

# BIOPROCESSING

## ANTIBODY PURIFICATION

# Alternatives to Protein A capture

Dev Baines, Sharon Williams, Steve Burton, ProMetic BioSciences, Cambridge, UK

➤ Monoclonal antibody biopharmaceuticals continue to represent a growth area for new treatments of a number of problematic diseases, particularly cancer. Of the 26 antibody-based products approved for use thus far, two are murine, five chimeric, thirteen humanised and two fully human monoclonal antibodies (mAbs).

Full length therapeutic monoclonal antibody molecules are expressed and produced in mammalian cells such as Chinese Ham-

ster Ovary (CHO) or mouse myeloma (NSO) cells, which provide the required post-translational modifications (glycosylation) of the

Fc region necessary for the effector function. The development of antibody engineering has led to new types of antibody therapeutics, including antigen-binding antibody fragments, multivalent high binding avidity antibodies and attachment by fusion technology or chemical conjugation to variety payloads such as cytotoxic agents. For antigen neutralisation or receptor blockade, the Fc-mediated effector is not required and it can be removed by partial digestion of the intact full length antibody with proteolytic enzymes, or excised at the DNA level, to yield Fab fragments. Even smaller molecules such as scFv can be expressed in microbial systems, usually *Escherichia coli*.

In the case of full length antibodies, Protein A-based adsorbents are by far the most widely used materials for capture and purification. Protein A is a natural cell surface protein anchored to the cell wall and membrane of *Staphylococcus aureus*. It binds to antibodies at the Fc region by an interaction which is predominantly hydrophobic with

New Books

## Project Management for the Biotech Industry

The biotech industry is a challenging business. Long lead times, high development expenses and high project failure rates are facts of life. An ever-changing market environment contributes to the uncertainties. In this book Johanna Holldack presents an approach for using project management in the biotech industry and elucidates the methodology and organization.


---


Project Management for the Biotechnology Industry  
€68.00, ISBN 3-928383-25-6

Tel. +49 (0)30/26 49 21-40, Fax +49 (0)30/26 49 21-11  
eMail [service@biocom.de](mailto:service@biocom.de)  
Web [www.biocom.de](http://www.biocom.de)



## Cell separation in biotech: scalable, disposable and easy to use





- widely scalable
- single use – fully disposable
- economical – no centrifuge
- convenient

Talk to us about our new

FILTRODISC BIO SD pat. pend.

Achema Hall 4.2  
Booth N16–N19

FILTROX AG, St. Gallen / Switzerland, +41 (0)71 272 91 11, [www.filtrox.ch](http://www.filtrox.ch)

EXPERTS IN FILTRATION. SINCE 1938.

## BIOPROCESSING

some hydrogen bonds and two salt bridges. Most of the Protein A used for bioseparation applications is now made by genetic engineering. Whilst the use of recombinant Protein A-based adsorbents for the purification of full length antibodies is common (due to their high selectivity and ever increasing IgG binding capacity) their use for engineered antibody fragments is limited due to their weak affinity for the Fab region. A cell surface protein isolated from *Peptostreptococcus magnus* (Protein L) interacts specifically with the mouse  $\kappa$  light chains and some human  $\lambda$  light chain subclasses, and has been used for the purification of engineered antibody fragments. However, Protein L-based adsorbents generally have a low binding capacity for antibody fragments, are not readily available in commercial quantities and are very costly.

### Alternative ligands for capture and purification of MABs

In addition to ongoing cost concerns, protein-based affinity ligands are generally considered to have poor stability with regard to the cleaning and sanitisation regimes required for process scale applications. Whilst some progress has been made by use of protein engineering to delete non-specific binding regions and increase the stability of antibody-binding polypeptide ligands, their resistance to sodium hydroxide, the favoured method of column cleaning and sanitisation, remains limited.

To circumvent the problems associated with proteinaceous ligands, there has been sustained interest in the development of small synthetic ligands which bind specifi-

cally to antibodies and antibody fragments. A number of such ligands were developed in the 1980's and early 1990's from a variety of chemical backbones, including histidine, dichloropyridine (Avid AL), thiophilic gel obtained from divinylsulphone activation, and a variety of mixed-mode ligand adsorbents possessing both hydrophobic and ionic groups. Many of these suffered from drawbacks, including lack of selectivity, low capacity and the need for inclusion of additives. However, these molecules demonstrated the promise of small synthetic ligands for antibody purification, particularly with respect to sodium hydroxide resistance and the ability to be reused many times without loss of performance.

Small peptide ligands have also been investigated for antibody purification applications. Linear peptides have shown some promise but remain susceptible to both chemical and biological (protease) degradation. Conformationally constrained, cyclised peptide analogs, termed peptidomimetics, offer increased stability, but these ligands are still subject to degradation due to the presence of peptide bonds. The relatively high cost and potential toxicity of peptide ligands remains a barrier to their wider use.

More recently, techniques such as computational chemistry and high-throughput screening have evolved which enable the design and selection of small affinity ligands with high selectivity and capacity for antibodies. One of the first examples to be reported used the crystallographic structure of the B domain of Protein A complexed with human IgG as a starting point for modelling a small synthetic ligand. A dipeptide motif (Phe-132:Tyr-133) was iden-

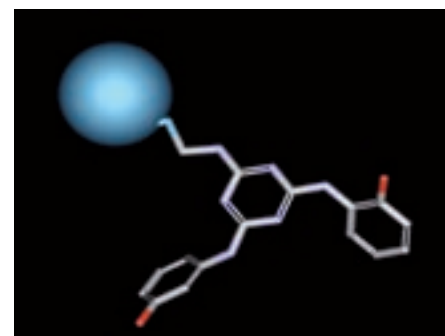


Fig. 2: Structure of MAdsorbent A2P on agarose beaded matrix

tified as the key component of Protein A responsible for binding to the Fc region of IgG and provided the template for design, synthesis and testing of equivalent mimic ligands (biomimetic ligands). The lead ligand and compound identified from this work was further refined by using combinatorial chemistry. Triazine ligand libraries produced by solid-phase chemistry were synthesised on agarose beads (Figure 1), assessed for binding to pure IgG and the selected ligands further tested for their ability to purify polyclonal IgG from human plasma. This work led directly to the development of the affinity adsorbent MAdsorbent<sup>®</sup> A2P (Fig. 2) which is used commercially for the purification of polyclonal IgG from plasma.

MAdsorbent<sup>®</sup> A2P can be used to purify polyclonal antibodies from different species and can withstand exposure to 1M sodium hydroxide, which is an important advantage over Protein A based adsorbents. However, MAdsorbent A2P has found only limited application for the purification of monoclonal antibodies due to

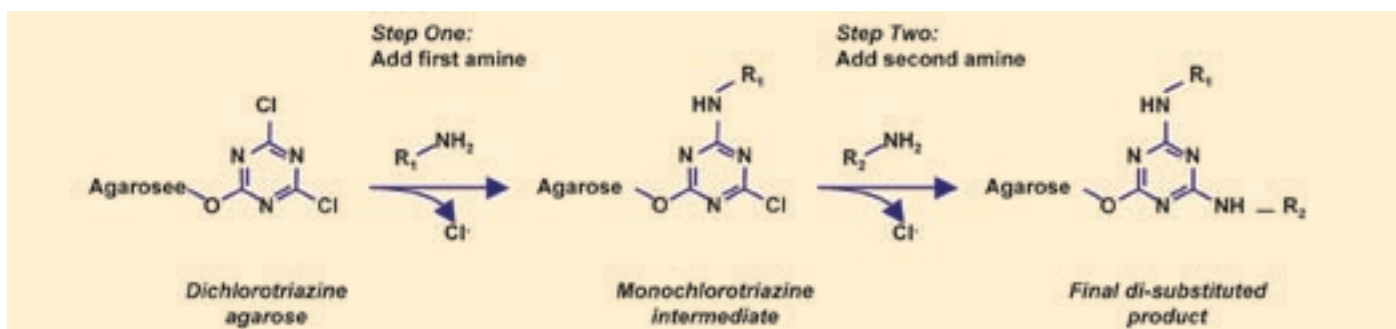


Fig. 1: Process of generating IgG binding chemical ligand libraries using chlorotriazine as the scaffold. The first chloride is displaced on reaction of cyanuric chloride with the PuraBead<sup>®</sup> 6XL, which is a 6% beaded agarose matrix. The 2<sup>nd</sup> and 3<sup>rd</sup> chlorines are displaced with various amines ( $R_1$  and  $R_2$ ) in a 'split and mix' combinatorial synthesis

# BIOPROCESSING

the presence of Pluronic F68 in most mammalian cell culture media (added to prevent shear-induced cell damage) which severely compromises the binding capacity of this adsorbent. If Pluronic F68 is removed first by chromatography or hydrophobic adsorption, then MAbsorbent A2P is effective for Mab purification. As part of the European FP6 Integrated Project to investigate improved approaches to downstream processing of biopharmaceuticals – Advanced Interactive Materials by Design (AIMs), chemical combinatorial libraries constructed on triazine scaffolds were screened for antibody binding from feedstocks containing Pluronic F68. A number of ligands suitable for whole molecule IgG capture and purification were

such as Fab, F(ab')<sub>2</sub> and scFV fragments. Unlike Protein L, this ligand binds to both κ and λ light chains. There is also some interaction with the Fc region, though conditions for elution differ for Fab fragments compared to Fc fragments, so both can be successfully separated and purified from papain digests (Fig. 3).

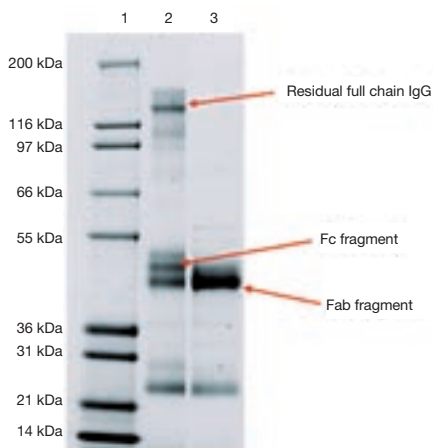
## Conclusion

Whilst Protein A-based adsorbents remain the materials of choice for purification of full length monoclonal antibodies, new generation adsorbents based on small, ultra-stable, synthetic ligands are emerging which are increasingly finding applications

in areas where Protein A is not applicable. Such applications include the purification of polyclonal IgG from mammalian plasma and the purification of antibody fragments devoid of the Fc region. However with continued development, synthetic ligands are likely to approach the performance of Protein A for full length antibodies, but with the added advantages of lower cost and significantly increased stability. ▼

### Contact

Dr Dev Baines  
 ProMetic BioSciences Ltd  
 211 Cambridge Science Park  
 Milton Road, Cambridge CB4 0WA, UK  
 dbaines@prometicbiosciences.com



**Fig. 3:** Capture and purification of Fab and Fc fragments on Fabsorbent F1P HF, Lane 1 - Molecular weight marker, Lane 2 - Papain digested IgG, Lane 3 - pH 4.0 elution. Human polyclonal antibody was digested with immobilised papain (Pierce) and applied to a column of Fabsorbent F1P HF at pH 7.50. Fab fragment was eluted at pH 4.0 and Fc fragment/intact undigested antibody elutes during the column cleaning procedure.

identified that gave high binding capacities in the presence of Pluronic F68. This work is currently continuing at ProMetic Biosciences.

Furthermore, a small synthetic ligand capable of binding to the antibody light chain has also been identified and the resulting adsorbent, Fabsorbent™ F1P HF, is capable of binding and purifying a wide range of antibody types, including antibody fragments that do not contain the Fc region

## BD Falcon™ FluoroBlok™ Insert Systems

Increase assay productivity

**Real-time fluorescence measurement of cell migration and invasion**

- Simplifies cell chemotaxis, migration and invasion assays
- Unique fluorescence-blocking membrane enables real time quantitative detection and kinetic analyses of cell migration without dismantling or destroying the insert
- Eliminates the need for manual cell scraping and counting: incubate and measure
- Different pore sizes available for various cell types
- Multiwell Insert Systems increase sample throughput via full automation compatibility
- ECM-coated inserts can be supplied to accommodate different cell and assay types

Helping all people  
 live healthy lives

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.  
 BD, BD Logo and all other trademarks are the property of Becton, Dickinson and Company. ©2009 BD  
 A468-01

**BD Biosciences**  
 Tel.: +32 2 400 98 95  
 Fax: +32 2 401 70 94  
 help.biosciences@europe.bd.com  
 bdbiosciences.com