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 $hIgG_3$

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

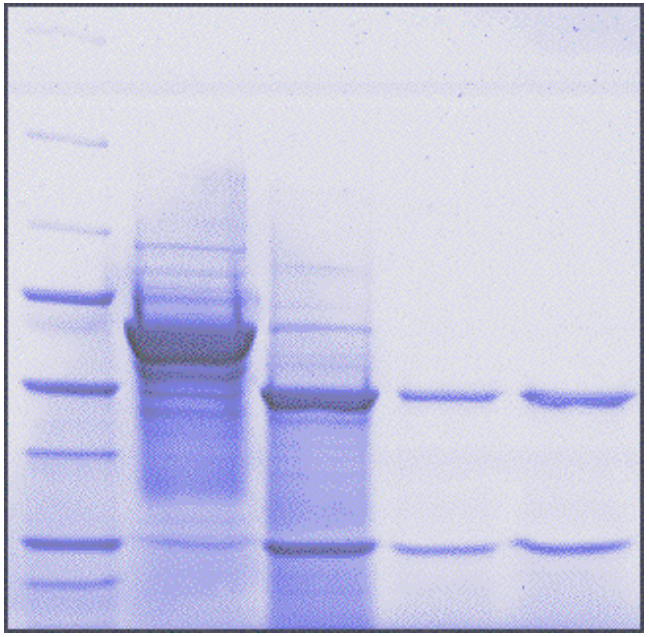
Analysis of protein A chromatography peak precipitates and approaches to reduce peak turbidity, 2006, S. Tobler, A. Noyes, J. Rajewski, R. Shpritzer, W. Piacenza, M. Tannatt, J. Coffman, S. Vunnum, B. Kelly, 232nd Meeting of the American Chemical Society, San Francisco

Anion exchange adsorption

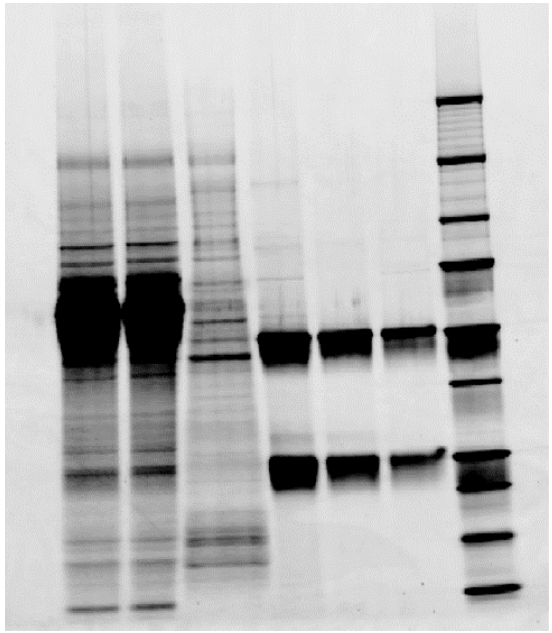
Strategies to address aggregation during protein A chromatography, 2005, A. Shukla, P. Hinckley, P. Gupta, Y. Yigsaw, B. Hubbard, *BioProcess International*, **3**(5) 36-44

Method improvements

Aggregate reduction, secondary wash buffers



protein A eluate
without 2° wash



2° wash eluate

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

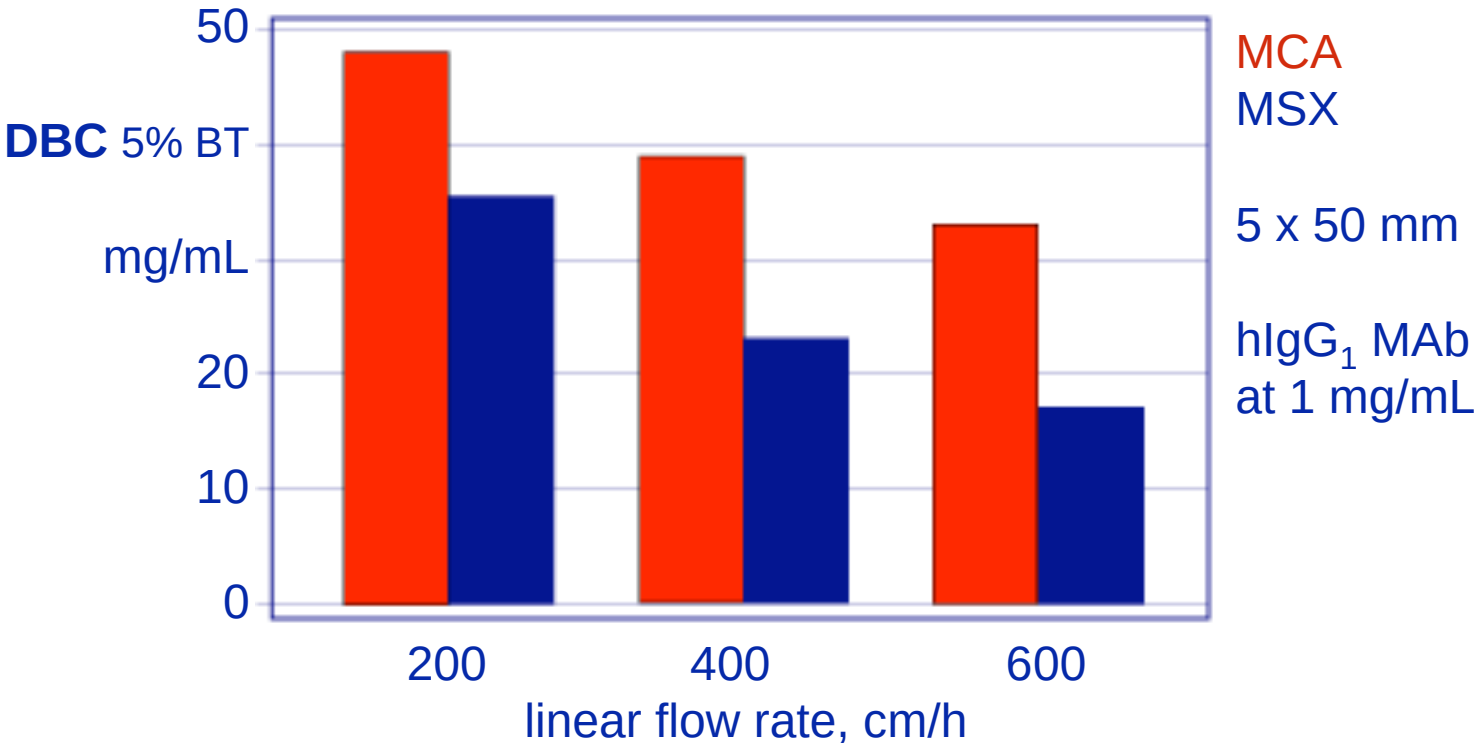
Arginine

- 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.

Arakawa, T., Philo, J.S., Tsumoto, K., Yumioka, R. and Ejima, D. (2004) Elution of antibodies from a Protein-A column by aqueous arginine solutions. Pro. Purif. Exp. 36, 244-248.
Ejima, D., Yumioka, R., Tsumoto, K., and Arakawa, T. (2005) Effective elution of antibodies by arginine and arginine derivatives in affinity chromatography. Anal. Biochem., 345, 250-257.
Arakawa, T., Kita, Y., Tsumoto, K., Ejima, D., and Fukada, H. (2006) Aggregation suppression of proteins by arginine during thermal unfolding. Protein Pept. Lett., 13, 921-927.

Media improvements

Capacity



Productivity improvements in the capture and initial purification of monoclonal antibodies, P. Gagnon and R. Richieri, 2006, 2nd Wilbio Conference on Purification of Biological Products, Thousand Oaks

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: 35mg 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%

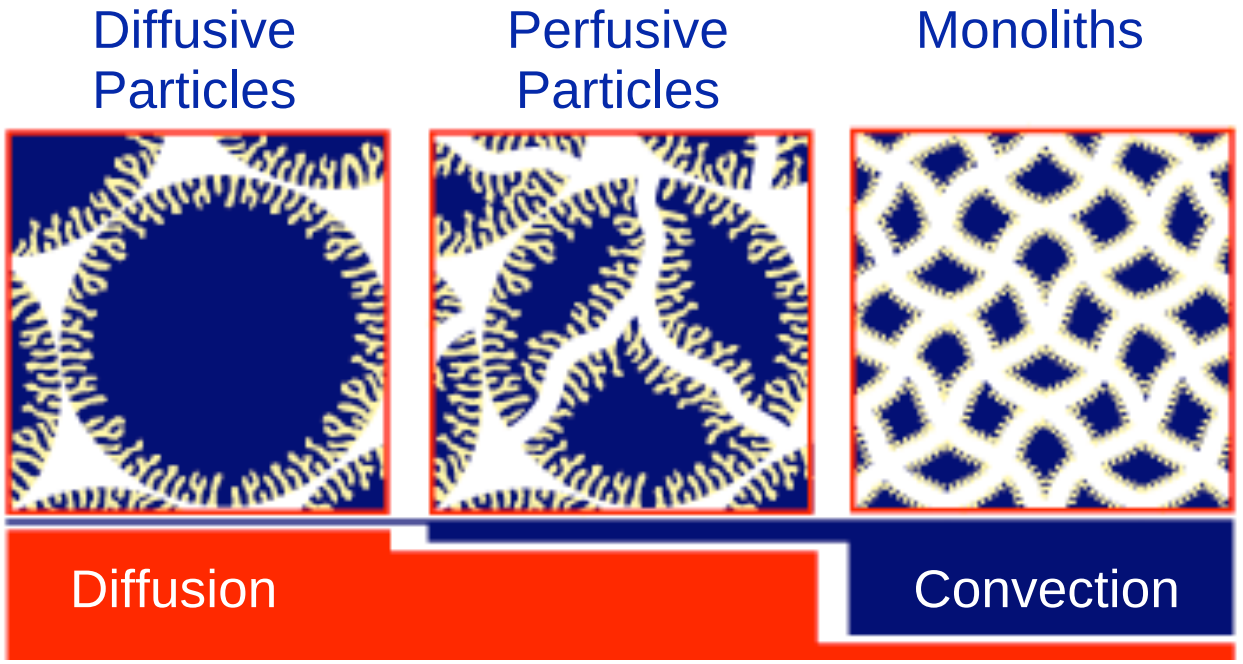
Total proc. vol. (sample + buffers): R1=1850 L R2=1620 L -12%

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

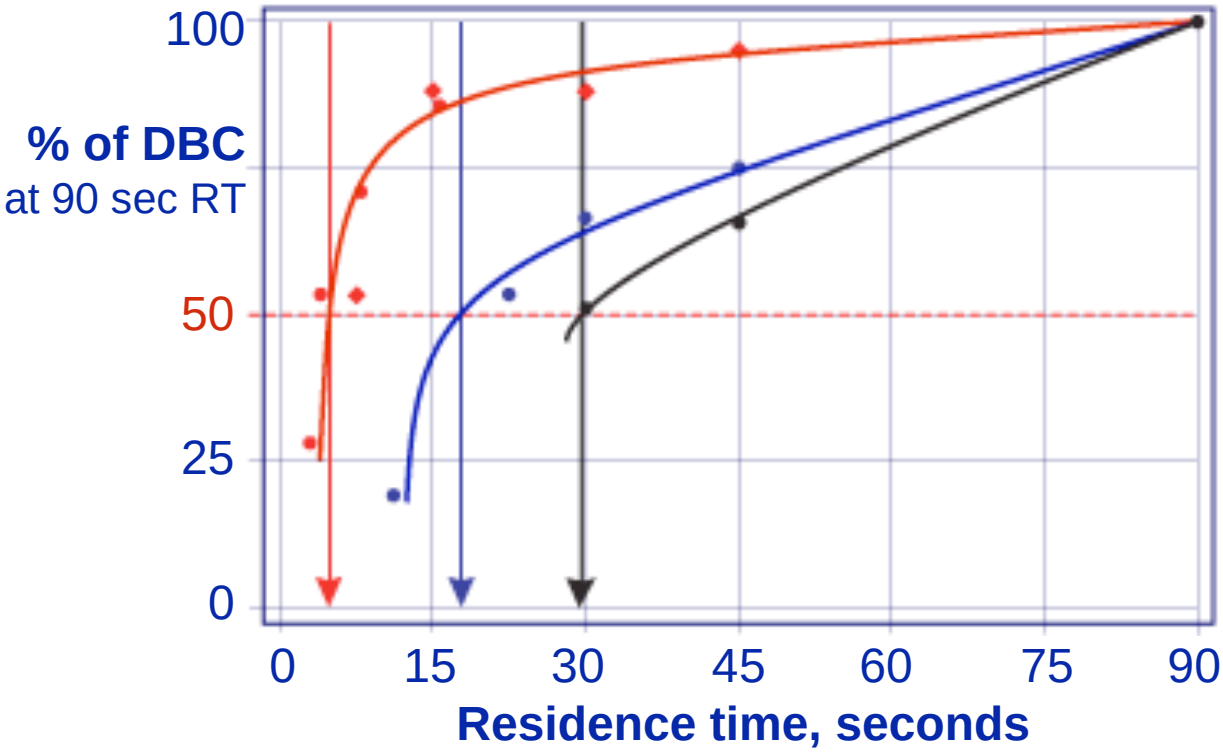


Blue: support matrix. Yellow: areas of diffusive flow. White: areas of convective flow

Productivity improvements in the capture and initial purification of monoclonal antibodies, P. Gagnon and R. Richieri, 2006, 2nd Wilbio Conference on Purification of Biological Products, Thousand Oaks

Media improvements

The influence of mass transport on residence time



Monolith
MCA
MSX

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

$hIgG_1$ 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

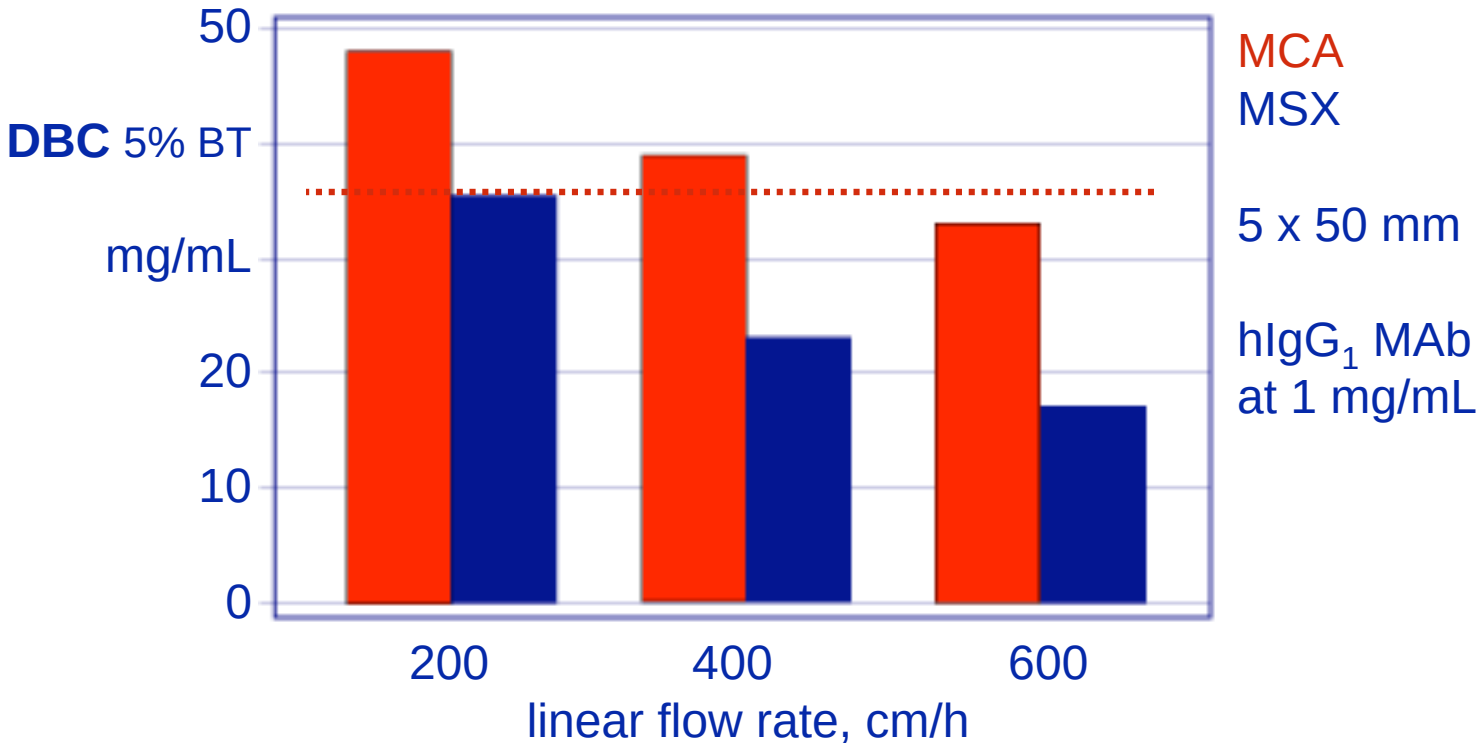
Process time: R1=9.7 hr, R2 =3.9hr -60% *(favorable)*

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



New application formats

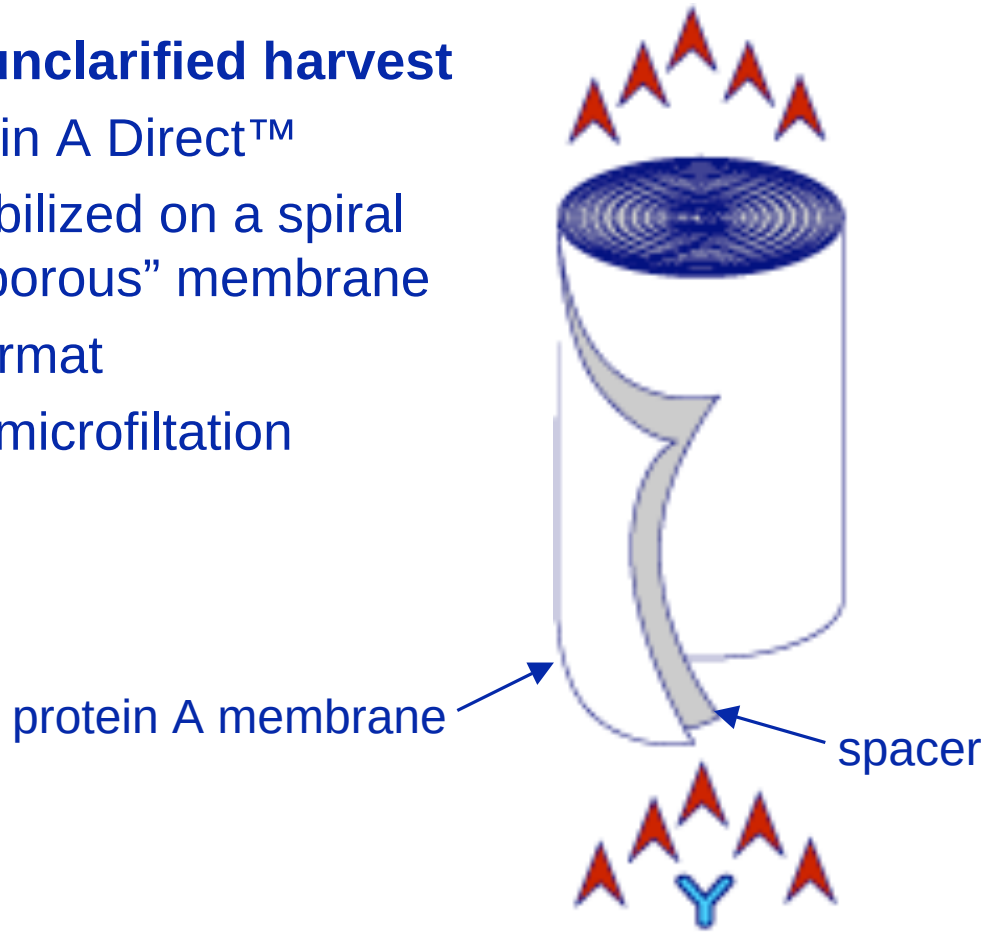
Capture from unclarified harvest

Sartobind Protein A Direct™

Protein A immobilized on a spiral wound “nonporous” membrane

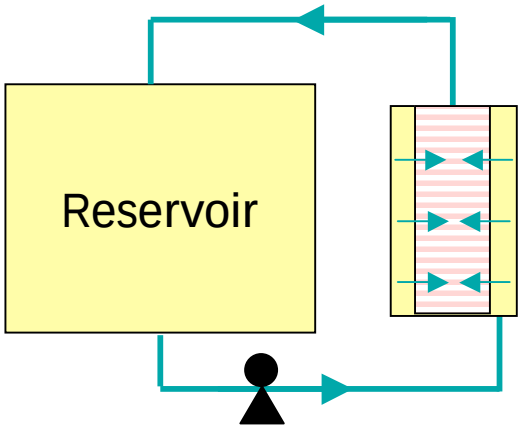
Recirculating format

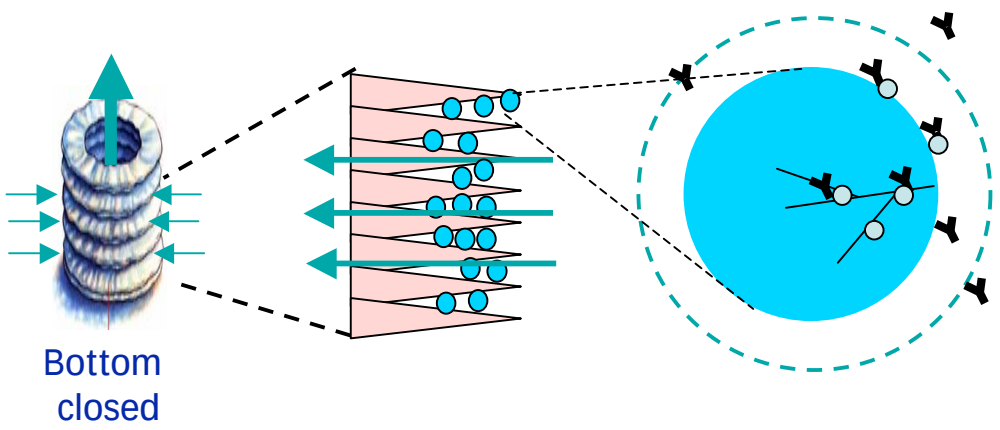
No clarification/microfiltation



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 m^2

external surface area 100 L of 10 µm particles: 38,400 m^2

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 m^2 per 100 L particles (bed height <0.7 mm)

Same frontal area as a column with 13.3 m diameter

3 cm/hr x 139 m^2 = 4,170 L/hr

Residence time at 3 cm/hr ~1.4 min

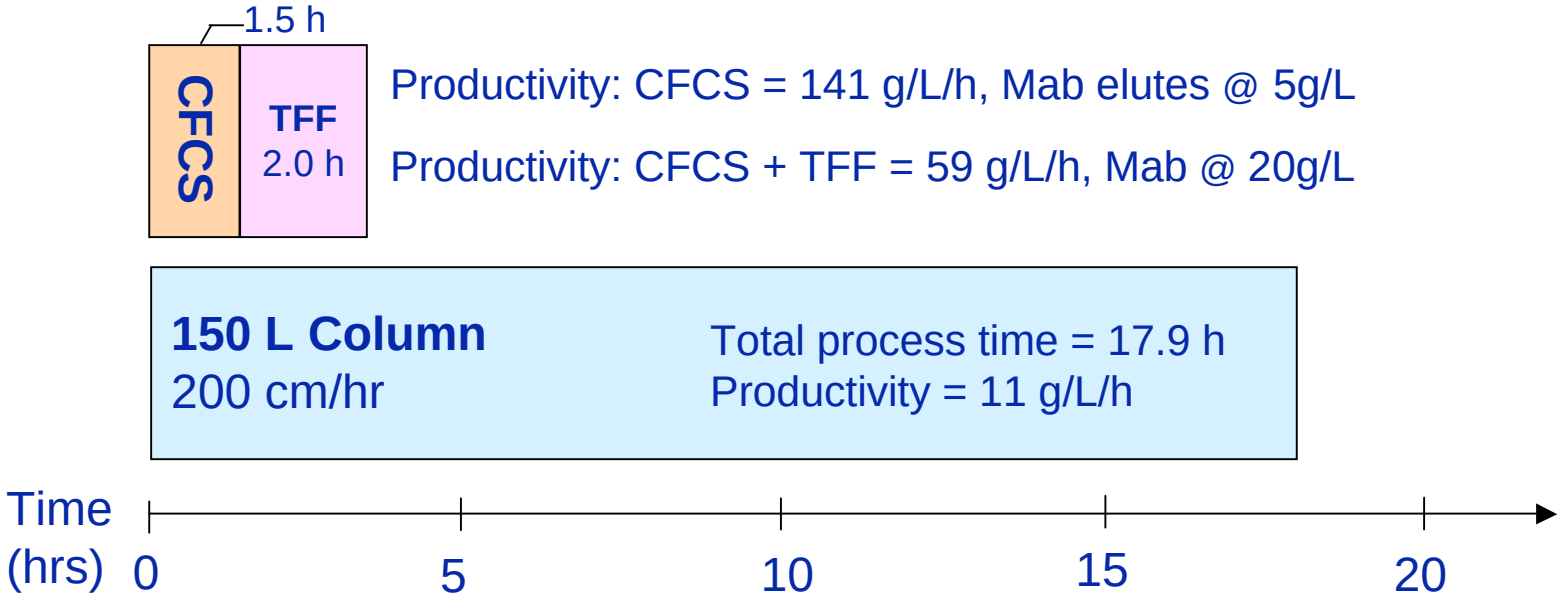
Shallow bed keeps backpressure less than 2 Bar

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



Graphics and calculations courtesy of 3M Bioprocessing Systems

New application formats

Simulated Moving Bed Chromatography

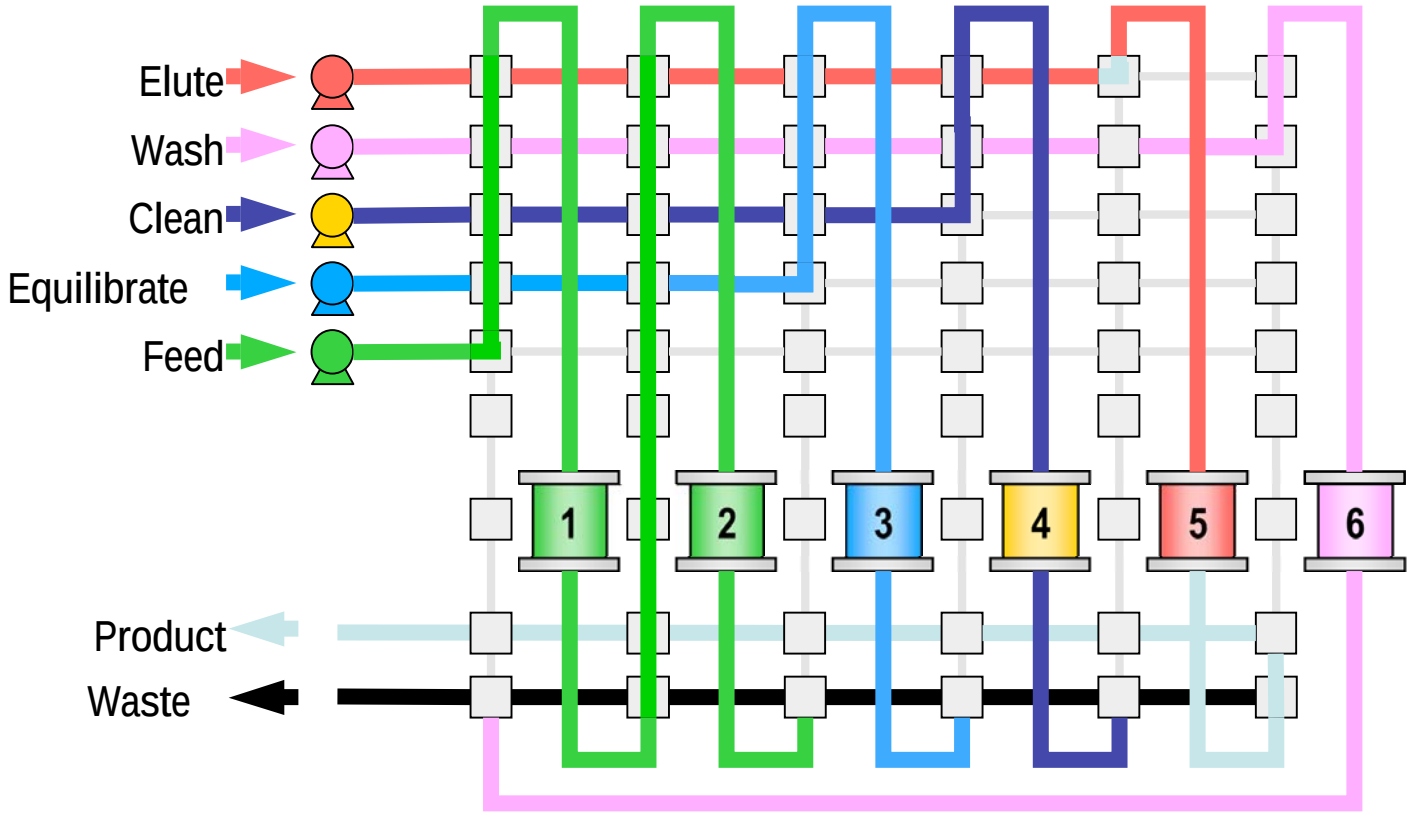


Diagram of BioSMB™ valve based column switching system, courtesy of Tarpon Biosystems, Inc.

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

System Type		Conventional	BioSMB
Sorbent			
Cost	*$/L CV*	$10,000	$10,000
Required residence time	*sec*	300	120
Maximum pressure	*bar*	2.0	7.0
Operational loading capacity	*g/L CV*	30	45
Geometry			
Bed diameter	*cm*	120	20
Bed length	*cm*	30	15
Bed volume	*L CV*	339	4.7
Total columns	#	1	16
Total bed volume	*L CV*	339	75
Process			
Cycles per batch	#	10	30
Total cycle time	*min*	119	32
Total batch time	*hr*	19.8	16.0
Buffer volume	*L/batch*	54,287	36,191
Costs			
Sorbent purchase cost	*$*	$3,392,920	$753,982
Single batch media cost	*$/g product*	$33.93	$7.54

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

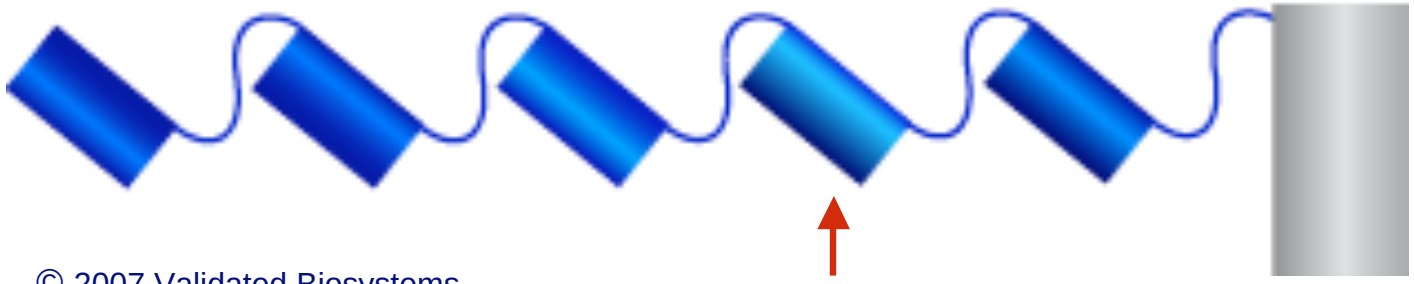
Reduced ligand leakage

Improved base resistance

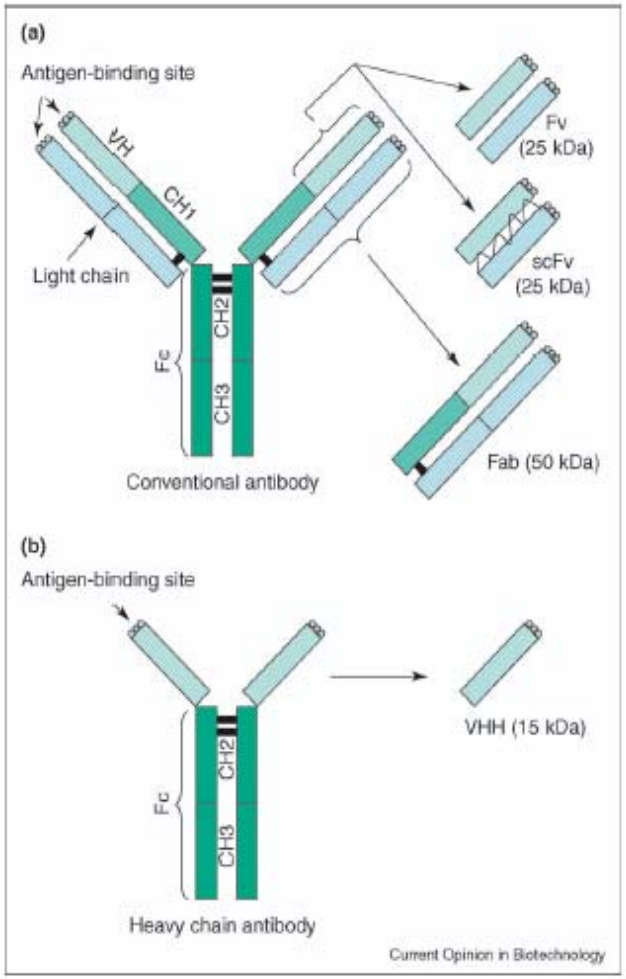
Improved protease resistance

functionally and
immunologically distinct

Protein A



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

Alternative ligands

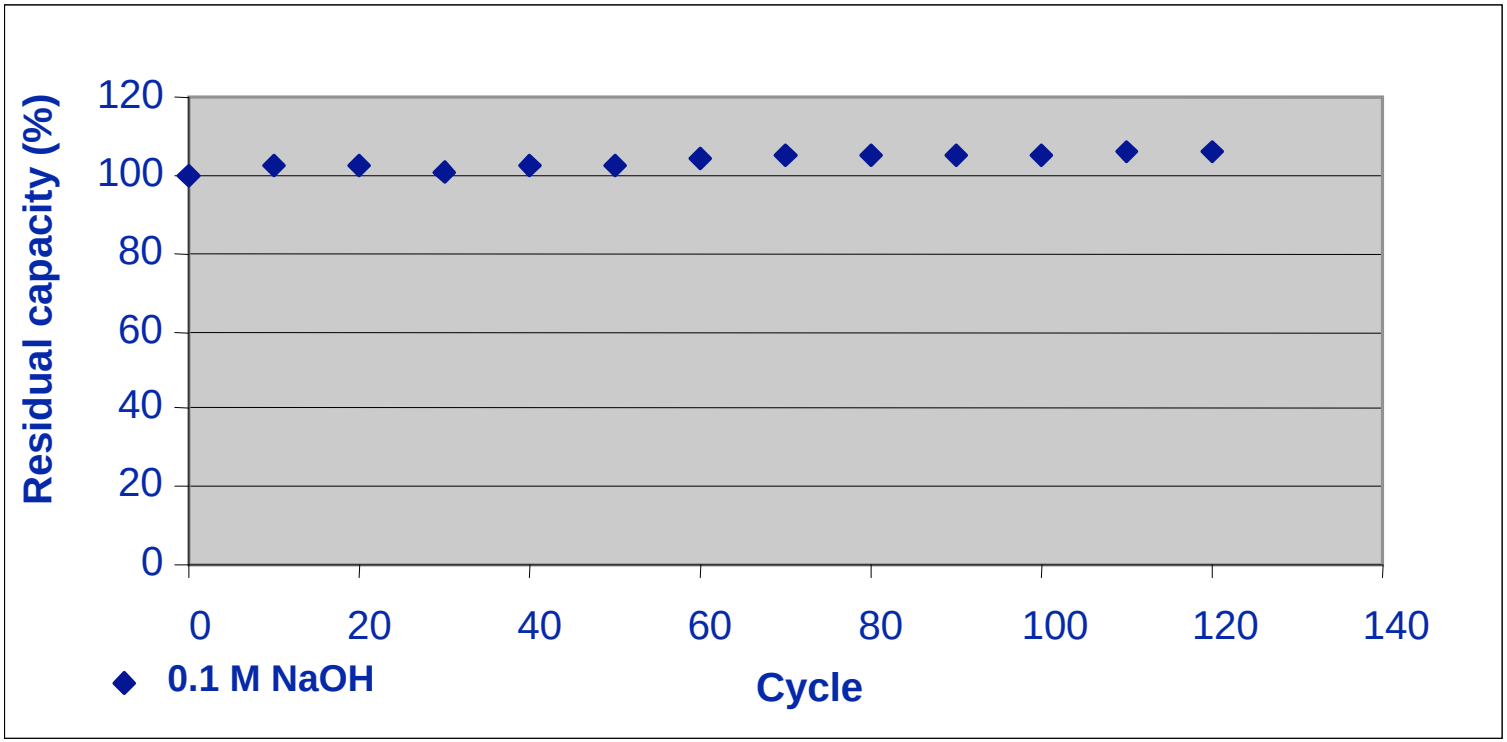
CaptureSelect, IVIG Recovery

	Cryo Rich Plasma (1)	Eluate Pool (1)	Cryo Rich Plasma (2)	Eluate Pool (2)
IgG 1	41.1%	42.7%	37.4%	42.3%
IgG 2	49.8%	52.2%	56.4%	50.9%
IgG 3	3.1%	2.1%	2.1%	2.1%
IgG 4	5.9%	3.1%	4.2%	4.8%

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
- bioceptor: 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

Conclusions

Opportunity dances with those on the dance floor.

–Anonymous

Acknowledgements

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