Use of Design-of-Experiments to Develop a Highly Productive Process for Manufacture of a Chimeric mAb in GS-CHO Cells

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Outline

- Initial mAb process
- Goals
  - Replacing soy hydrolysate
  - Titer improvement
    - Composition
    - Dosage and timing
  - Manipulation of glycan distribution
    - Galactose supplementation
- DOE design
  - Factor screening
  - Level optimization

Initial process

- *Molecule:* Chimeric IgG1 mAb
- *Cell line:* GS-CHO expression system
  - qP = 10 pg/cell-day
  - IVC = 37E6 cell-day/mL
- *Process mode:* Fed-batch (glucose feed)
- *Cell culture medium:* Basal containing 10 g/L soy hydrolysate
- *Process productivity:* 0.5 g/L in 17 days

Goals

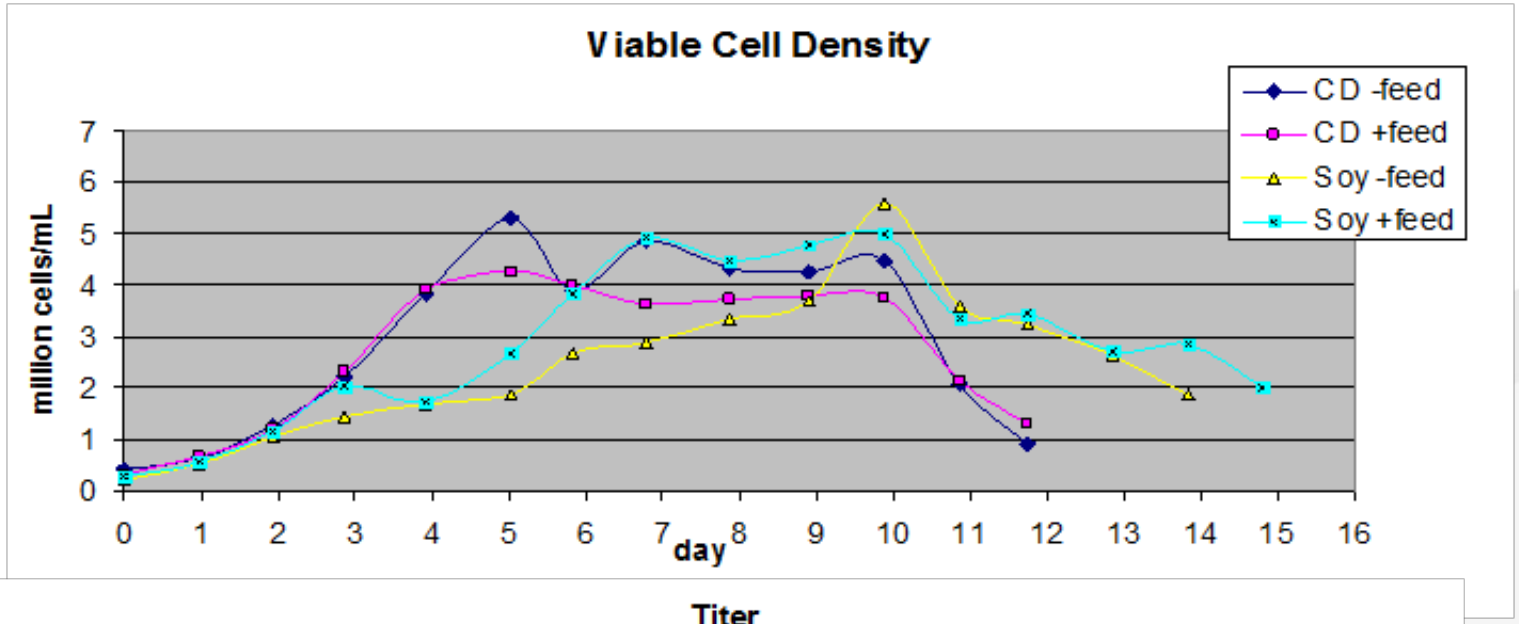
- Improve process
  - 3-fold increase in productivity in a shorter process
    - 1.5 g/L in 14 days
  - No change in basal medium
  - Chemically defined supplements
- Maintain oligosaccharide profile
- Develop toolbox
  - Screen medium components to identify factors
  - Use DOE to confirm effect on titer and quality
  - High throughput culture system for screening and optimization (Tubespin)

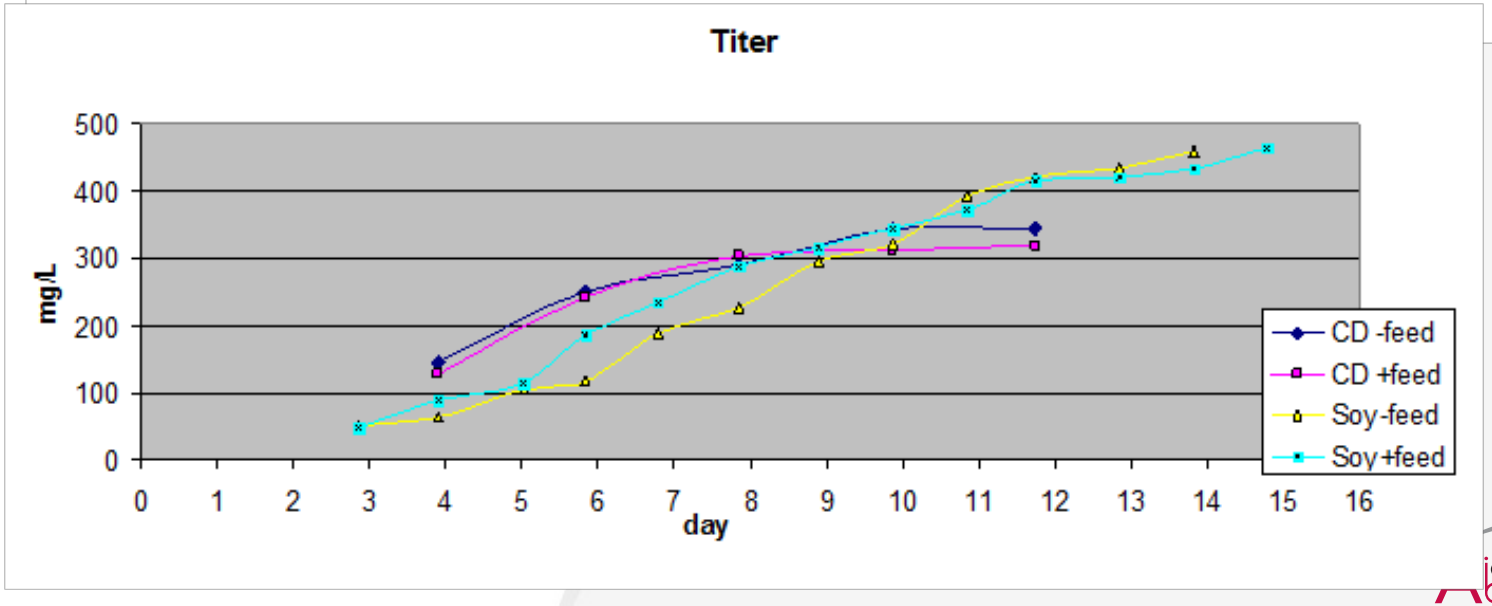
Replacing Soy Hydrolysate

Chemically defined hydrolysate substitute

- ExCell CD Hydrolysate Fusion (SAFC)
  - Recently introduced chemically defined hydrolysate substitute
  - Tested as soy replacement
- Results
  - Overall productivity somewhat lower
  - Promoted initial rapid growth
  - Boosted initial productivity

Initial cell growth more rapid…





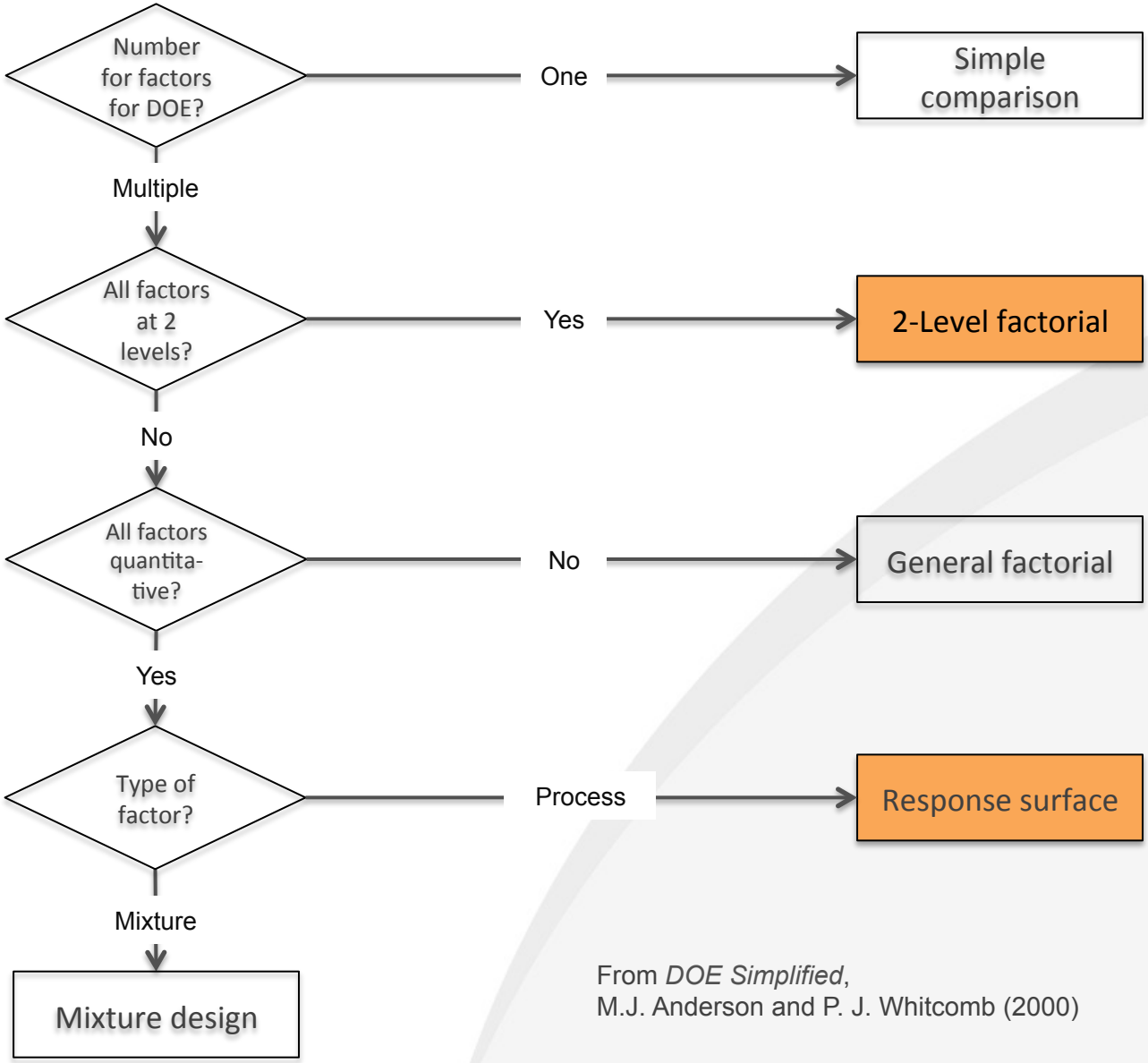
…With somewhat lower titer

	As initial supplement	As initial supplement and feed	Avg. Titer mg/L
Soy	+		460
		+	465
CDH	+		343
		+	318

Basal medium supplemented with either soy hydrolysate (control) or CDH Fusion; average of duplicates

Design-of-Experiments Studies to Improve Titer

Flowchart for applying DOE



From *DOE Simplified*,
M.J. Anderson and P. J. Whitcomb (2000)

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DOE approach

1. Identify variables, ranges, and type and response
2. If >5 variables, do a screening design (fractional factorial)
3. Identify 3-4 most important variables
4. Run response surface methodology to optimize key variables
   - Response Surface Methodology (RSM)
     - »Central Composite
     - »Box-Behnken
5. Get a predictive model of the process for optimization
6. Do check-point trials to verify model and predicted results

Custom basal medium concentrates

- Vitamins and minerals
- Amino acids
- Lipids

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Factorial design
Screen of concentrates

Supplementation with 2x concentrates	Vit. & Min.	Amino acids	Lipids	Titer (mg/L)
A (control)	-	-	-	556
B	+	-	-	601
C	-	+	-	1054
D	+	+	-	990
E	-	-	+	574
F	+	-	+	652
G	-	+	+	1043
H	+	+	+	965
Primary effect on titer (mg/L)	-4.7	417	7.7	
Average fold-increase	0.99	1.70	1.01	

Amino acid concentrate increased titer by 1.7x

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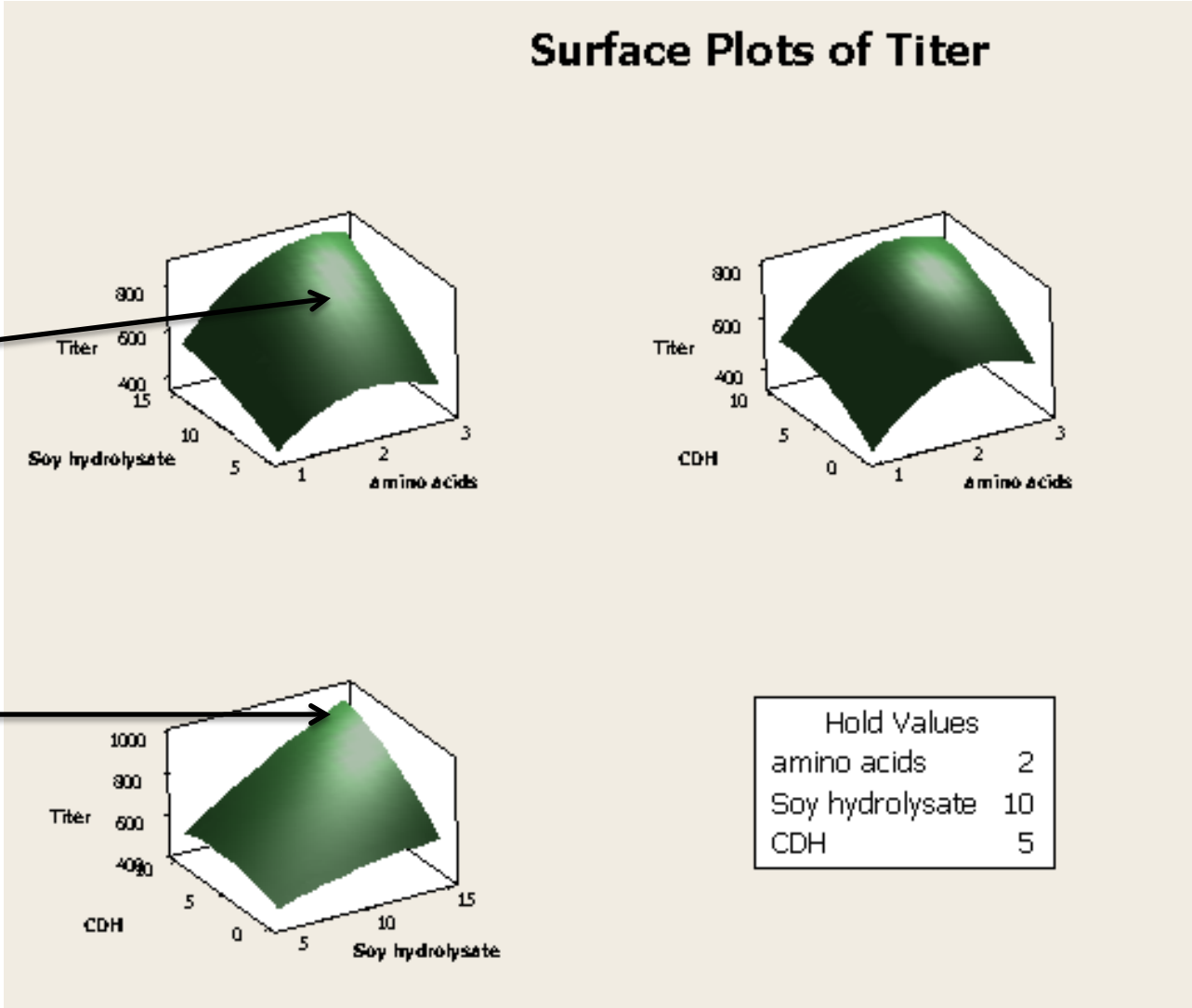
RSM analysis for titer optimization

- Amino acids are optimal at ~2.3x
- Soy and CDH increase titer, but only in the context of amino acids >1x
- Productivity limited to <1 g/L



First check-point trial: Composition

- Bioreactor verification run
  - 4 L, 15 day
  - Basal medium
    - Supplemented with CDH Fusion, basal AA concentrate, and soy hydrolysate
    - Fed glucose

- Results
  - qP = 14 pg/cell-day
  - IVC = 69E6 cell-day/mL
  - Titer = 1.05 g/L

Can We Increase Beyond 1 g/L Titer?

Additional supplements and feeds screened

- Commercially available and custom
  - Supplements
  - Medium concentrates
  - Productivity enhancers
- Design: fractional factorials with center points for screening
- Results:
  - Confirmed previous factors with positive effects
  - Identified new factors with significant positive effects
    - Commercial medium concentrate
    - Amino acid supplement

Second check-point trial: Composition

- Bioreactor verification run
  - 4 L, 14 day
  - Basal medium
    - Formulated without soy hydrolysate and supplemented with CDH Fusion, basal medium AA concentrate, and fed with a commercial medium concentrate, and a custom 2 amino acid mix
- Results
  - qP = 21 pg/cell-day
  - IVC = 65E6 cell-day/mL
  - Titer = 1.35 g/L

Summary of major factors
Composition of supplements and feeds

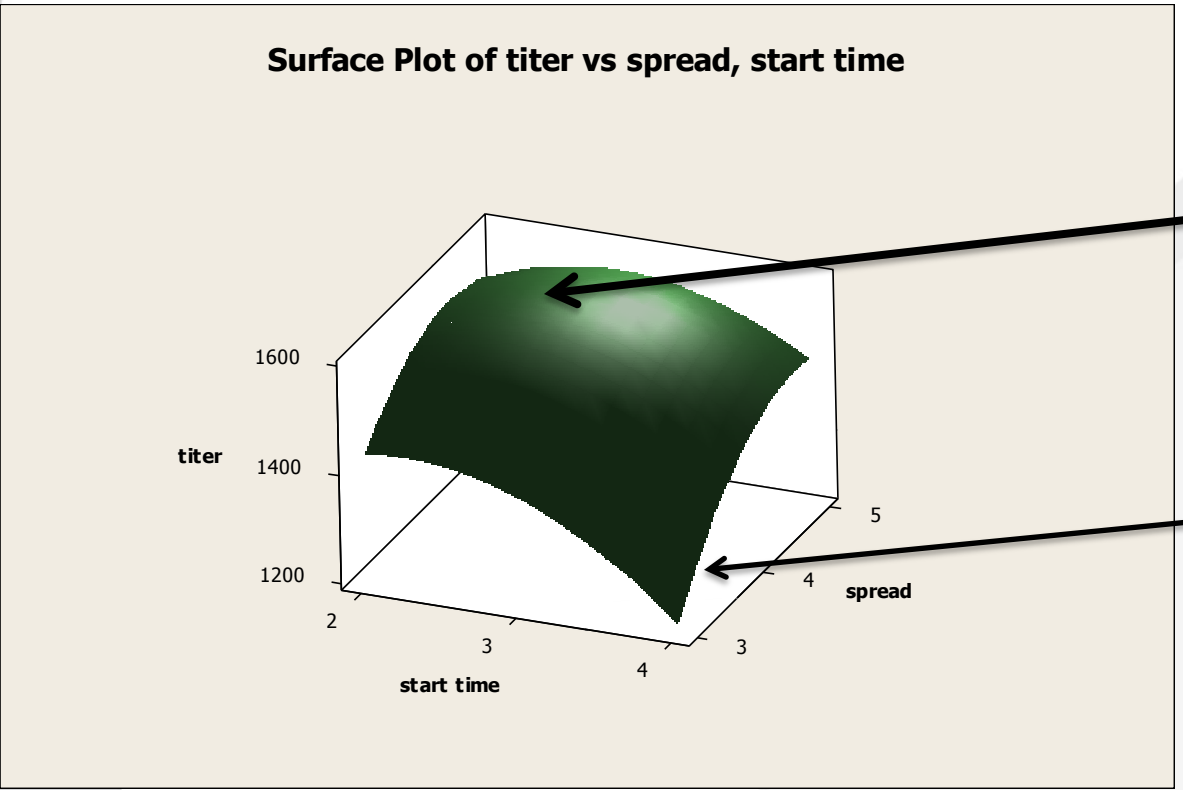
- Major factors
  - Commercial medium concentrate feed
  - 2-amino acid mix
  - Basal medium AA concentrate
  - CDH Fusion

Dosage and timing

- Experimental design
  - 20 mL TubeSpin Bioreactor format
  - Chemically Defined Medium & Feed
    - Basal medium
      - Custom without soy hydrolysate, supplemented with optimized feed mixture at 10% (v/v) initially
  - Dosage and timing of feed explored using RSM
    - Start date: Feed initiated on Day 2, 3, or 4
    - Duration: 20 mL of feed delivered over 2, 3, or 4 days

Dosage and timing of feed
RSM analysis: Titer

- Response Surface: Central Composite Design with duplicates and center point replication
- Model-predicted titer at optimum = 1581mg/L



Titer Optimum:
1581 mg/L
D2.6 over 4.5 days

Late, rapid feeding:
1200 mg/L

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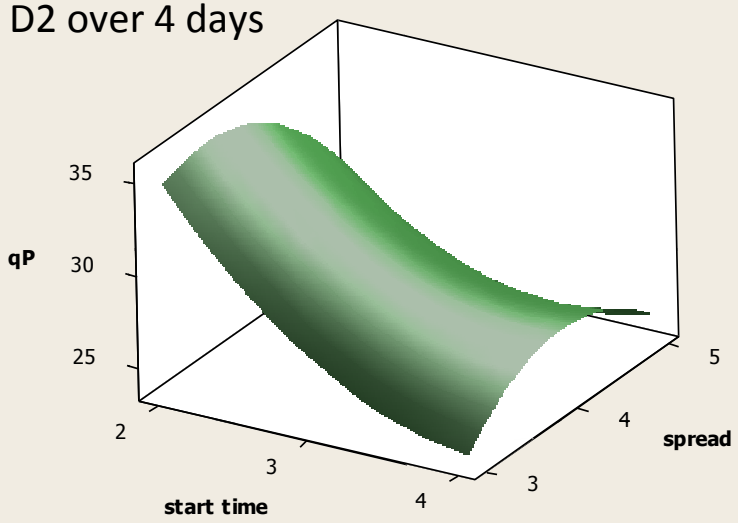
Dosage and timing of feed
RSM analysis: qP and IVC

Note: Titer = qP x IVC

Surface Plot of qP vs spread, start time

qP Optimum:

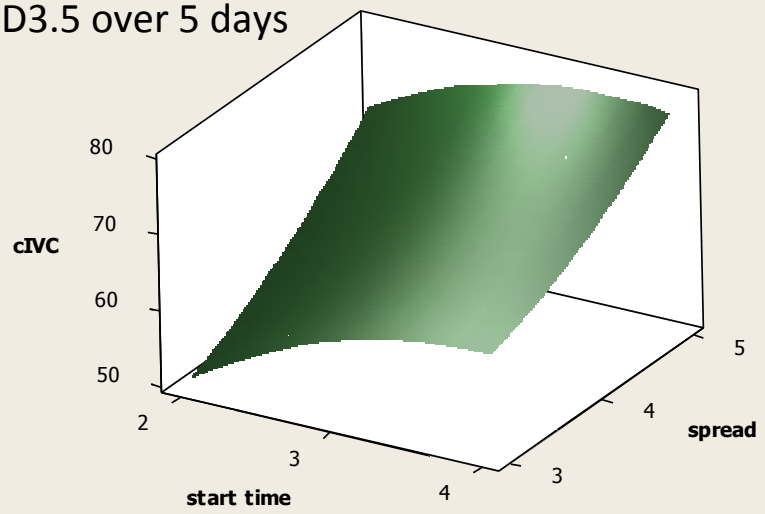
D2 over 4 days



Surface Plot of cIVC vs spread, start time

IVC Optimum:

D3.5 over 5 days



Third check-point trial planned: Dosage and timing

- Bioreactor verification run in progress
  - 4 L, 14 day
  - Basal medium
    - Formulated without soy hydrolysate, supplemented at 10% (v/v) with optimized feed (CDH Fusion, medium concentrate feed, 2 amino acid custom mix, basal medium AA concentrate)
  - Feed initiated on Day 3
  - Feed duration 4 days
- Anticipated results (extrapolated from RSM analysis)
  - qP = 28 pg/cell-day
  - IVC = 70E6 cell-day/mL
  - Titer = 1.5 g/L

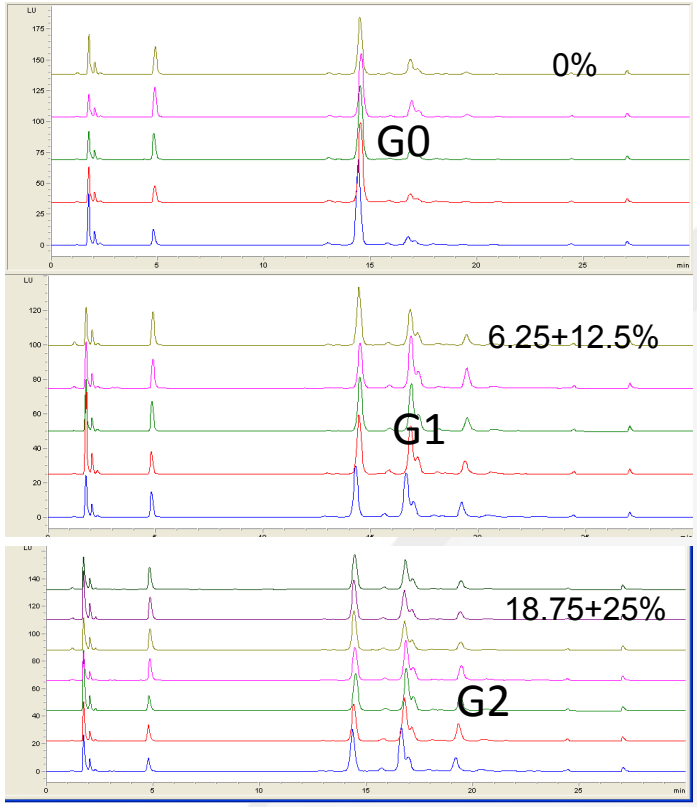
Design-of-Experiments Studies to Manipulate Glycan Distribution

Glycan control

- Initial process generated mAb with G1=G0>G2
- With increasing titer, glycan profile shifts G0>G1
- Several factors reported to impact glycosylation were tested using a fractional factorial
  - Gal, GlcNAc, mannose, uridine, Mn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{3+}, NH^{4+}
- Results showed glucose/galactose feed mixtures boosted the abundance of G1/G2 forms

Galactose supplementation
Effect on glycan distribution

- Glucose/galactose mixtures at 0, 6.25, 12.5, 18.75 and 25% galactose were used as feed. 0% galactose is plotted below as the control. 6.25+12.5% and 18.75+25% were separately combined and analyzed.



Glucose/galactose feed mixtures ↑ G1/G2 forms

mAb upstream development
Summary

- Used DOE to identify significant variables, developing an understanding of their effects and interactions
- Eliminated soy hydrolysate from basal medium
- More than doubled the titer to 1.35 g/L of mAb in a 4 L bioreactor in a 14-day process. Increased qP from 10 to 21 pg/cell-day. Nearly doubled IVC (from 37 to 65E6 cell-day/mL).
- Verification to achieve 1.5 g/L (3x goal) in progress
- Made significant progress toward offsetting the effect of a higher titer process on mAb glycans by incorporating a galactose feed
- Successfully implemented the TubeSpin culture system as a high throughput method for process development studies

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