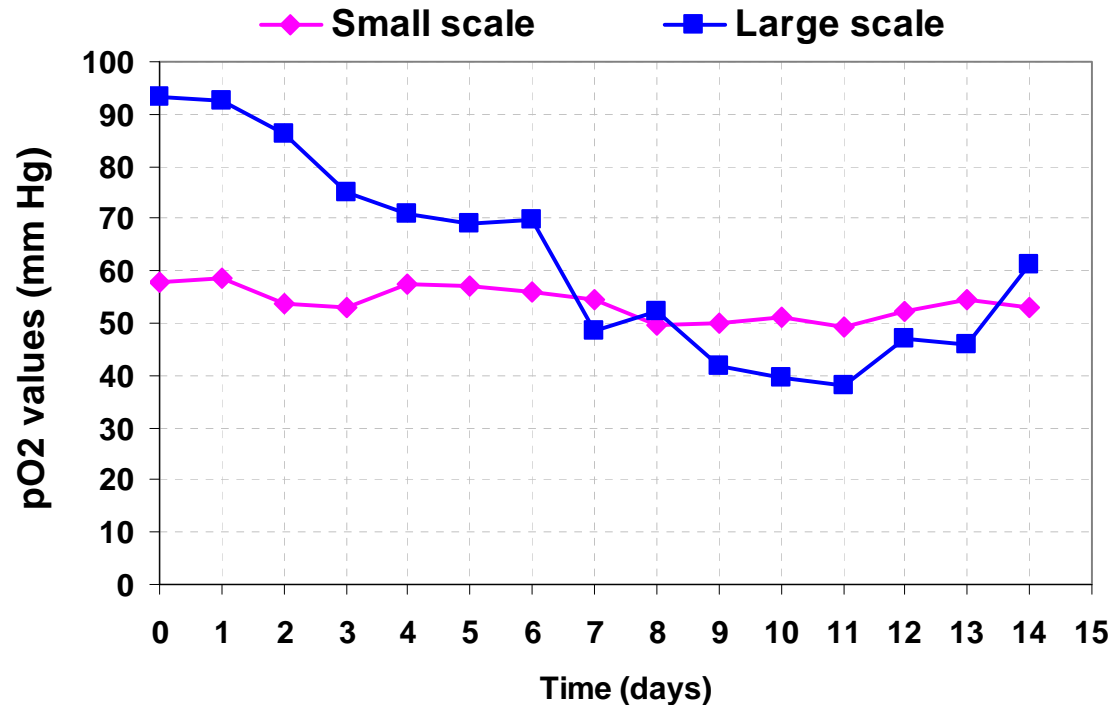
- Increased osmolality at small scale due to feed volumes
 - Feed additions do not account for sampling volume



- Alignment of osmolality does not improve protein quality comparison

Dissolved gas concentrations (pO₂)

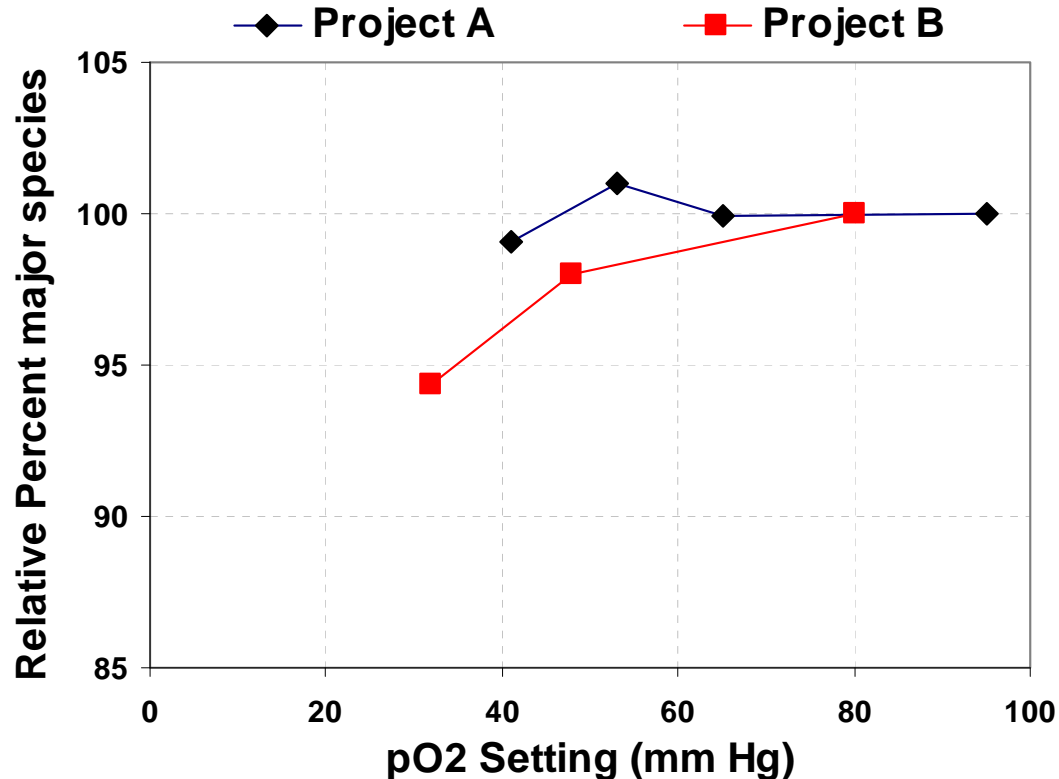
- Increased pO₂ with scale with same DO setpoint



- Increase DO setpoint at small scale to match pO₂ levels

Dissolved gas concentrations (pO₂)

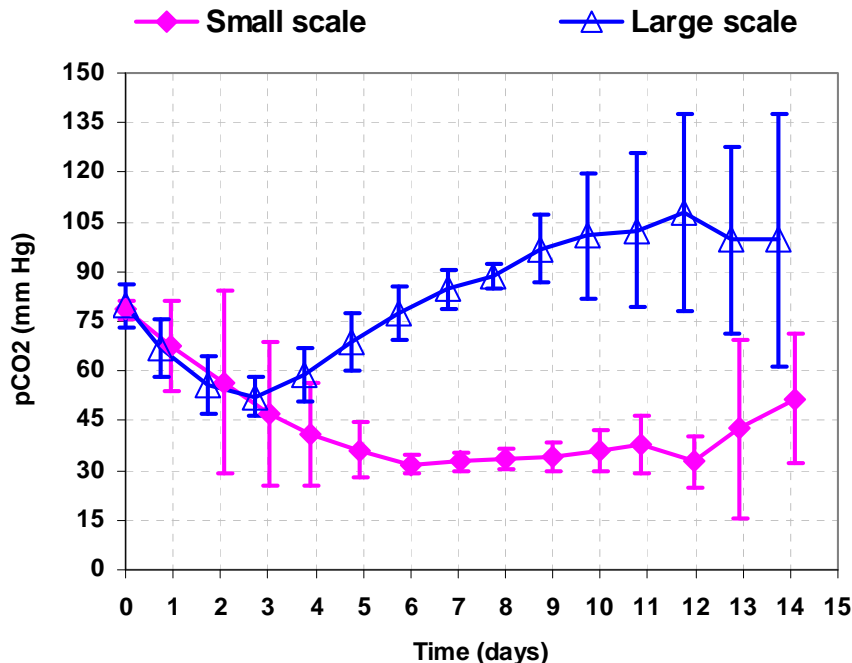
- pO₂ impacts oligosaccharide distribution for 1 of 2 Mabs



- Increase DO setpoint at small scale to match pO₂ levels

Dissolved gas concentrations (pCO₂)

- pCO₂ concentrations increase with scale



- Equipment limitations to evaluation of large scale agitation/aeration
- Experiments showed no impact of pCO₂ on either antibody
- Agitation and airflow values can not be directly scaled from bench scale models
 - Use pilot or existing knowledge

Model comparison improved, but not perfect

Online Parameter	Initial	Improved
Temperature	Green	Green
pH	Yellow	Green
Dissolved oxygen	Green	Green
Mass transfer	Green	Green
Gas flows	Yellow	Yellow
Base Consumption	Yellow	Yellow
Offline Parameters	Initial	Improved
Growth and viability	Green	Green
Glucose	Green	Green
Osmolality	Yellow	Green
pO ₂ , pCO ₂	Red	Yellow

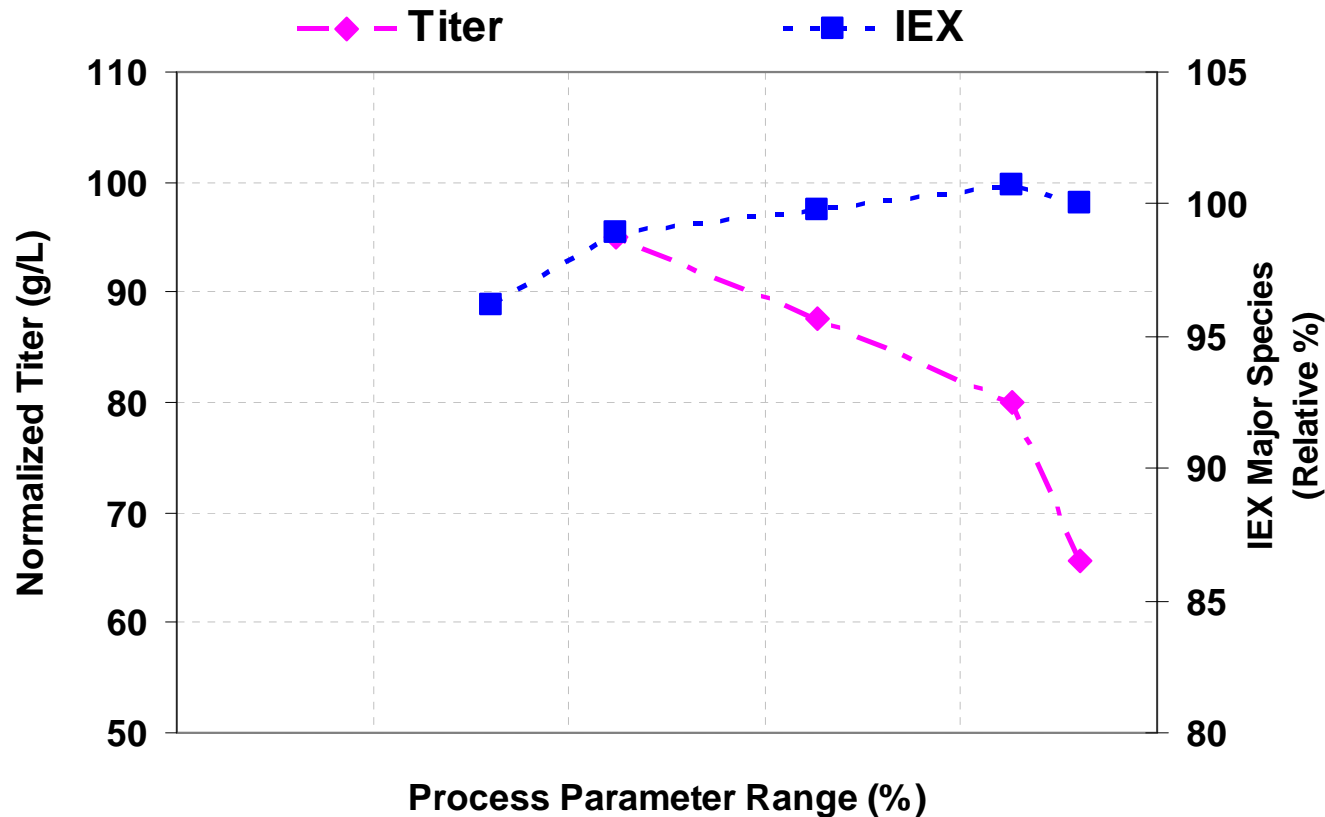
Can the model be used to predict large scale data?



The proof of the pudding is in the eating!

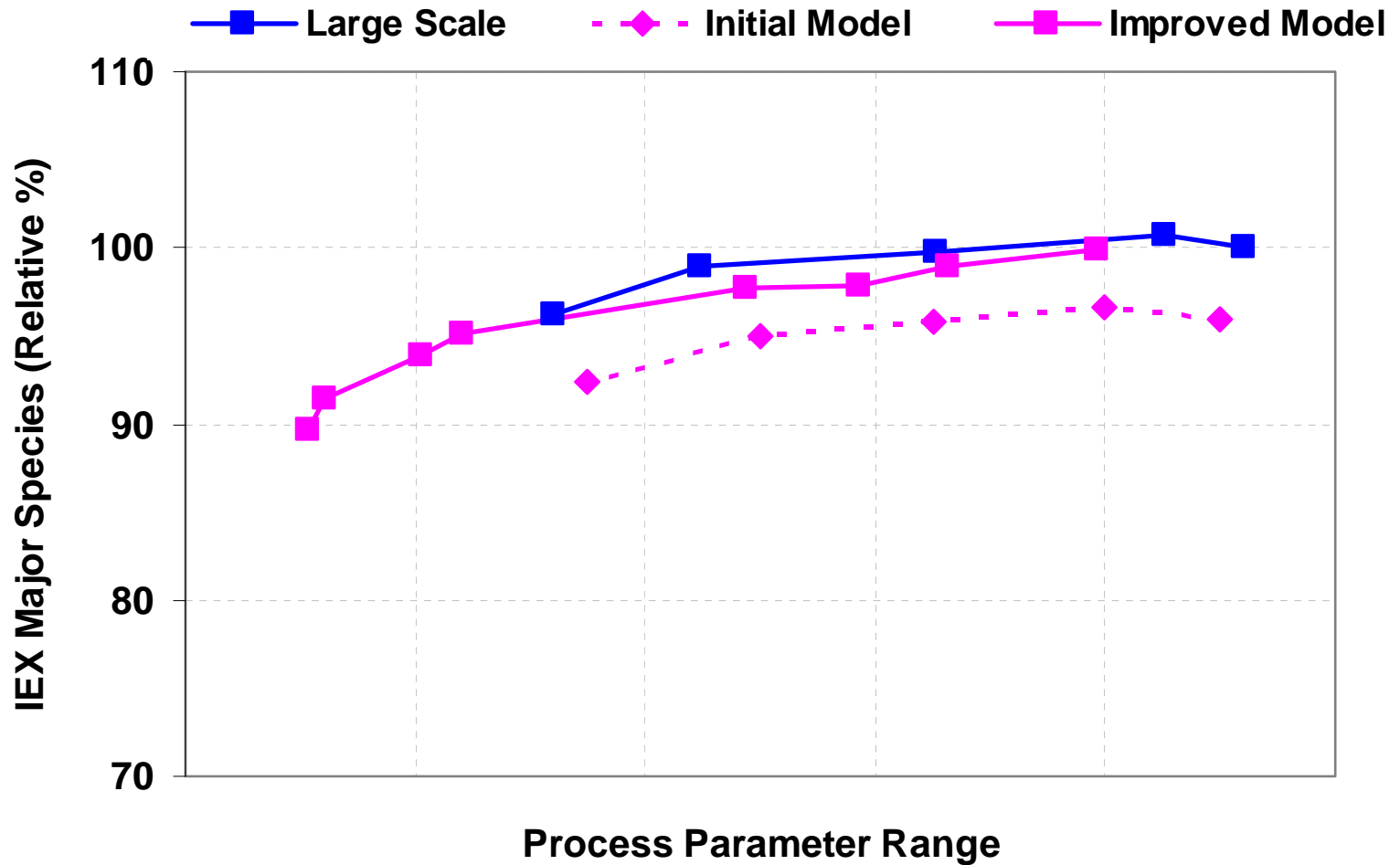
Optimization of key process variable

- Data review identified key parameter for productivity and quality

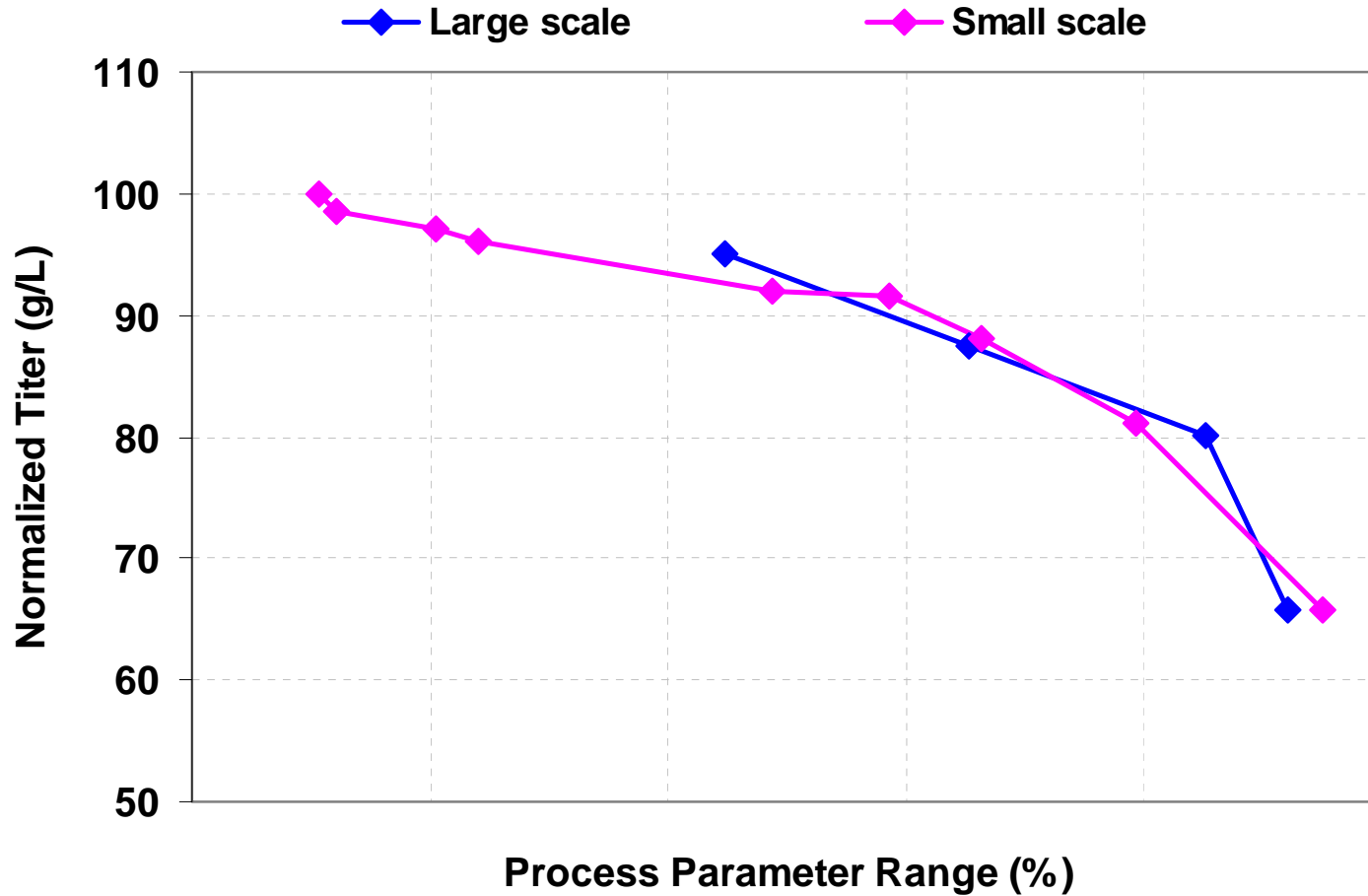


- Can we use improved model to optimize process parameter?

PQ comparison over expanded range



Titer still good too!



Summary

- Assessment of initial scale down model
 - Excellent prediction for growth and productivity
 - Prediction of protein quality trends, absolute differences vs large scale data
 - Cell line differences observed for protein quality prediction
- Small scale comparison for two monoclonal antibody processes
 - Improved comparison with identification of key parameters
 - Gained understanding of model limitations and impact on protein quality
- Evaluation of key parameter for large scale process
 - Improved scale down model used to model process impact
 - Increased confidence in process with less development at scale

Acknowledgements

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 - Technical Operations