Bioprocessing Challenges:
High-Titer Mammalian-Based Cell Systems

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Business Drivers

• Elements influencing the way Biologics may be manufactured /supplied in the future

- Cost of Raw materials
- Advent of Biosimilars
- Reimbursement pressures
- Squeeze in Profit Margins
- Drives Reduction in Costs
- Advent of Enhanced Cell line screening & Productivity
- Process /Facility Bottlenecks
- Drive to Debottleneck and Compress entire Process Train
- Expanding & Emerging Products Market
- Supply Reliability
- Drive Process, Facility and Supply Flexibility
Cost Impacts

Profit Margins for new and existing drugs will be squeezed
- Increasing Costs of Operations, Raw materials
- Competition between Innovator and Biosimilar products
- Drive for reduction in Healthcare costs
  - Reimbursement pressures
  - Consolidation of Health providers

Mandates for Bio manufacturing Operational Changes
- Reduce Cost per unit mass of product produced

Future state of Biologics processing

Current/Future state of Cell Culture:
- technology has evolved and high titer processes (>5 g/L) are norm

Current state of biologics process:
- Increased time in Production Reactor
- bottlenecks in processing at >5 g/L
- buffer volumes too large
- excessive column cycling
- column capacity exceeded
- filtration Areas & Processing time Increase

* Data from Thomas Ryll, IBC 2009
Impact of Production Reactor titer on Throughput

<table>
<thead>
<tr>
<th>Production Scale</th>
<th>15K Liters</th>
<th>2K Liters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production reactor Titer (g/L)</td>
<td>Amount Produced annually (Kg)</td>
<td>Amount Produced annually (Kg)</td>
</tr>
<tr>
<td>0.1</td>
<td>41.6</td>
<td>5.2</td>
</tr>
<tr>
<td>1</td>
<td>416</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>2080</td>
<td>260</td>
</tr>
<tr>
<td>10</td>
<td>4160</td>
<td>520</td>
</tr>
</tbody>
</table>

Product Demands
- Shift to smaller production reactors
- Process redesign to fit existing facility infrastructure

Assumptions:
- 2 Bioreactor production trains
- 1 Purification train
- 7 day thaw rate
- No operational, Facility, Equipment bottlenecks

Impact of Harvest Titer on Facility Throughput

- Actual Productivity
- Productivity w/ no Throughput Constraints
- Processing time Technology Bottlenecks

Single Product in three Large-scale Biomanufacturing Trains and Two Purification Suites
Presentation Overview

• Reshape Conventional Biologic Manufacturing processing steps to address
  1. Bottlenecks associated with increased Production time and Downstream operations constraints
  2. Process Space Compression to address increased titers
  3. Increase Throughput Capacity
  4. Cost Pressures

• Review Technologies / Capabilities that address the above drivers
  – Production Reactor throughput
  – Downstream Capacity and Process Compression

Fed-Batch Mammalian Process

• Typical mammalian cell culture process including innoculum train, fed-batch production reactor
• Cell clarification via centrifugation, microfiltration and/or depth filtration
• Initial Capture Chromatography (Bind-Elute) for majority of Purification
• Secondary Chromatography – Polishing step for product variant, aggregate removal
• Viral Filtration – Robust Virus removal
• Ultrafiltration/Diafiltration - Buffer exchange - Formulation
Typical Large Scale Fed-batch Cell Culture MFG Process

Cell Bank

Thaw

Wave Reactors

N-4 Batch
60-L BR
3 days

N-3 Batch
3-5 days

N-2 Batch
3-5 days

N-1 Batch
3000-4000L BR
3-5 days

Production #1
Fed-batch
15K-20K Liter BR
11-20 days

Nutrient Feed In

Production #2
(≥3 days after #1)
Fed-batch
15K-20K Liter BR
11-20 days

Nutrient Feed In

N-1 Cell Retention Debottlenecking

- Shift growth phase to N-1 stage
  - Very high-seed production cultures to shorten culture duration
  - More batches in the same amount of time
  - Increase production capacity by more efficiently utilizing the ratio between N-1/N stage bioreactors
  - No changes in production (N) media or volumetric capacity
**N-1 Bioreactor Modes of Operation**

- **Batch N-1**
  - Fresh Basal In
  - Cell Return
  - Waste Basal Out
  - Waste Accumulates
  - No New Nutrients Added

- **Perfusion N-1**
  - Cells Retained
  - Nutrients Added
  - Waste Removed

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**Big Impact on Manufacturing Capacity**

- **Assumptions**
  - Campaign length is 1 year, maximum number of batches per 365-day campaign slot, 2-day turnaround time per bioreactor
**Limitations of Current Purification Platform**

Capacity: process volumes limit throughput for titers > 4-5 g/L

- Resin binding capacity
  - Large columns x multiple cycles = large volumes
- Protein A most concerning
  - 30-40 g/L capacity
  - Polishing steps flow-through mode

~5 g/L at 20,000L Scale

1.6 m Protein A column (400L resin, 6 cycles, 45,000 L buffer)
- Protein A eluate volume ~ 7100 L
- Polishing Chrom eluate volume ~10,500L

**High Titer Processing: Strategies Capture**

- New Capture Resins that provide improved capacity and/or Productivity
- Alternative non ProA Capture resins eg IEX, HyperCel
- Factors that drive one technology over another:
  1. COGs
  2. Platformability,
  3. Scalability,
  4. Facility/Engr Retrofit,
  5. Validation-complexity,
- Alternative technologies (eg Precipitation, Expanded Bed)
**High Capacity Process Alternative Capture Step**

- Protein A improvements
  - New suppliers offering lower cost
  - New higher binding capacity resins
  - New modes of operation
- Protein A Replacement
  - B/E mode followed by one or two polishing F/T steps
  - \( \geq 270 \text{ g/L} \) dynamic binding capacity to reduce process volumes by \( \geq 2X \)
  - Better resin cleaning and lifetime
  - Eliminate high cost of protein A

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**High Capacity Resins: Protein A**

- For a high titer, shorter duration production bioreactor, the Protein A capture step with \( \sim 35-40 \text{ g/L} \) loading capacity has been identified as a potential throughput bottleneck
  - Many column cycles
  - Large buffer requirements
  - Large intermediate process volumes
- Process modeling has shown that increasing capture column binding capacity to \( 60 \text{ g/L} \) combined with buffer concentrates will alleviate potential bottleneck

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**Leverage higher capacity resins:**

MAbSelect SuRe LX vs MabSelect SuRe

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Ghose et al., Biotech Progress, 20(3), 2004
Maximizing Capacity on Protein A

MAbSuRe LX

New higher capacity version of resin

Dual flow rate operation

Stepping down flowrate during load optimizes for mass transport

DBC (10 % BT) of 60-70 g/L

Comparable performance with a ~50% increase in binding capacity

<table>
<thead>
<tr>
<th>Product</th>
<th>Resin</th>
<th>Load g/L</th>
<th>Yield %</th>
<th>HCP ppm</th>
<th>Pr A ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CONTROL</td>
<td>35</td>
<td>&gt; 95</td>
<td>500</td>
<td>2.1</td>
</tr>
<tr>
<td>A</td>
<td>SuRe LX</td>
<td>55</td>
<td>&gt; 95</td>
<td>488</td>
<td>3.2</td>
</tr>
<tr>
<td>B</td>
<td>CONTROL</td>
<td>35</td>
<td>&gt; 95</td>
<td>300</td>
<td>2.5</td>
</tr>
<tr>
<td>B</td>
<td>SuRe LX</td>
<td>55</td>
<td>&gt; 95</td>
<td>690</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Tangential Flow Filtration Solution

- Membrane modules in-parallel
- Concentration over time; requires several membrane passes
- Product held in recirculation vessel

- Lower cost, smaller footprint
- Uses conventional UF modules and could be fully disposable
- Higher recovery, lower shear
- Allows in-line operation
Downstream Process Intermediate Volumetric Constraints

- Chromatography Improvements and SPTFF enable HT Processing
  - Reduces downstream volumes in a platform process (FT columns)
  - Facilitate high titer MFG processes (> 5g/L) within existing MFG constraints

Effect of Using Buffer Concentrates

10g/L Harvest Conditions
- Case 1 – All chromatography buffers at 1x
- Case 2 – All chromatography buffers at 5x

<table>
<thead>
<tr>
<th>Case</th>
<th>Total # of Buffer Preps</th>
<th>Purification Cycle Time (days)</th>
<th>Upstream Cycle Time (1.train) (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50-60</td>
<td>(\tau)</td>
<td>(\tau \approx 1.5) days</td>
</tr>
</tbody>
</table>

Without concentrates, Purification becomes the bottleneck
Integrated High Capacity Upstream Downstream processing

Fed Batch Mammalian Cell Production Reactor
Centrifugation
High Capacity Depth Filtration
N-1 → N Optimization

Depth Filtration
High Capacity capture column
Buffer Concentrates

Protein-rA AEX
TFF: process intermediate volume reduction

Viral Filtration
High Capacity Viral Filtration
SPTFF: UF2 for HC DS
Ultrafiltration

High Capacity Resin
Buffer concentrates
N-1 Optimization

Technology changes on Facility Throughput

Annual Productivity (Metric Tons /Year)

• Actual Productivity
• Productivity w/ no Throughput Constraints

Single Product in three 15K Trains and Two Purification Suites
Economic Analysis

Scope:
Includes processes between the LSM Bioreactor and the Final Bottling Step

Production Cost Contributions

Assumptions:
- Model current Purification technology
- Same facility Fit
- Enhanced bioreactor turnaround
- All analysis assumed no labor or utility constraints

- Increased number of batches correlates with increased in Productivity
- Allow for Resource utilization optimization reduce COGs by 23%
Summary

• Facility bottleneck for 1-5g/L Fed batch processes at large scale is production bioreactor (with three trains). Shift in Bottlenecks occur at Downstream as one approached 10g/L

• Integration of advances in N-1 Perfusion, High capacity resins, Buffer concentrates, and intermediate volume reduction allows for throughput increase by 2x as compared to no change in technology
  – Allow the avoidance of expanding facility Footprint

• As annual output and scale increase with titer increase, the relative importance of different cost categories are expected to change
  – Overall cost of goods/gram product decreases by >70%

Acknowledgements

• N-1 Perfusion:
  – William Yang
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  – Jennifer Zhang (Protein A)

• SPTFF:
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  – Alex Brinkmann (SPTFF)

• Buffer Concentrates, Throughput
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