The Protein A Paradigm
Can it be improved?
Can it be replaced?

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What is the protein A paradigm?

Established safety record with numerous injectable products

Versatility: broad compatibility with a variety of downstream steps

Platform-ability: consistent performance with a wide range of antibodies

Sample preparation limited to clarification

(Relatively) simple method development

High purification factor in a single step, including clearance of DNA, endotoxin, and virus

Protects downstream steps from foulants
What is the protein A paradigm?

High procurement cost
Productivity bottleneck
  Fair capacity
  Long residence time
Elution conditions can cause or enhance product aggregation, insolubility, and/or instability
Immunotoxic leachate/removal
Fair base resistance
  Limited media life
  Unaggressive sanitization
Doesn’t bind most hIgG₃
Advances and alternatives

1. Method improvements
2. Media improvements
3. New application formats
4. Alternative ligands
Method improvements

Aggregate reduction through sample preparation
Flocculation with calcium phosphate

Anion exchange adsorption
Strategies to address aggregation during protein A chromatography, 2005, A. Shukla, P. Hinckley, P. Gupta, Y. Yigsaw, B. Hubbard, BioProcess International, 3(5) 36-44
Method improvements

Aggregate reduction, secondary wash buffers

Courtesy of Bio-Rad Laboratories
Method improvements

Aggregate reduction, secondary wash buffers
Common components:

**NaCl**: (0.1 - 1.0 M) to damp nonspecific electrostatic interactions

**Urea**: (1.0 - 2.0 M) to damp nonspecific hydrogen bonding and hydrophobic interactions

**Propylene glycol**: (5 - 20%) to damp nonspecific hydrophobic interactions

**EDTA**: 2 - 5 mM to dissociate metal complexants

*Secondary washes can also enhance removal of DNA, endotoxin, virus — and proteases!*

A. Grönberg, E. Monié, HJ. Johansson, 2006, Screening of intermediate wash buffers for protein A chromatography using a 96-well plate, 232nd Meeting of the American Chemical Society, San Francisco
Method improvements

Aggregate reduction, elution conditions
Moderation of elution pH
Temperature reduction
Conductivity at least physiological
Urea
Arginine

0.1 - 0.2 M Arginine, pH 3.8, in addition to reducing aggregation, prevents the loss of solubility encountered at ~pH 6.5 with many antibodies.

Media improvements

Media improvements

Productivity and the Capacity Paradox

Higher capacity translates to reduced column volume. Conservation of residence time requires conservation of column length and linear flow rate. Reduction of column volume must therefore be achieved by reducing column diameter. Reducing column diameter reduces volumetric flow rate, constricting the productivity bottleneck that already exists at the capture step.
**Media improvements**

**Higher capacity, equal residence time**

Sample: 1000 L supernatant with 1 g Mab/L (1 kg Mab)

Capacity/CV: Resin 1: 35 mg MAb/mL (CV=28.5 L)
Capacity/CV: Resin 2: 48 mg MAb/mL (CV=20.8 L)  -27%

Bed height (both): 30 cm

**Bed diameter: R1=35 cm, R2=30 cm**  -14%

Linear flow rate (both): 200 cm/hr

Residence time (both): 9 minutes

**Vol. flow rate: R1=190 L/hr   R2=138 L/hr**  -27% (unfavorable)

Buffer volume 10CV each, EQ, wash, El: R1=850 L  R2=620 L  -27%

Total proc. vol. (sample + buffers): R1=1850 L  R2=1620 L  -12%

Process time: R1=9.7 hr, R2 =11.8 hr  +22% (unfavorable)

Productivity: g Mab/L media/hr: R1=3.62, R2=4.10  +14%
Media improvements

Improvements in Mass Transport

Diffusive Particles  Perfusive Particles  Monoliths

Diffusion  Convection

Blue: support matrix. Yellow: areas of diffusive flow. White: areas of convective flow

Media improvements

The influence of mass transport on residence time

Residence time, seconds

% of DBC
at 90 sec RT

Monolith
MCA
MSX

12 x 9 mm
5 x 50 mm
5 x 50 mm

hIgG, MAb
at 1 mg/mL
**Media improvements**

**Equal capacity at different flow rates**
- Sample: 1000 L supernatant with 1 g Mab/L (1 kg Mab)
- Capacity (both): 35mg Mab/mL (at flow rates indicated below)
- Bed volume (both): 28.5 L
- Bed height (both): 30 cm
- Bed diameter (both): 35 cm

**Linear flow rate:** R1=200 cm/hr, R2=500 cm/hr  \( +250\% \)

**Residence time:** R1=9 min, R2=3.6 min  \( -60\% \)

**Vol. flow rate:** R1=190 L/hr, R2=475 L/hr  \( +250\% \)

Buffer volume (both) 10CV each, EQ, wash, El: 850 L

Total proc. vol. (sample + buffers, both): 1850 L

**Process time:** R1=9.7 hr, R2=3.9 hr  \( -60\% \) (favorable)

**Productivity:** g Mab/L media/hr: R1=3.62, R2=9.00  \( +249\% \)

*Productivity increases linearly with the inverse of residence time.*
Media improvements

The Capacity Paradox Resolved

DBC 5% BT

mg/mL

MCA MSX
5 x 50 mm
hlgG1 MAb at 1 mg/mL

linear flow rate, cm/h

200 400 600
New application formats

Capture from unclarified harvest
Sartobind Protein A Direct™
Protein A immobilized on a spiral wound “nonporous” membrane
Recirculating format
No clarification/microfiltration

protein A membrane
spacer
New application formats

Cartridge Filter Chromatography System™ (CFCS, 3M)

pleated cartridge filter  10 µm capture beads

Graphics courtesy of 3M Bioprocessing Systems
New application formats

Cartridge Filter Chromatography System
Large surface area of small particles enhances binding
  external surface area 100 L of 60 µm particles: 6,400 m²
  external surface area 100 L of 10 µm particles: 38,400 m²

  Provides direct access to a higher proportion of the
  diffusive pore volume within each particle.

  Increases the film transfer coefficient

  Provides a larger surface for convective mass transfer

Calculations courtesy of 3M Bioprocessing Systems
New application formats

**Cartridge Filter Chromatography System**

Particle distribution over large capture filter surface area permits high volumetric flux at low linear flow rates.

- 139 m² per 100 L particles (bed height <0.7 mm)
- Same frontal area as a column with 13.3 m diameter

3 cm/hr × 139 m² = 4,170 L/hr

Residence time at 3 cm/hr ~1.4 min

Shallow bed keeps backpressure less than 2 Bar

Data and some calculations courtesy of 3M Bioprocessing Systems
New application formats

Cartridge Filter Chromatography System

10,000 L clarified harvest at 3 g Mab/L
150 L of CFCS protein A beads

Productivity: CFCS = 141 g/L/h, Mab elutes @ 5g/L
Productivity: CFCS + TFF = 59 g/L/h, Mab @ 20g/L

Total process time = 17.9 h
Productivity = 11 g/L/h

150 L Column
200 cm/hr

Graphics and calculations courtesy of 3M Bioprocessing Systems
New application formats

Simulated Moving Bed Chromatography

Diagram of BioSMB™ valve based column switching system, courtesy of Tarpon Biosystems, Inc.
New application formats

BioSMB, Cost and Risk Reduction

Material Costs
- Reduction in media usage
- Reduction in buffer usage
- Reduction in cleaning water usage

Capital Costs
- Elimination of large scale columns
- Reduction in system footprint for given throughput
- Potential integrated buffer blending eliminates tanks and transfer systems
- Reduced WFI usage & peak demand

Operational Costs & Risks
- Elimination of column packing, testing, unpacking, cleaning & storage
- Elimination of column & system cleaning validation
- Simplification of process changeover

Courtesy of Tarpon Biosystems, Inc.
New application formats

**BioSMB**, economics with disposable cartridge columns

<table>
<thead>
<tr>
<th>System Type</th>
<th>Conventional</th>
<th>BioSMB</th>
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<tbody>
<tr>
<td><strong>Sorbent</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost $/L CV</td>
<td>$10,000</td>
<td>$10,000</td>
</tr>
<tr>
<td>Required residence time sec</td>
<td>300</td>
<td>120</td>
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<tr>
<td>Maximum pressure bar</td>
<td>2.0</td>
<td>7.0</td>
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<tr>
<td>Operational loading capacity g/L CV</td>
<td>30</td>
<td>45</td>
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<tr>
<td><strong>Geometry</strong></td>
<td></td>
<td></td>
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<tr>
<td>Bed diameter cm</td>
<td>120</td>
<td>20</td>
</tr>
<tr>
<td>Bed length cm</td>
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<td>15</td>
</tr>
<tr>
<td>Bed volume L CV</td>
<td>339</td>
<td>4.7</td>
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<tr>
<td>Total columns #</td>
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<td>16</td>
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<tr>
<td>Total bed volume L CV</td>
<td>339</td>
<td>75</td>
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<tr>
<td><strong>Process</strong></td>
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<td></td>
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<tr>
<td>Cycles per batch #</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Total cycle time min</td>
<td>119</td>
<td>32</td>
</tr>
<tr>
<td>Total batch time hr</td>
<td>19.8</td>
<td>16.0</td>
</tr>
<tr>
<td>Buffer volume L/batch</td>
<td>54,287</td>
<td>36,191</td>
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<tr>
<td><strong>Costs</strong></td>
<td></td>
<td></td>
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<tr>
<td>Sorbent purchase cost $</td>
<td>$3,392,920</td>
<td>$753,982</td>
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<tr>
<td>Single batch media cost $/g product</td>
<td>$33.93</td>
<td>$7.54</td>
</tr>
</tbody>
</table>

20,000 L bioreactor
5 g/L expression level
100 kg product/batch
Protein A affinity

Courtesy of Tarpon Biosystems, Inc.
Alternative ligands

**SuRe** (GE)
MabSelect SuRe™

- Exclusive Fc specificity
- Reduced ligand leakage
- Improved base resistance
- Improved protease resistance

functionally and immunologically distinct

Protein A

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Alternative ligands

CaptureSelect™
Camelid VHH fragments specificities for:
- hIgG$_1$
- hIgG$_2$
- hIgG$_3$
- hIgG$_4$
- hFab Kappa
- hFab Lambda
- IgA, IgM, IgE
- Chimeras
- Multi species IgG

Graphic courtesy of BAC
### CaptureSelect, IVIG Recovery

<table>
<thead>
<tr>
<th></th>
<th>Cryo Rich Plasma (1)</th>
<th>Eluate Pool (1)</th>
<th>Cryo Rich Plasma (2)</th>
<th>Eluate Pool (2)</th>
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<tbody>
<tr>
<td>IgG 1</td>
<td>41.1%</td>
<td>42.7%</td>
<td>37.4%</td>
<td>42.3%</td>
</tr>
<tr>
<td>IgG 2</td>
<td>49.8%</td>
<td>52.2%</td>
<td>56.4%</td>
<td>50.9%</td>
</tr>
<tr>
<td>IgG 3</td>
<td>3.1%</td>
<td>2.1%</td>
<td>2.1%</td>
<td>2.1%</td>
</tr>
<tr>
<td>IgG 4</td>
<td>5.9%</td>
<td>3.1%</td>
<td>4.2%</td>
<td>4.8%</td>
</tr>
</tbody>
</table>

Data courtesy of BAC and Baxter
Alternative ligands

CaptureSelect, Base stability

Data courtesy of BAC
Alternative ligands

Mixed Mode Capture

Attempts to design an effective small molecule ligand for IgG began with T-gel (thiophilic adsorption) ~1985
Followed by Abx™ ~1989, and Avid-AL™ ~1991
Subsequent attempts by ProMetic Biosciences to design protein A mimetics (MAbsorbent® A1P, A2P) ~1995
More recent efforts with charged heterocyclics
  Pall: MEP Hypercel™ - and others
  bioceceptor: Synduced-Fit
  UpFront: Yet-to-be-named dark horse
And with CHT™ ceramic hydroxyapatite
Alternative ligands

**Mixed Mode Capture**

Presentations at major conferences, past 12 months:

Hydroxyapatite as a capture method for purification of monoclonal antibodies, 2006, P. Gagnon, S. Zaidi, and S. Summers, IBC World Conference and Exposition, San Francisco (CHT to AIC to HIC)

EBA cascade capture for industrial scale protein isolation, R. Noel, A. Lihme, MB. Hansen, I. Vaast, 2006, IBC World Conference and Exposition, San Francisco (MM to AIC)

Optimizing downstream purification platform to produce monoclonal antibodies for preclinical and early clinical studies, 2006, J. Chen, The Waterside Conference, Chicago (MEP to CHT)
Alternative ligands

**Mixed Mode Capture, EBA FastLine Pro, 800 L**

- Near neutral pH elution
- 150 cm diameter x 45 cm settled bed height
- Operating FR: **900 cm/hr**
- **200,000 L** whey/day (7-8 cycles)
- Clean: **50°C, 0.5M NaOH** + detergent
- Working capacity: 10-20 g Ig/L, >90% recovery
- 13 kg Ig/cycle

Photo and data courtesy of UpFront Chromatography
Alternative ligands

Mixed Mode Capture: potential benefits

*Base resistance*

60 minutes 1M NaOH at 60°C (hundreds of cycles)*

- Longer column life
- More effective sanitization

Lower price

Elimination of leaching/removal

Expanded bed format bypasses clarification

*UpFront EBA media. CHT: more than 15,000 hrs in 1M NaOH at 23°C.
Other mixed mode products may have less base resistance.

EBA media data courtesy of UpFront Chromatography. CHT data courtesy of Bio-Rad Laboratories.
Alternative ligands

Mixed Mode Capture: challenges
Capacity
Selectivity
  especially re: viral clearance
Complexity of method development
Platform-ability
Conclusions

The dogmas of the quiet past are inadequate to the stormy present.

–Abraham Lincoln
Opportunity dances with those on the dance floor.

—Anonymous
Thanks to Avid BioServices for providing MAb supernatants to perform experimental work. Thanks to Applied Biosystems for providing beta samples of POROS® MabCapture A™, GE Healthcare for providing MabSelect Xtra™, and BIA Separations for providing analytical protein A monoliths. Thanks to Sartorius for providing information on Sartobind Protein A Direct; to UpFront Chromatography for providing information on their mixed mode and EBA systems; to BAC for providing data on CaptureSelect ligands; to Tarpon Biosystems for providing information on BioSMB; to 3M Bioprocessing Systems for providing information on their Cartridge Filter Chromatography System, and to Bio-Rad Laboratories for PAGE gels illustrating the importance of secondary wash buffers. Additional thanks to all of the above suppliers for invaluable editorial suggestions during development of this presentation. The diagram of SuRe is an artist’s conception without official endorsement by GE Healthcare.