A High-Throughput Approach To Quantify Fab Yields In a Heterogeneous Mixture Of Monomer and Dimer

Phil Barish, PhD | VP, Operations
April 21, 2016
Next-Generation Antibody Platform

AbSci’s production technology, SoluPro™, embodies the best of E. coli and mammalian expression systems

- Streamlined Drug Discovery Platform
- All-In-One Protein and Antibody Expression
- Consistent High-Quality Protein Product
- Scalable for Manufacturing
Current *E. coli* production processes lead to protein aggregation into inclusion bodies

- Extensive processing is required to liberate soluble protein
Inducer concentration proportional to induction in _each_ cell
Able to optimize stoichiometric ratios for maximal expression

 Achives g/L expression for complex proteins in *E. coli*
Flow Cytometry Confirms Homogeneous Induction

**araBAD Expression of YFP**

**prpB Expression of RFP**

**T7 Expression of RFP**

<table>
<thead>
<tr>
<th>Arabinose</th>
<th>75 μM</th>
<th>1.5 μM</th>
<th>0.75 μM</th>
<th>0.375 μM</th>
<th>0.1 μM</th>
<th>0.05 μM</th>
<th>0 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionate</td>
<td>0 μM</td>
<td>0.025 μM</td>
<td>0.1 μM</td>
<td>0.225 μM</td>
<td>0.5 μM</td>
<td>0.75 μM</td>
<td>1.5 μM</td>
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<tr>
<td>IPTG</td>
<td>400 μM</td>
<td>40 μM</td>
<td>22 μM</td>
<td>4 μM</td>
<td>2.2 μM</td>
<td>0.9 μM</td>
<td>0.4 μM</td>
</tr>
</tbody>
</table>
The SoluPro Approach

Homogeneous Induction

Oxidized Cytoplasm

High Titers (g/L)
and
Short Fermentations (24-48hr)
Antibody fragment (Fab)

- 48 kDa heterodimer
- Antigen: TNFα

araBAD → Fab HC → prpB → Fab LC

Anti-human Fab-CH1 Sensor

Protein L Sensor

8x8 titration of inducers
Initial Approaches

Quantitation in Crude Lysates

- Detect heavy chain (Fab-CH1) or light chain (ProL)
**Initial Approaches**

**Quantitation in Crude Lysates**

- Detect heavy chain (Fab-CH1) or light chain (ProL)

**Standard Curve**

**Experimental**

**Problem: Binding of monomeric heavy or light chain to sensor**
Initial Approaches

Quantitation Using Antigen - TNFα

- Custom anti-TNFα biosensors
  - Biotinylated TNFα with Streptavidin (SA) sensors
    - Load offline, detect in quantitation mode
    - Difficulty regenerating sensor
  - 6x His tagged TNFα with Nickel charged (Ni-NTA) sensors
    - Load and detect online in kinetics mode
    - Regenerate by removing TNFα

- Problem: Non-specific binding
  - E. coli membrane protein binds to TNFα
  - Association of monomeric species with TNFα
High-Throughput Purification

Crude lysates

Purification using Fab-CH1 resin

Flow through

E. coli

Detection using Protein L Sensor

Elution

Fab

HC

LC

Protein

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1-Day Fab HT Screening

Cell Culture and Expression: 16 hours
• Culture in 24 deep-well plates in minimal media
• Induce at OD$_{600} = 0.7$, harvest at 14 hours
• Screen 24 different Fabs or conditions per plate

Cell Lysis and Purification: 3-4 hours
• Cell homogenization followed by purification
• Single-step 96-well plate purification
• Purify 4 deep-well plates simultaneously

Quantitation: 1 hour
• BLI using Protein L biosensors
• Octet reads 96 samples per plate
• Kinetics can be evaluated using purified product
Fab Inducer Titration

Inducer Optimization to Achieve High Yields of Soluble and Active Protein at Low Cell Densities

- 64 point, 8x8 titration of arabinose and propionate
**Fab Inducer Titration**

Inducer Optimization to Achieve High Yields of Soluble and Active Protein at Low Cell Densities

**Fab Yields (mg/L)**

<table>
<thead>
<tr>
<th>Propionate (mM) - Light Chain</th>
<th>Arabinose (μM) - Heavy Chain</th>
<th>0</th>
<th>0.666</th>
<th>2.1</th>
<th>21.06</th>
<th>41.12</th>
<th>66.61</th>
<th>421.2</th>
<th>2106</th>
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<tbody>
<tr>
<td>0</td>
<td></td>
<td>0.8</td>
<td>1.3</td>
<td>2.1</td>
<td>2.0</td>
<td>1.8</td>
<td>1.9</td>
<td>1.0</td>
<td>0.0</td>
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<td>2</td>
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<td>2.3</td>
<td>8.9</td>
<td>18.6</td>
<td>15.4</td>
<td>14.3</td>
<td>11.9</td>
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<td>20.1</td>
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<td>1.5</td>
<td>4.3</td>
<td>8.5</td>
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<td>34.8</td>
<td>21.5</td>
<td>7.8</td>
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<tr>
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<td></td>
<td>1.6</td>
<td>4.6</td>
<td>8.6</td>
<td>34.2</td>
<td>36.2</td>
<td>35.1</td>
<td>17.0</td>
<td>7.5</td>
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<tr>
<td>50</td>
<td></td>
<td>3.8</td>
<td>5.5</td>
<td>8.5</td>
<td>33.4</td>
<td>29.7</td>
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<td>15.0</td>
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<tr>
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<td>4.7</td>
<td>8.0</td>
<td>24.0</td>
<td>25.9</td>
<td>22.7</td>
<td>12.3</td>
<td>6.2</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>2.1</td>
<td>4.2</td>
<td>7.3</td>
<td>19.0</td>
<td>21.1</td>
<td>22.6</td>
<td>7.3</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Low [0, 0.666, 2.1]  | 21.06, 41.12, 66.61, 421.2, 2106]  | High

araBAD → Fab HC → prpB → Fab LC
Conclusions

Summary of Fab Expression

Optimized Deep-Well Expression of **53.9 mg/L**
- Approximately **12-14%** of total cellular protein

Fab Structure and Activity Confirmed
- Fab binding kinetics (K_D) confirmed using Octet
- Structure confirmed through disulfide bond mapping

Takeaway

Having the right analytical tools is key to leveraging advance protein expression platforms
Thank You

Phil Barish
pbarish@abscibio.com
(503) 208-7882
www.abscibio.com

Acknowledgements
Emily Robinson
Sean McClain
Logan Garrett
Jeff Mihailoff
Terence Hui