Development of Selective Ligand Adsorbents for use in Downstream Processing of Biotherapeutics

Dev Baines
Director, Research & Development
ProMetic BioSciences Ltd

SEPARATION SCIENCE & TECHNOLOGY GROUP
Mixed Mode Chromatography – A Multimodal Separation Technique
for Biopharmaceutical Purification
Wednesday 27th June 2007

ProMetic Biosciences Ltd (PBL)

PBL is an innovative company bringing new technologies and solutions that enable the production of higher quality, safer and less expensive therapeutic products.

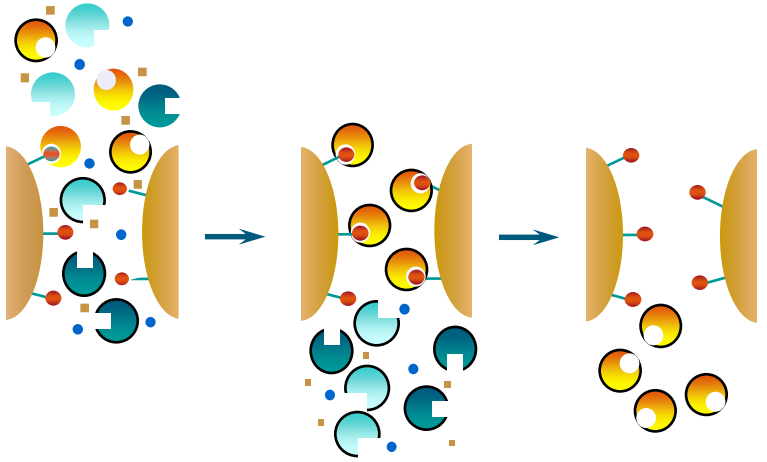
- Materials and technology for the purification of biological products.
- Removal of impurities, contaminants and pathogens.
- Development of purification processes.

ProMetic Biosciences

PBL - Technology

A Bio-recognition based - approach

- Affinity technology improves yields & purity with fewer process steps, with lower costs.
- Works with all protein products (plasma, rDNA, monoclonal antibodies etc.).
- Purification of target protein **or** specific removal of undesired compounds/pathogens.



CAPTURE **WASH** **RECOVER**

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Need for DSP performance improvements

- Cost of goods pressure (yield improvements/cost reductions)
- Processes developed for production of early-phase clinical material not always appropriate for large-scale manufacturing
- Product safety (increased purity/contaminant removal)
- Limited biomass availability (yield improvements)
- Improvements in protein expression levels (binding capacity)
- New biological products in development (established platforms may not be applicable to future biological products)
- Follow-on Biologics (process improvements/cost reductions)
- Novel Proteins

PROMETIC BIOSCIENCES

Biotherapeutics in development based on novel scaffolds

Pharma consolidates its grip on post-antibody landscape

Company	Molecule	Scaffold, clinical status
Ablynx	Nanobodies	Llamma heavy chain, phase 1
Adnexus Therapeutics	Adnectins	Fibrinonectin domains, phase 1
Affibody	Affibodies	Protein binding domain of Protein A, preclinical
Aptanomics	Peptide aptamers	Synthetic peptides, preclincal
Avidia	Aimers	A-domain derived cell surface receptors, phase 1
Biorexis Pharmaceuticals	Transbodies	Transferrin, phase 1
Borean Pharma	unnamed	Trimerized tetnectin domains, preclincal
Domantis	Domain antibodies	Heavy and light chain antibodies, preclinical
Evogenics Therapeutics	Evibodies	Derived from V-like domains of T-cell receptors, preclinical
ESBTech	scFV fragments	Stable single chain antibody fragments, preclinical
Genmab	Unibodies	Monovalent IgG4 mAbs fragments, preclinical
Micromet	BiTEs	Bispecific, T-cell activating single chain antibody fragments, preclinical
Molecular Partners	DARPins	Designed ankyrin repeat proteins, preclinical
Pieris	Anticalins	Derived from lipocalins, preclinical
Scil Proteins	Affilins	Derived from human lens protein gamma crystalline, preclinical
Trubion Pharmaceuticals	SMIPs	Custom-designed small modular immunophramaceuticals

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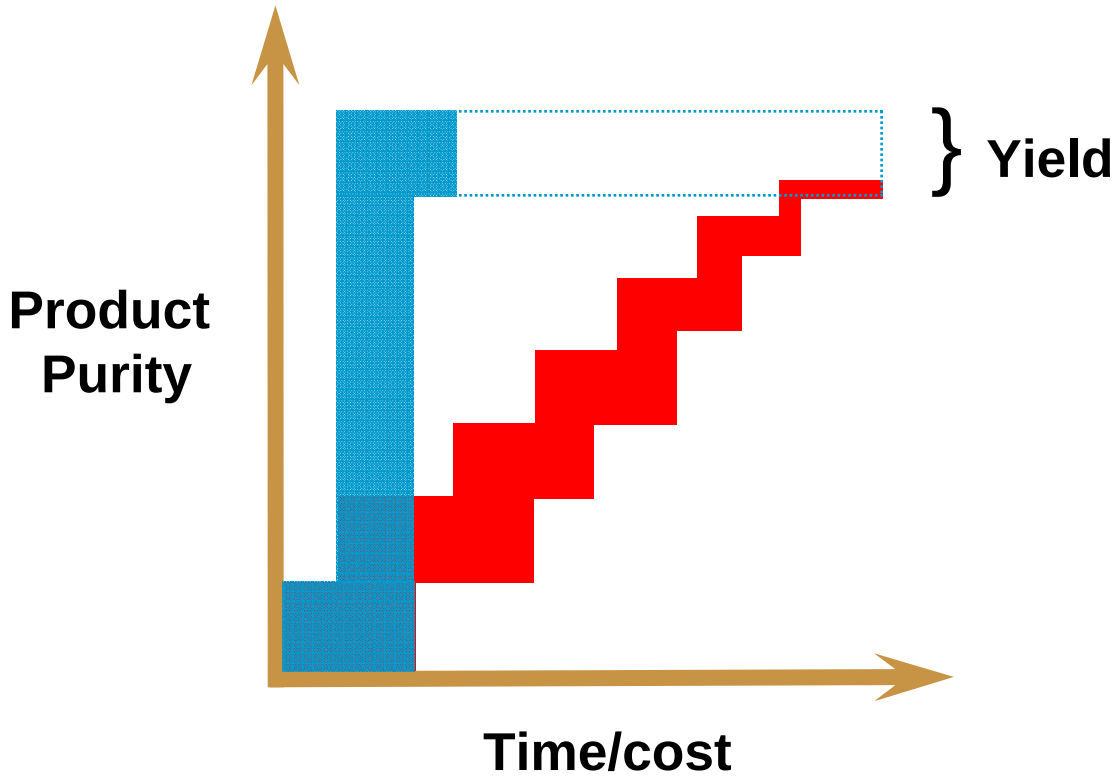
PROMETIC BIOSCIENCES

Advantages of Target Selective chromatography



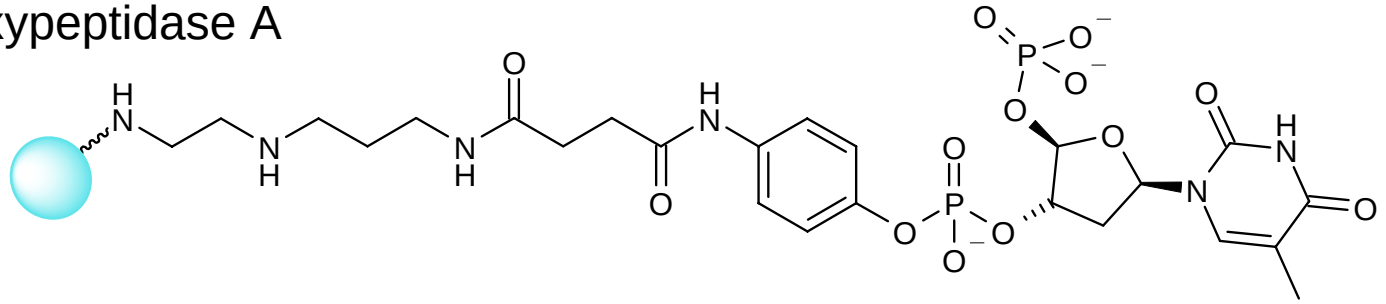
PROMETIC BIOSCIENCES

Advantages of Target Selective chromatography

AC is not a new technique!
It is a method in which biospecific and reversible interactions are used for the selective purification of biologically active material from crude samples (Wilcheck, 2004, *Protein Science,* **13**, 3006, *My life with Affinity)*

❑ First reported example: Starkenstein, 1910, *Biochem. Z.,* **24**, 210
- used starch to purify ά-amylase

❑ Modern AC is attributed to Cuatrecasas, Wilchek and Anfinsen, 1968, *PNAS,* **61**, 636, *Selective Enzyme Purification by Affinity Chromatography*
– general principles and potential application of affinity chromatography illustrated by purification of staphylococcal nuclease, ά –chymotrypsin and carboxypeptidase A



3'-(4-aminophenylphosphoryl)-deoxtthymidine-5'-phosphate

PROMETIC BIOSCIENCES

Advantages of Target Selective Chromatography

AC is not a new technique!
But, known ligands/inhibitors that bind to the target molecule are usually immobilised to the chromatographic support

- Designer Ligands 'biomimetics' – Lowe *et.al.* 1986, *J. Chromatogr.,* **376**, 111
  Lowe *et.al.* 1992 *Tibtech.,* **10,** 442

- Screening Chemical Combinatorial Libraries – 1995 (Novo Nordisk A/S/ ProMetic/Cambridge University – protein targets included immunoglobulins, insulins, FVIII and human growth hormone – WO 97/10887 – based on the concept that the selectivity of the ligands can be increased by increasing the spatial geometry and orientation of the ligand structures

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Advantages of Target Selective Chromatography

- Mimetic Ligands exploit complimentary binding groups and the spatial orientation at specific binding site on the target protein

- Mixed Mode Ligands exploit gross physical properties of the target protein through enhanced hydrophobic and ionic interactions

- Both are synthetic ligands

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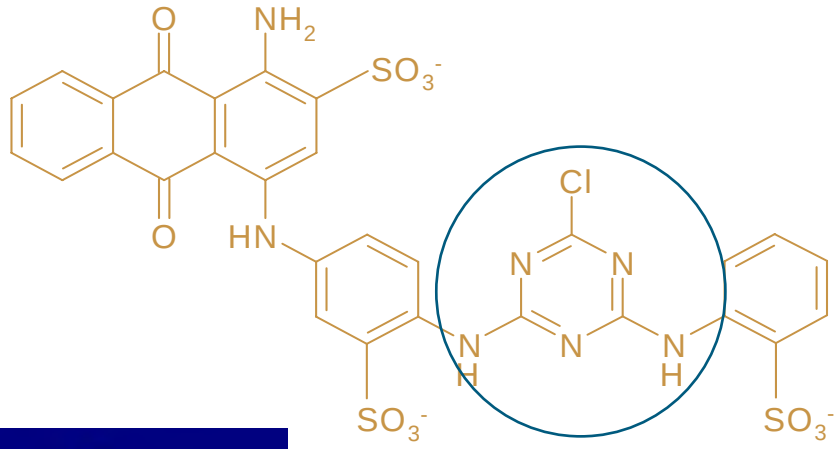
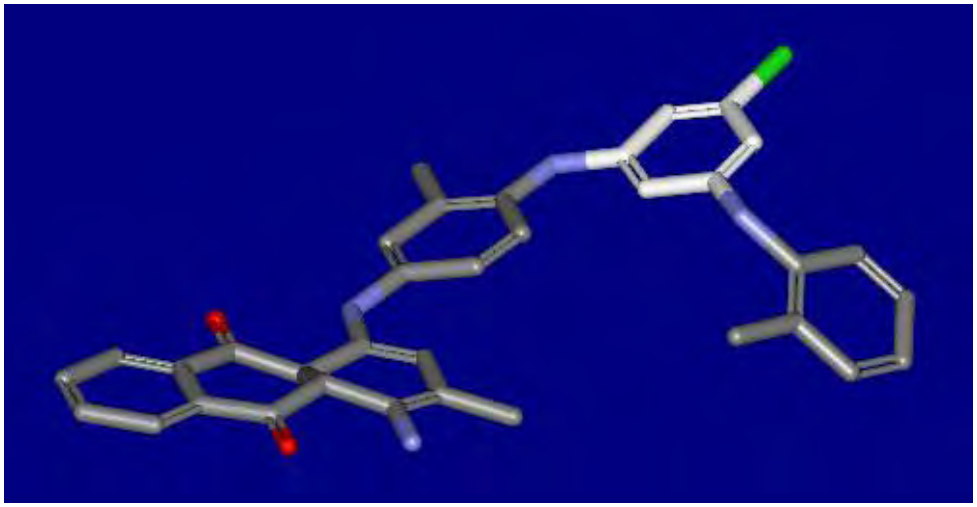
Synthetic *vs* biological ligands

Criterion	Synthetic/biomimetic ligand	Biological/specific ligands
Cost	Inexpensive	Usually expensive, e.g. monoclonal antibodies/protein A
Availability	Organic synthesis by adsorbent manufacturer	Biological origin, e.g. ascites, fermentation etc.
Synthesis	Facile	Often complex and purification needed
Specificity	Moderate to high	Usually high
Capacity	High (up to 40 mg protein/mL adsorbent). >10% ligand utilisation	Low. Typically <1% ligand utilisation
Scale-up	Large scale use: columns at >100 litre scale	Limited application
Sterilisation	High	Mostly low or not sterilisable

PROMETIC BIOSCIENCES

Advantages of Target Selective chromatography





C.I. Reactive Blue 2 → Mimetic Blue SA

Optimisation of ligand structure and coupling chemistry

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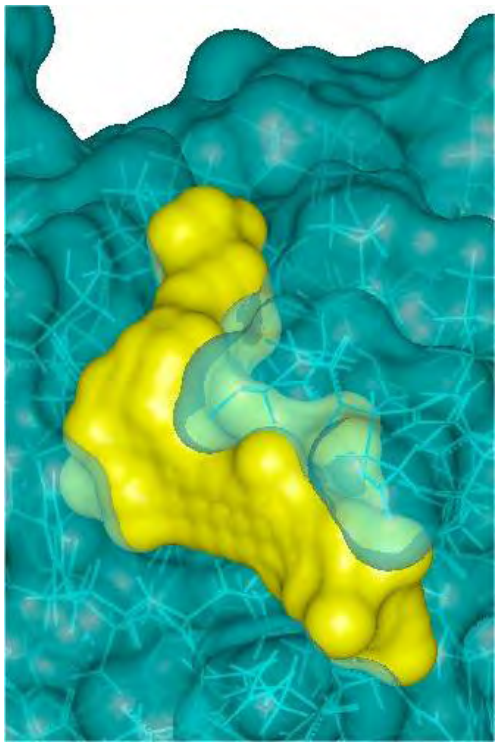
Approaches to ligand discovery



- Optimisation of existing ligands (analogue synthesis**)**
- Rational design (computer modeling of new ligand structures)
- Combinatorial libraries (systematic screening of ligand arrays)

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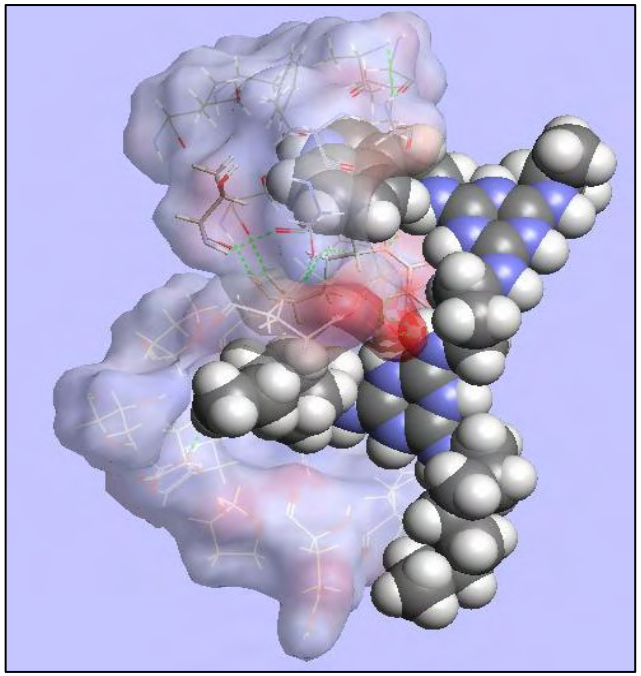
Ligand modeling and in-silico design



- Use structural info when available
- Model and refine
- Ligand structure data base
- Search virtual and real spaces
- Design for bioprocess or proteomics
- Design for medical devices
- Design for therapeutics

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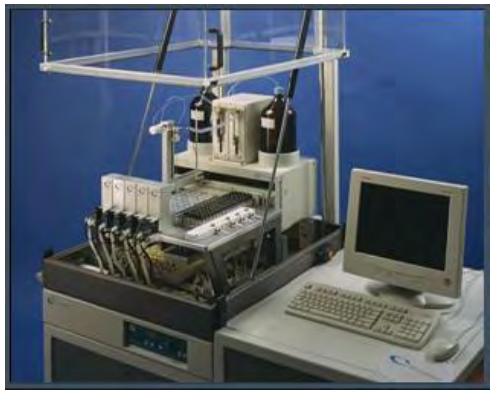
Ligand library synthesis and screening

- Library design
- Combinatorial synthesis
- Library verification
- Library screening
- Ligand selection
- Development & Scale-up



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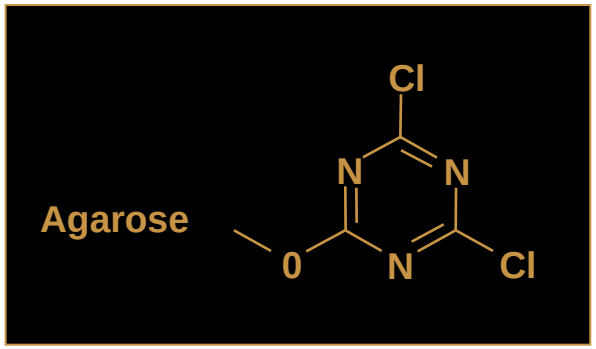
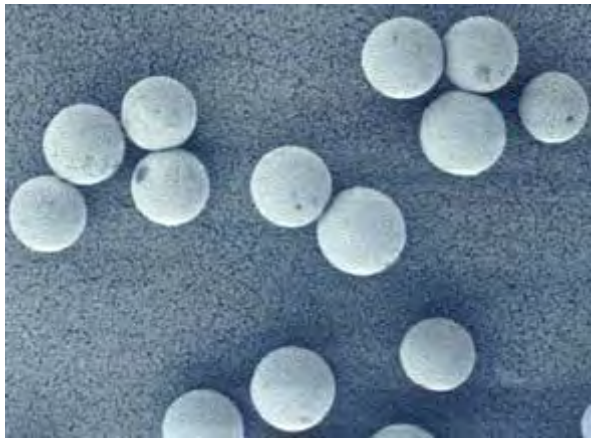
Chemical Combinatorial Libraries™: triazine ligands on agarose support matrix

- Trivalent
- Base stable
- Aqueous-phase chemistry
- Easy to monitor ligand level
- Well characterised



PuraBead® 6XL (6HF)

- Agarose, 6%, cross-linked
- Near mono-dispersed
- Green, continuous process



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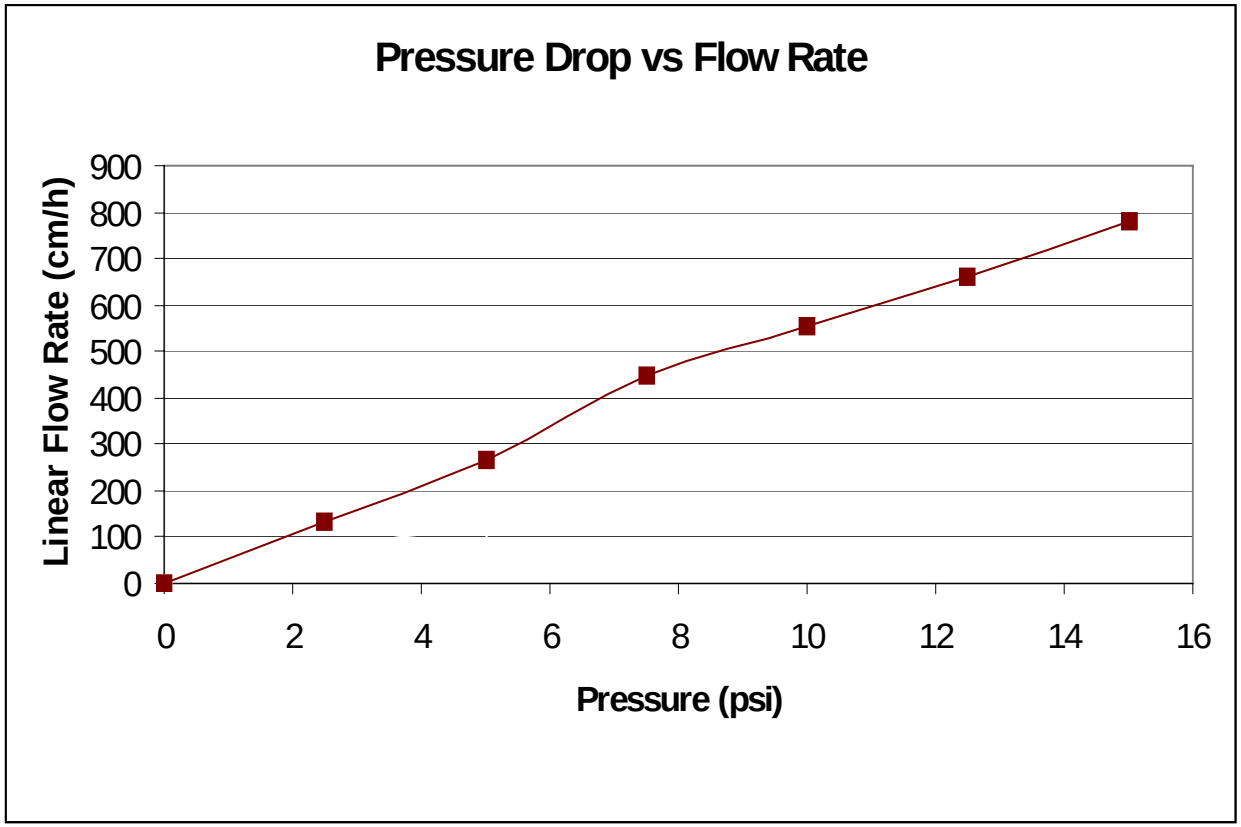
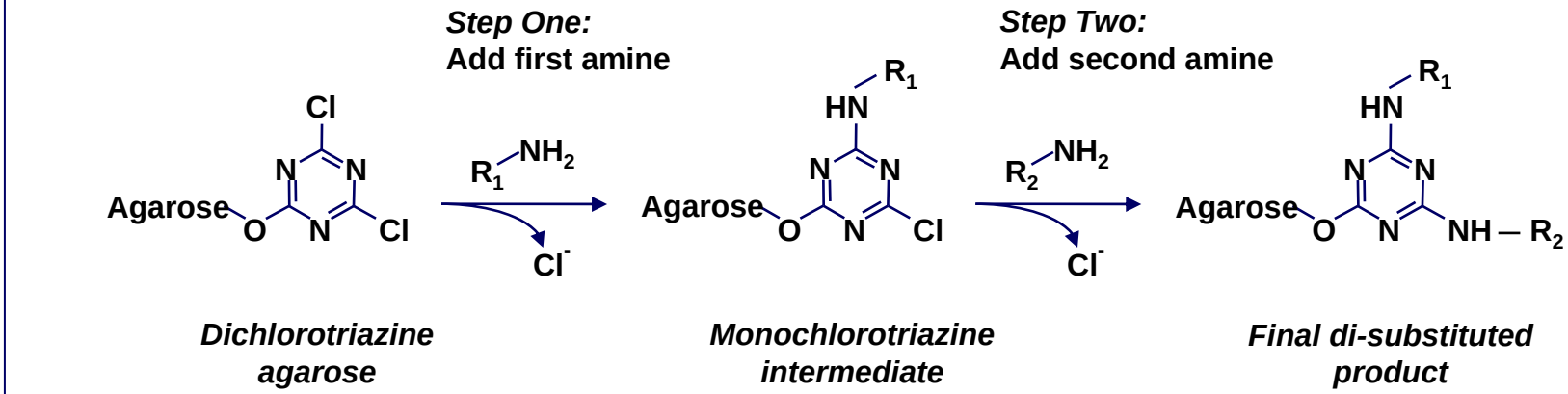
Purabead 6HF Flow Properties



PROMETIC BIOSCIENCES

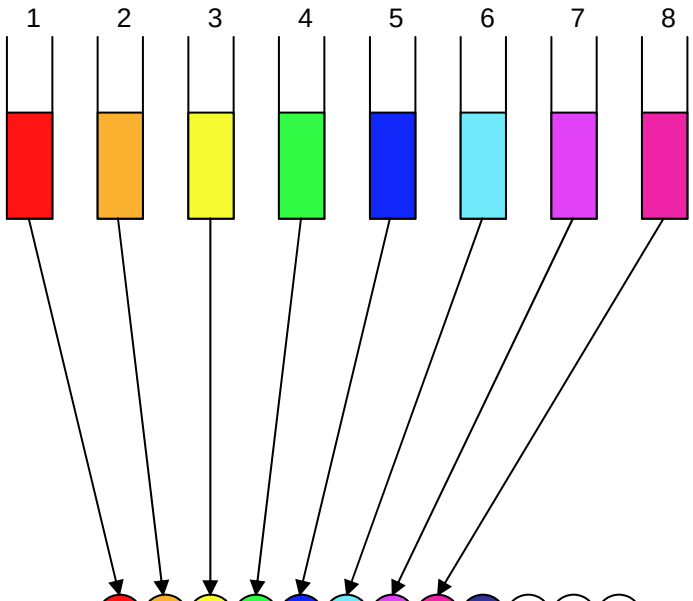
Ligand synthesis



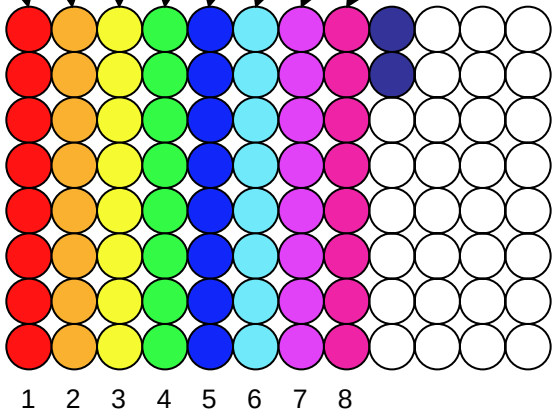
PROMETIC BIOSCIENCES

Library Synthesis

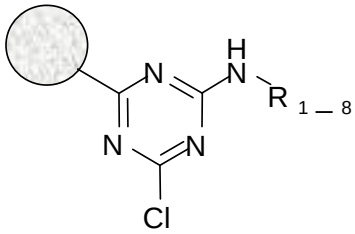
Reactor vessels in the robot



96 well, fritted block



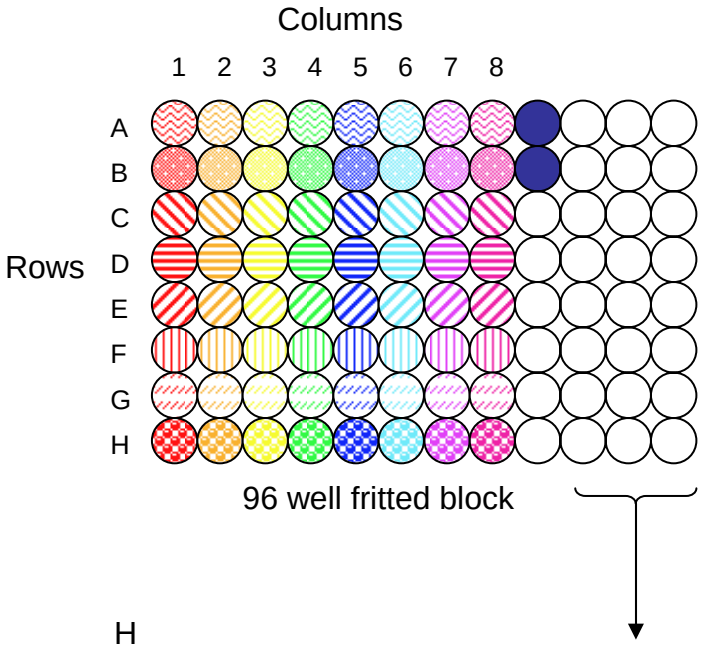
Intermediates

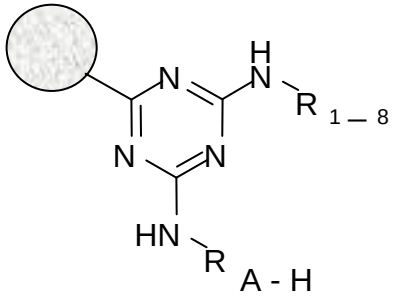


PROMETIC BIOSCIENCES

Library Synthesis

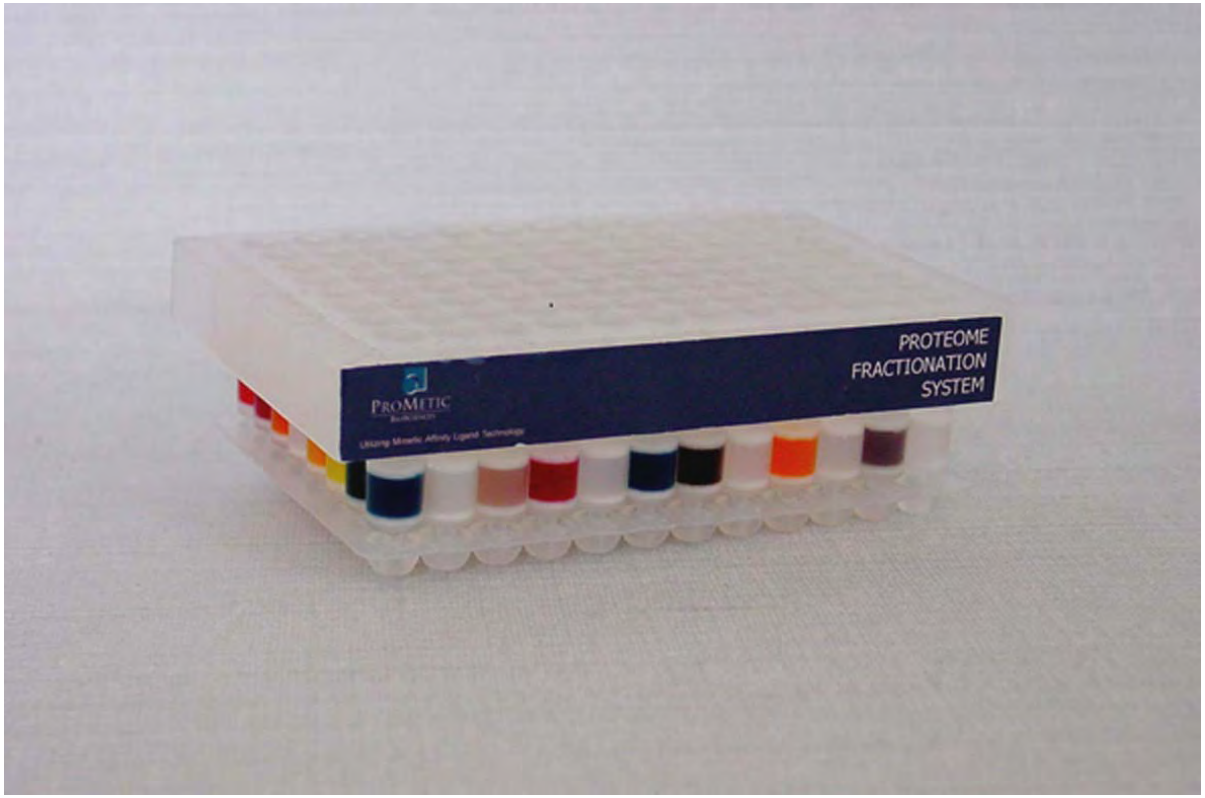
Addition of Second Amines to rows A-H





PROMETIC BIOSCIENCES

96-well library format



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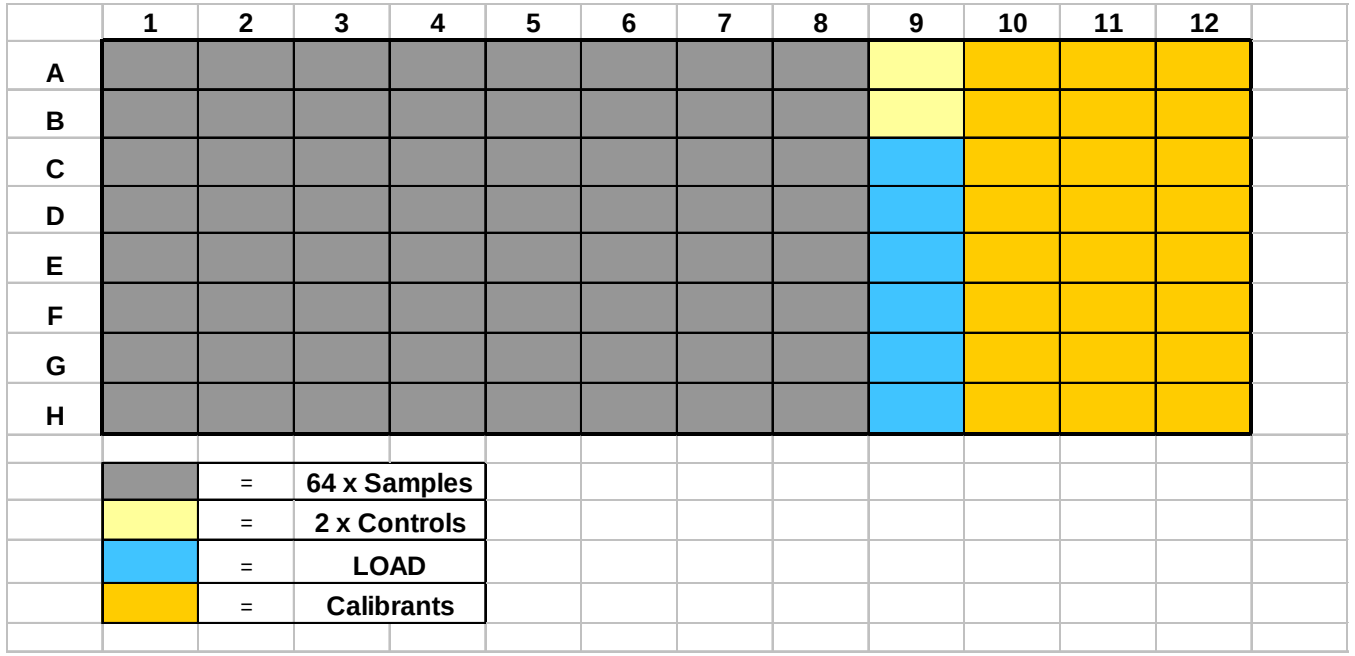
Library Screening



Techniques Available:

ELISA
SDS PAGE (E-PAGE)
Western Blot (E-PAGE)
Total Protein
Activity Assays

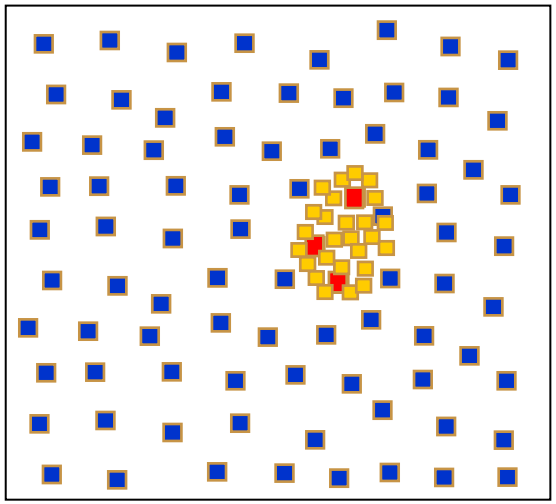
PROMETIC BIOSCIENCES

Chemical Combinatorial Libraries®: screening strategy

Virtual Library: **All possible ligands that can be made from available amine intermediates**

General library: **Ligands selected for synthesis from a virtual library**

Sub-Library: **Focused library developed on basis of leads from general library screen**



Virtual library space

General library

Leads from general library screen

Sub-library

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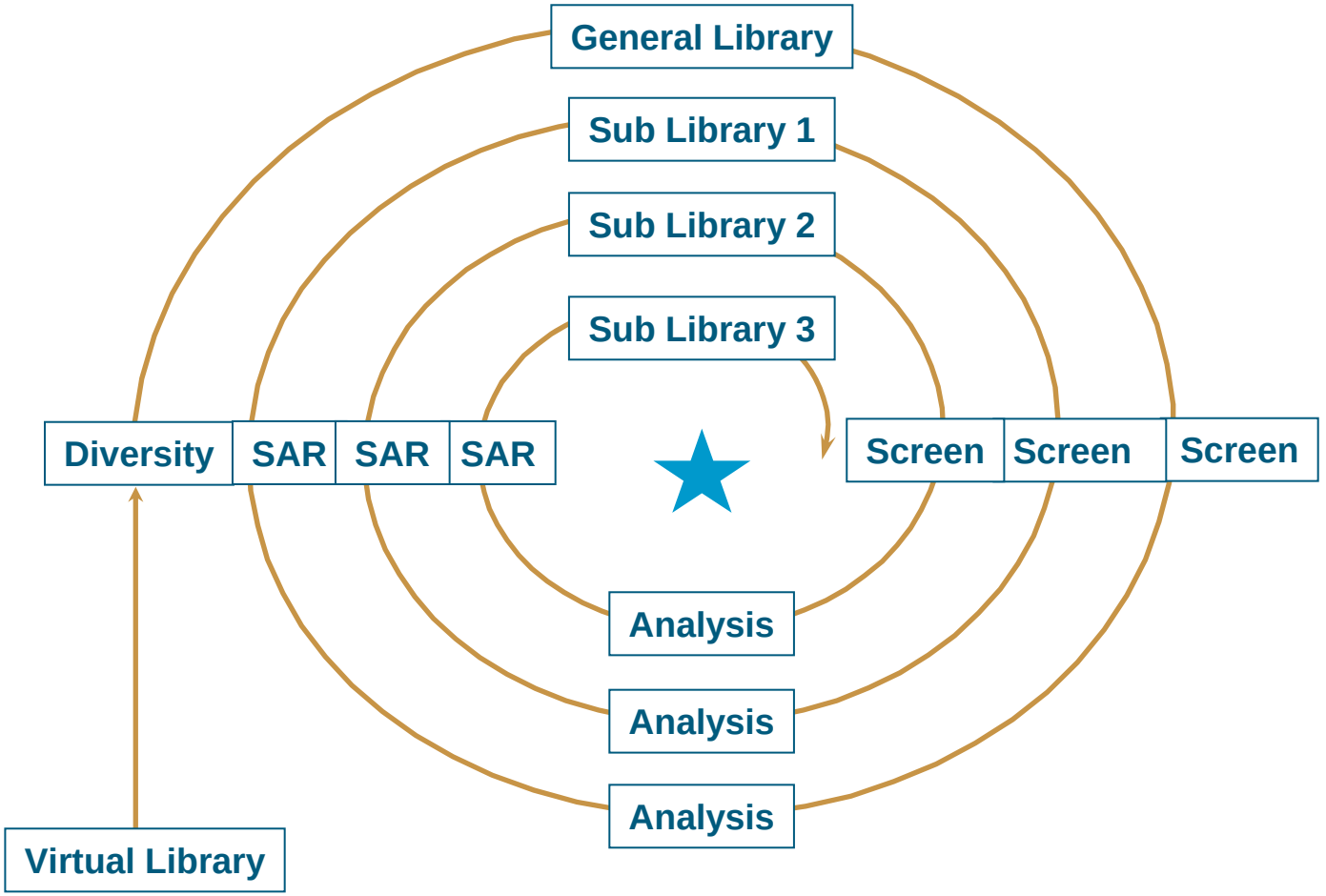
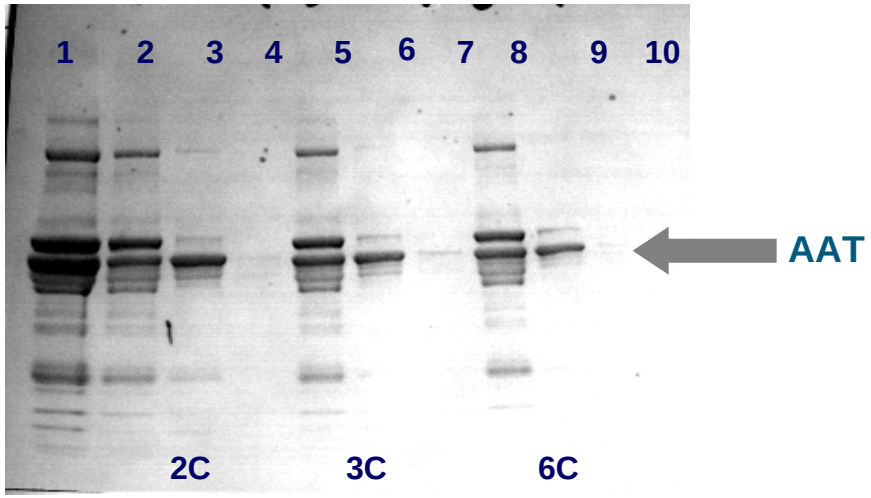
Chemical Combinatorial Libraries® Screening Strategy

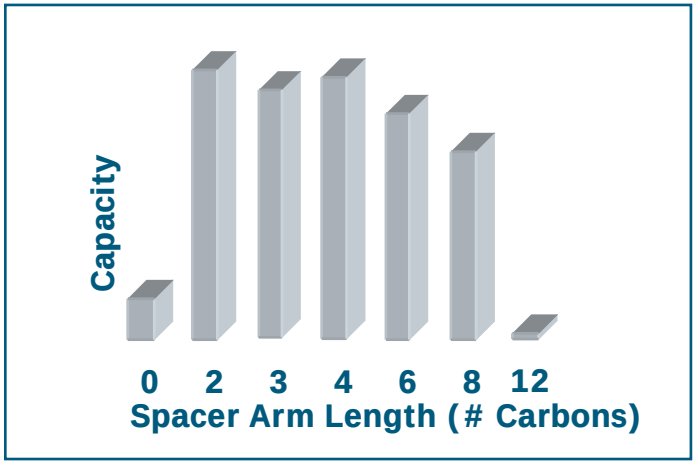


PROMETIC BIOSCIENCES

Ligand/adsorbent development

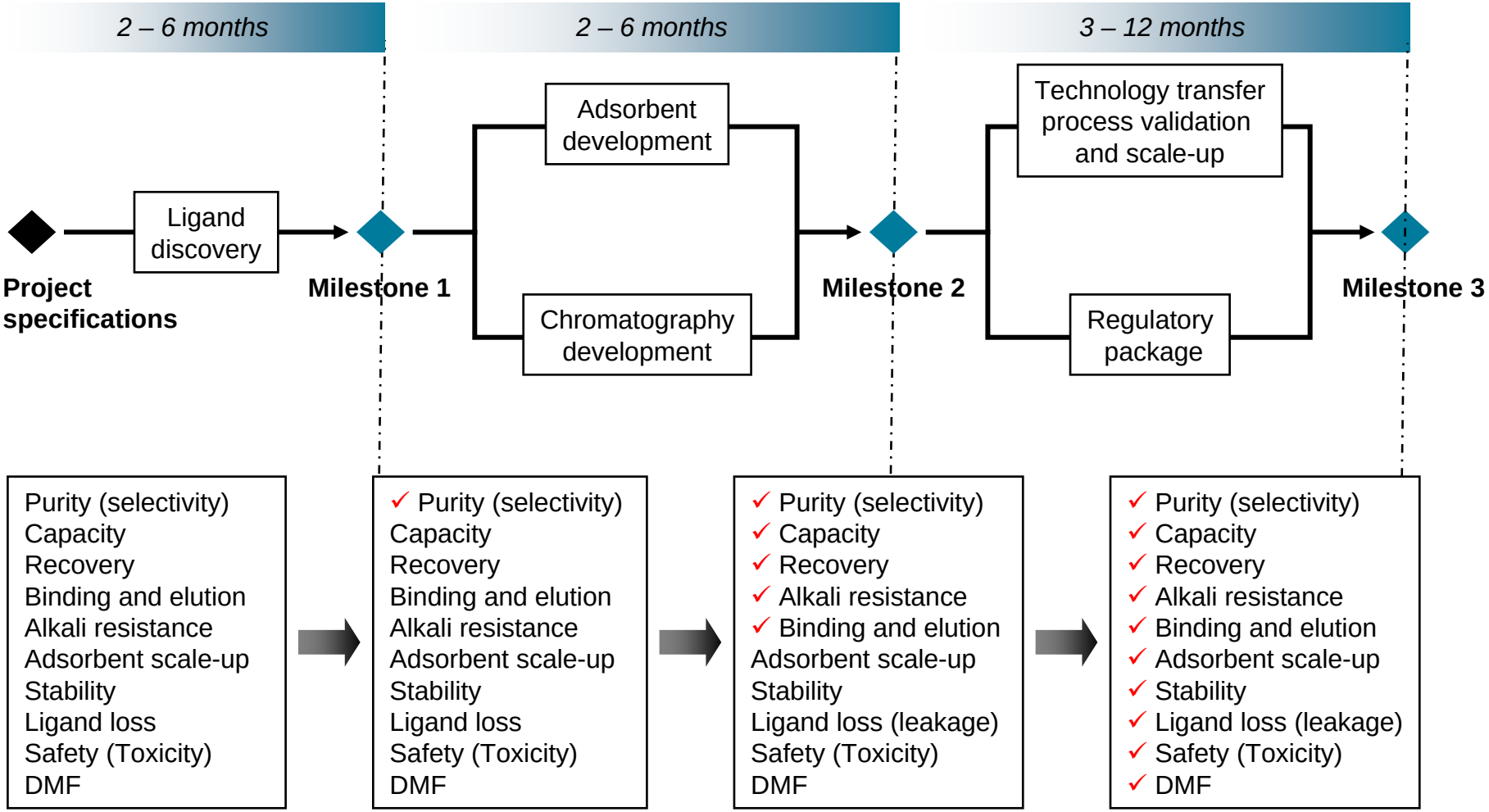
- Ligand chemistry optimisation
- Attachment chemistry –spacer arm
- Ligand concentration (density)
- Matrix & cross-linking
- Performance optimisation (including optimisation of chromatographic parameters)
- Suitability for manufacture
- Safety studies





PROMETIC BIOSCIENCES

Typical ligand discovery programme



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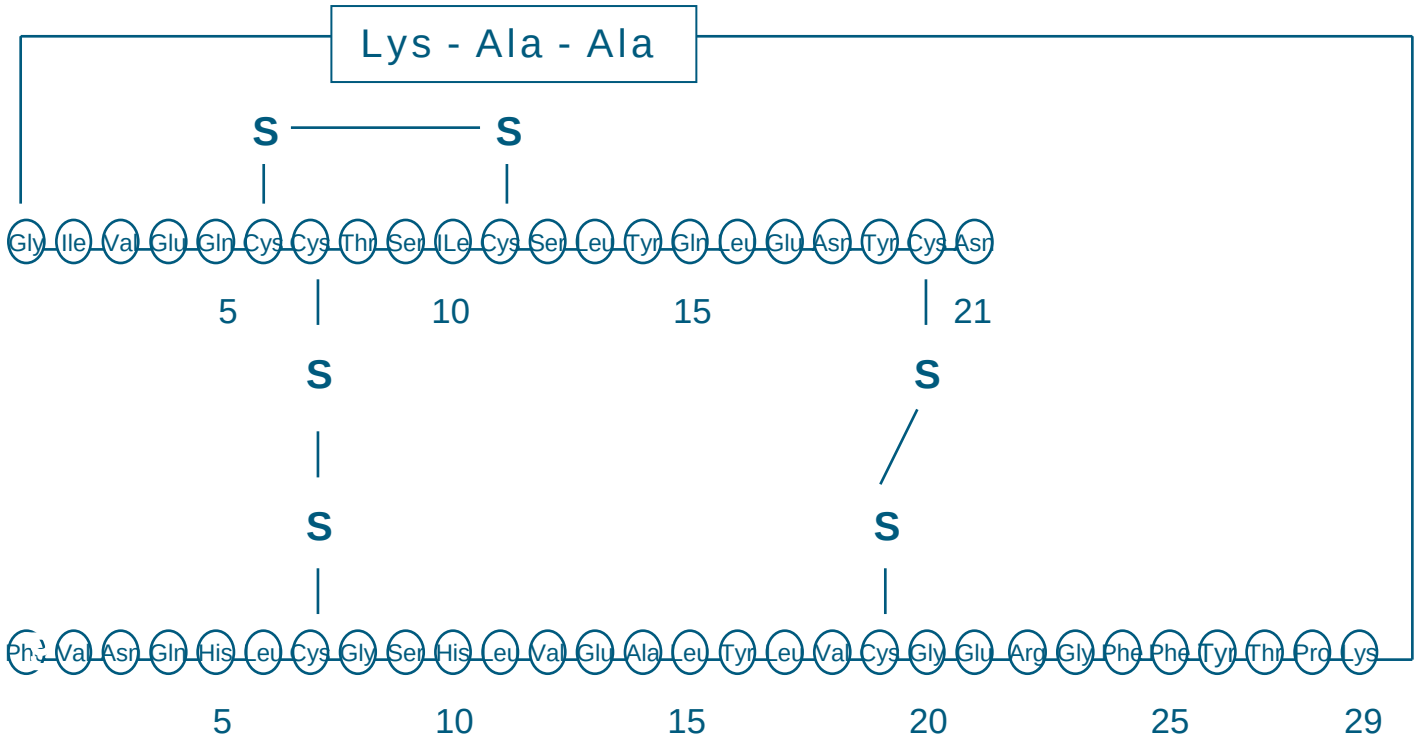
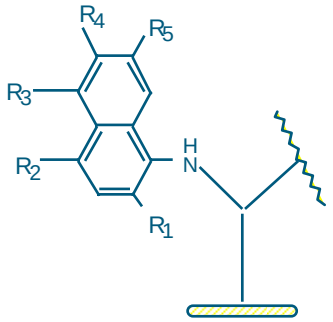
Discovery of an affinity ligand for the purification of MI3 insulin precursor



Substituent effects



Ring Pos.	Substituents	MI3 Binding
R_1	OH	bad
R_2	OH NO_2	bad
R_3	OH	good
R_4	OH	good
R_5	OH	good

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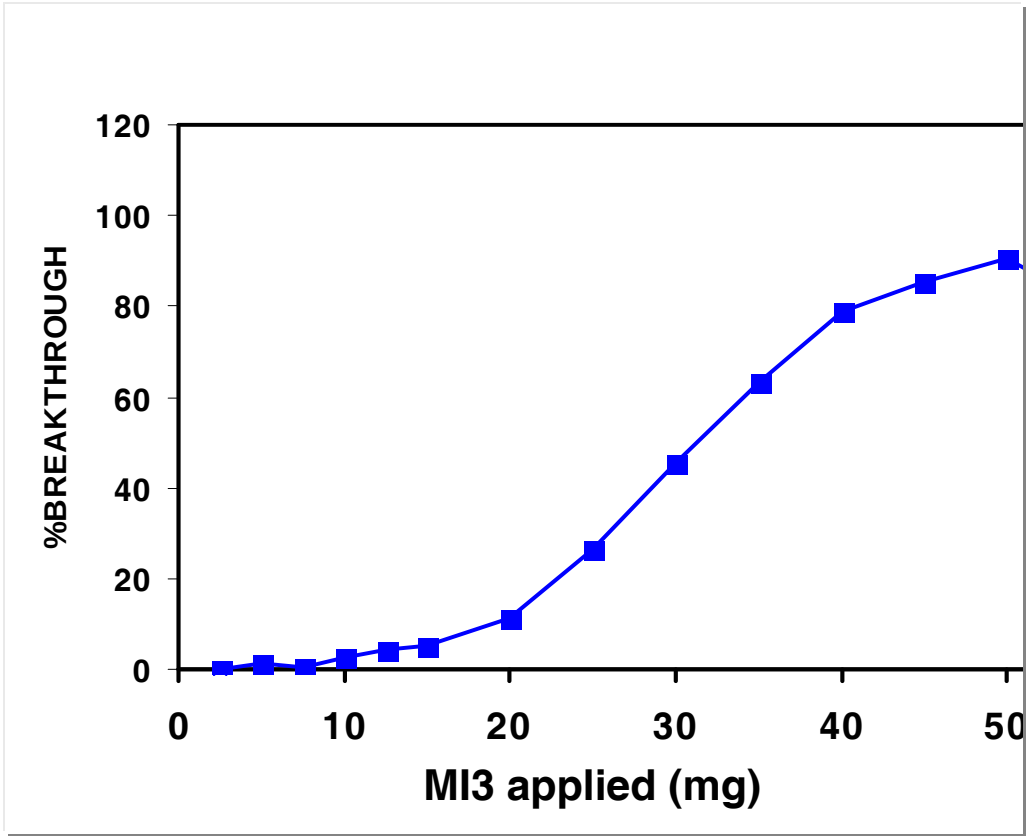
Conditions for MI3 purification

EQUILIBRATION
0.2 M Na-acetate pH 5.5
APPLICATION
Broth adjusted to pH 5.5
WASH
0.1 M Na-acetate 5.5
ELUTION
0.1 M acetic acid
C.I.P.
0.5 M NaOH

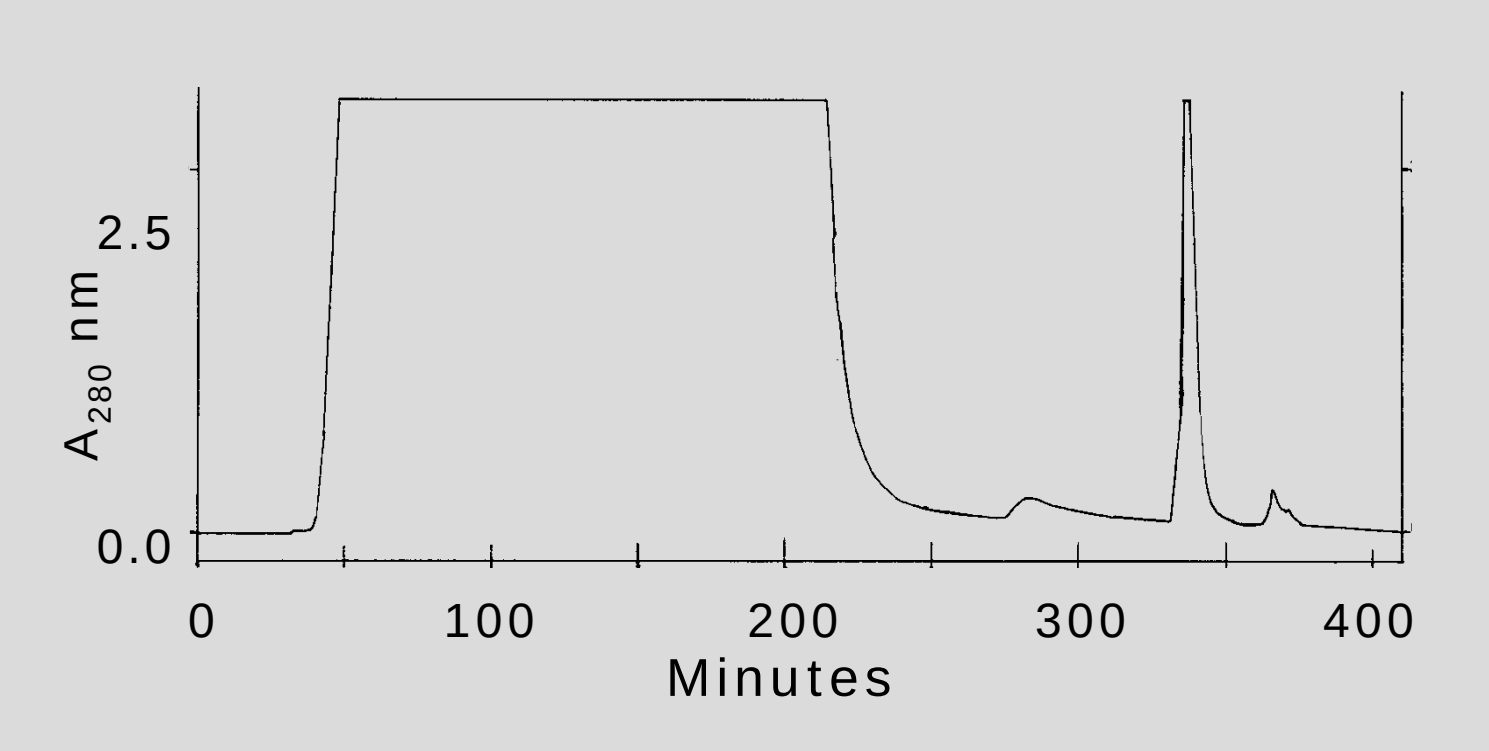
PROMETIC BIOSCIENCES

Breakthrough curve for MI3 on 2/2 agarose



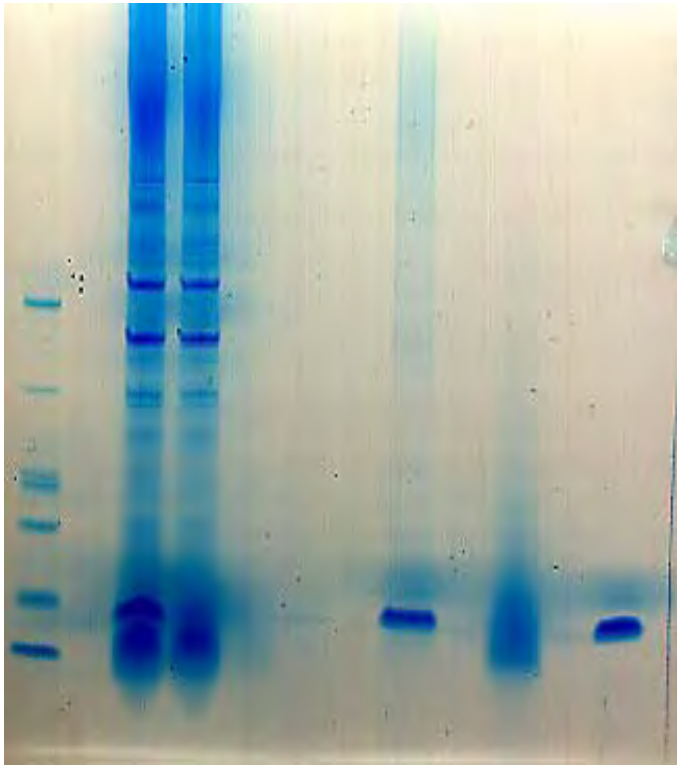
PROMETIC BIOSCIENCES

Purification of MI3 insulin precursor from fermentor broth



PROMETIC BIOSCIENCES

Purification of MI3 Insulin precursor from fermentor broth: SDS PAGE



1. Mw standards
2.
3. Application
4. Flow through
5. Wash
6. Wash
7.
8. Pool
9.
10. C.I.P
11.
12. MI3 Standard

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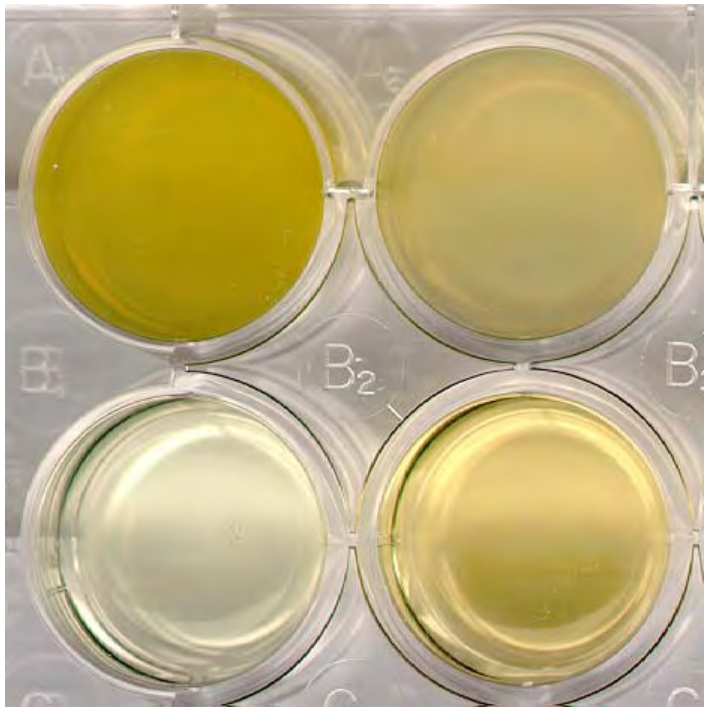
Purification of MI3 insulin precursor from fermentor broth: visual appearance

Application

Flow through



Elution Pool

CIP

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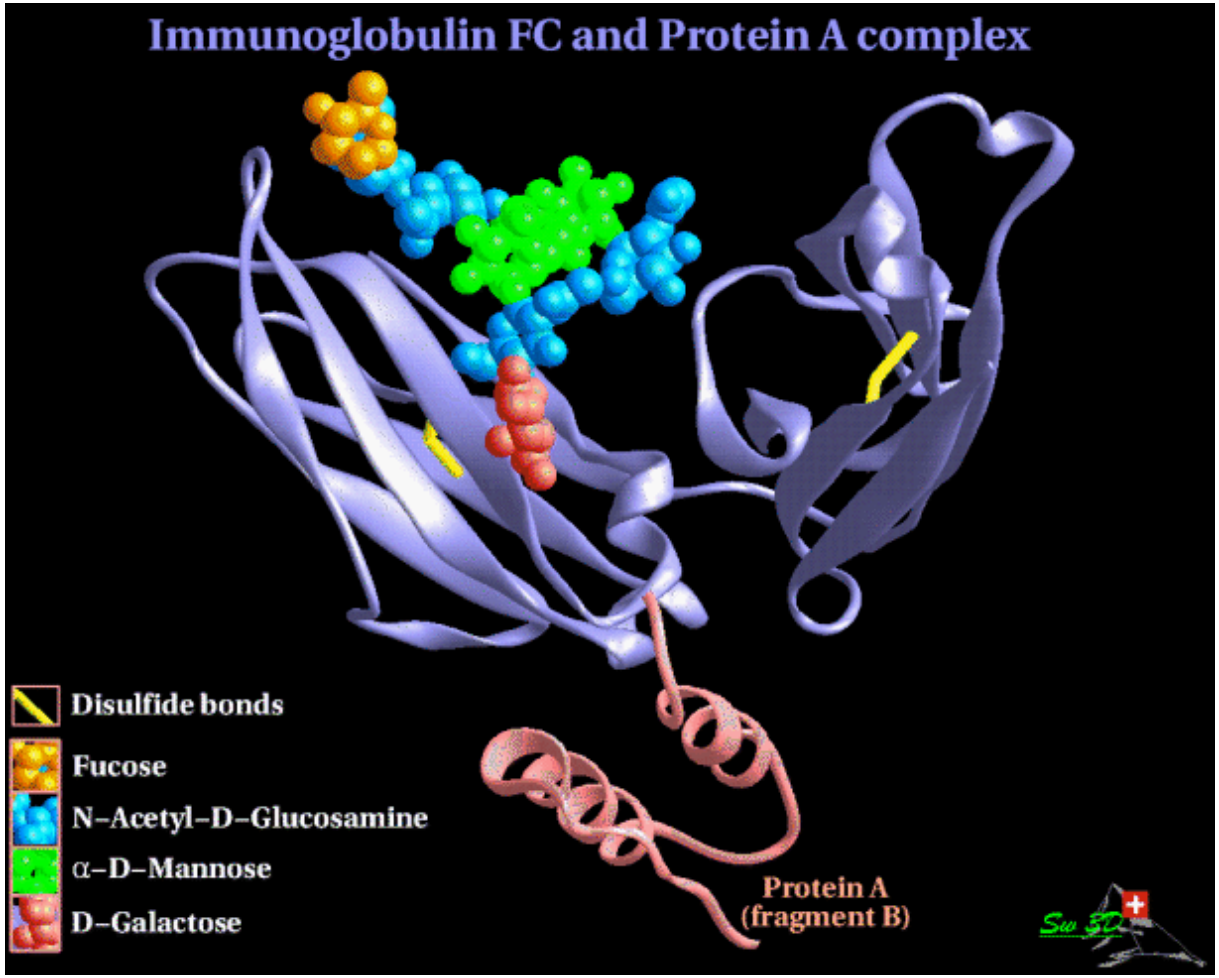
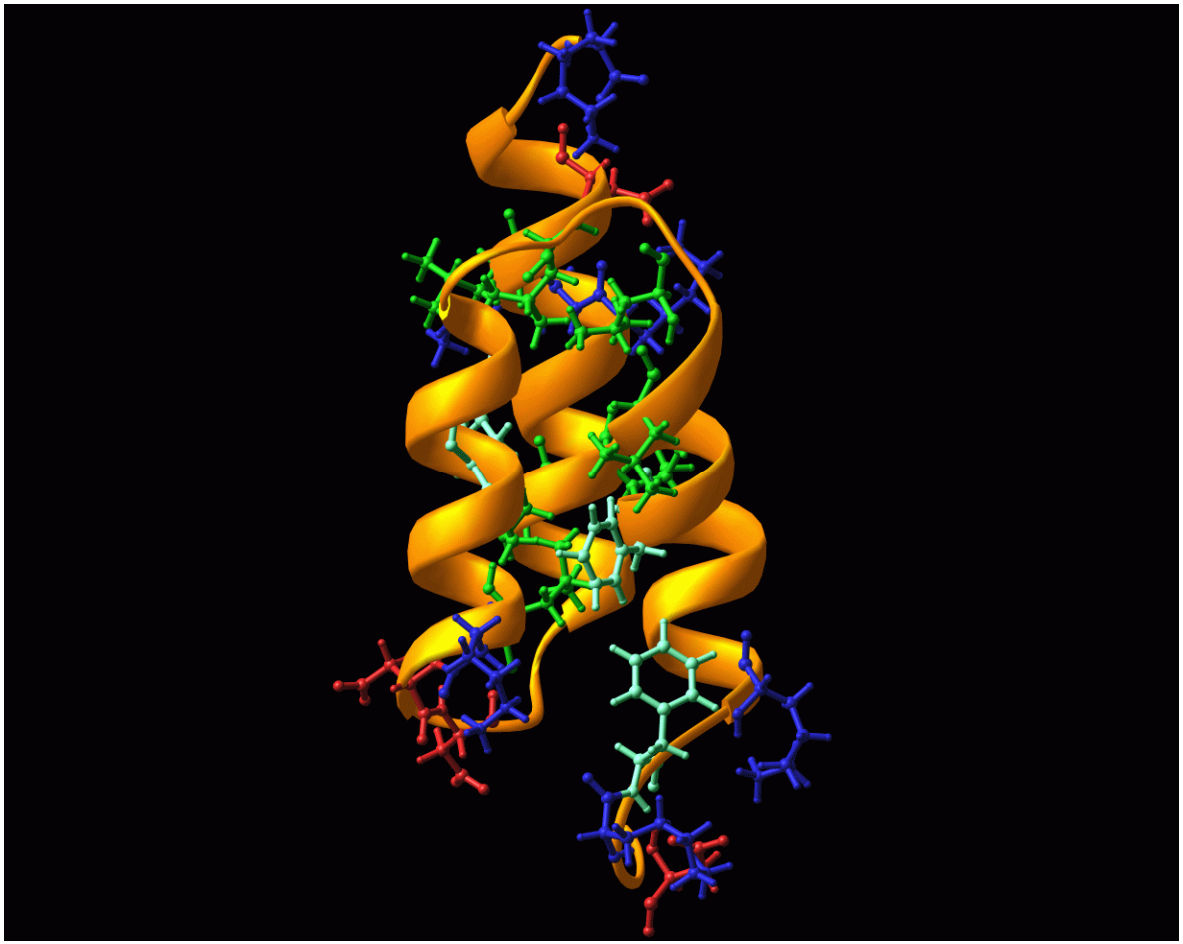
Protein A interaction with Fc domain



www-nmr.cabm.rutgers.edu

PROMETIC BIOSCIENCES

Protein A – B Domain



B Domain

www-nmr.cabm.rutgers.edu

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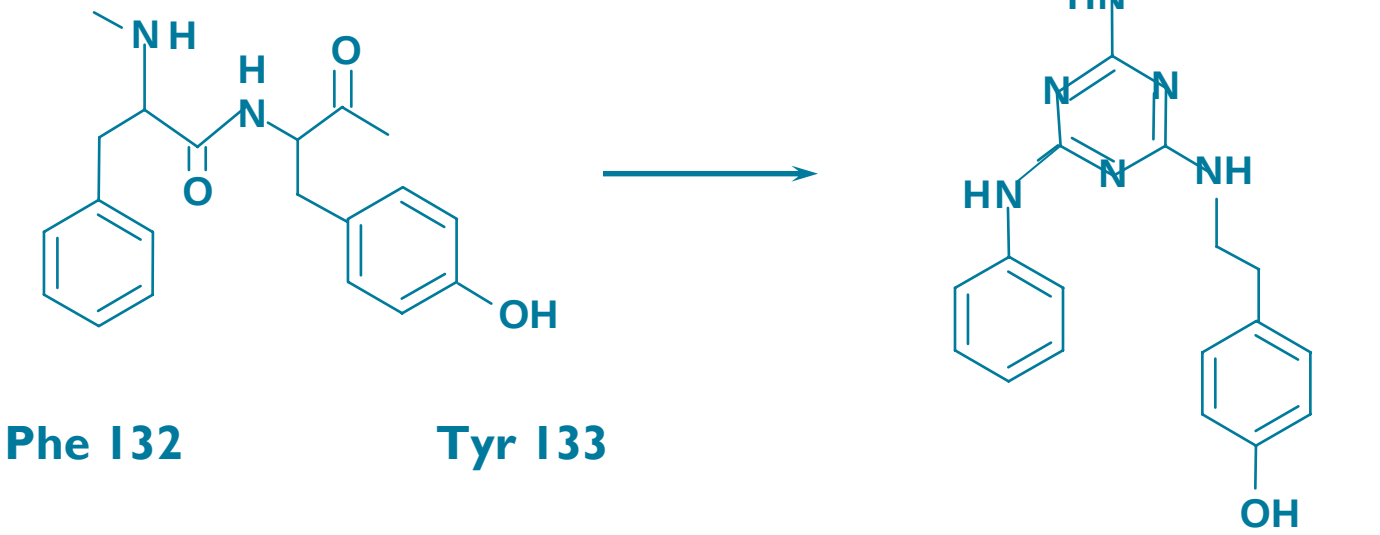
Rational design of synthetic affinity ligands for IgG purification



Protein A **Protein A Mimic**

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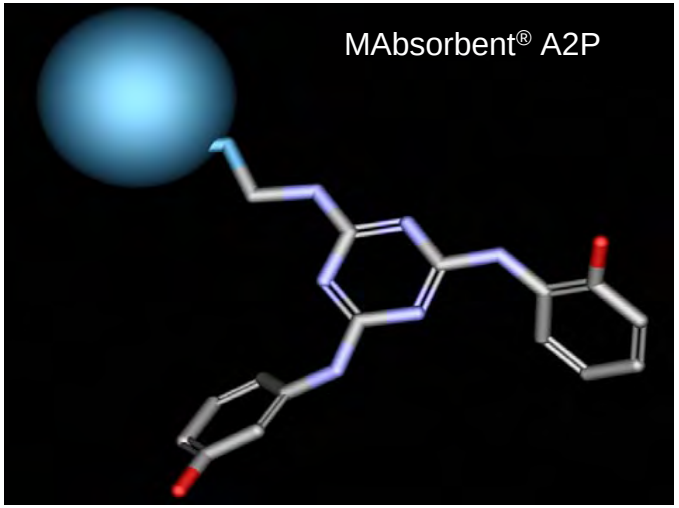
MAbsorbent® A2P

- MAbsorbents® were developed to mimic the Phe-132, Tyr-133 dipeptide binding site in the hydrophobic core of Protein A.
- MAbsorbent® A2P was developed by screening triazinyl scaffold based chemical ligand libraries.
- MAbsorbent® A2P-HF has been developed with improved cross-linking chemistry giving a very rigid but hydrophilic base matrix that resists shrinking and swelling and permits high operational flow rates.



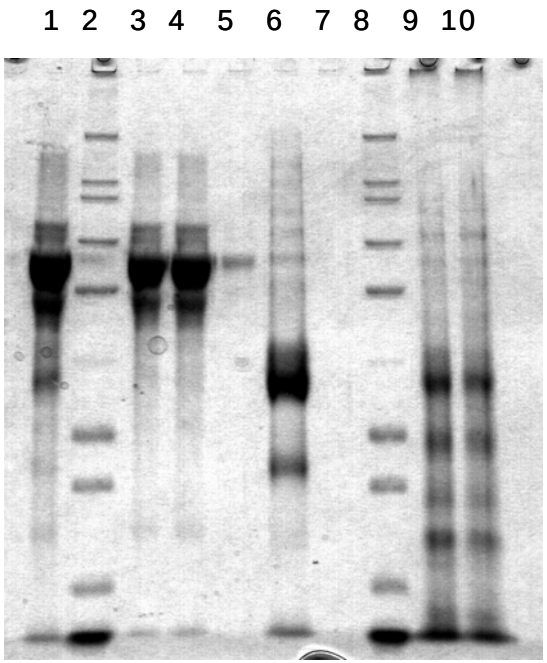
MAbsorbent® A2P

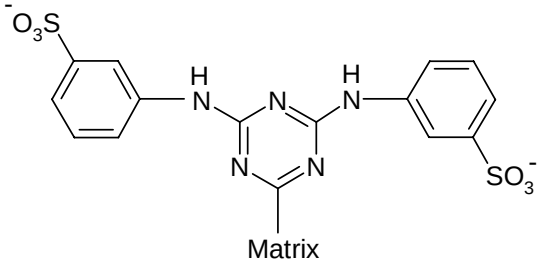
- Binds IgG at the Fc region of the molecule
- Binds human and murine IgG
- Binds all human IgG subclasses
- Binds IgG at neutral pH and is salt tolerant
- Mild elution of IgG (acidic pH or ethylene glycol)
- Can be sanitised with 1 M NaOH
- Can be autoclaved

Identification of an affinity ligand for purification of a tPA-Urokinase fusion protein

check

Ligand #23/B2 Analogue 1



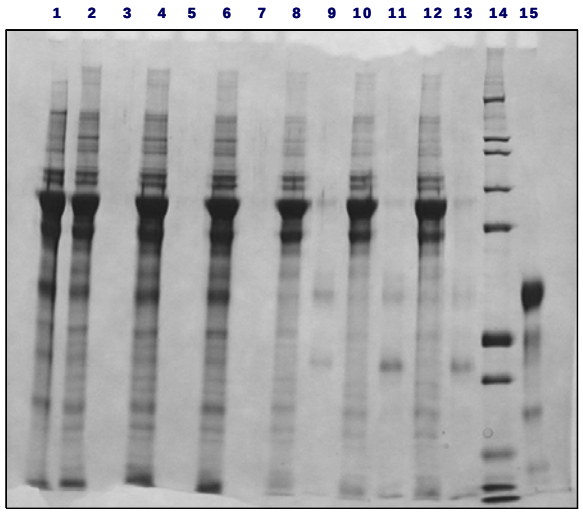


Lane	Fraction
1	Load (cell culture fluid)
2	MW Marker
3	Flow through (1)
4	Flow through (2)
5	Wash
6	Elution (2)
7	Sanitisation
8	MW Marker
9	Lysine Affinity elution P007/01
10	Lysine Affinity elution P008/01

PROMETIC BIOSCIENCES

Identification of an affinity ligand for purification of a tPA-Urokinase fusion protein

Library #23 Flow Through Fractions								
Ligand	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
A	43	42	45	37	24	40	2	9
B	43	2	42	41	5	41	2	1
C	41	43	41	37	26	42	7	6
D	38	38	39	40	19	36	3	3
E	21	2	27	15	1	32	1	1
F	44	42	40	39	10	43	4	5
G	27	5	40	30	2	36	1	1
H	28	2	39	35	2	38	1	1

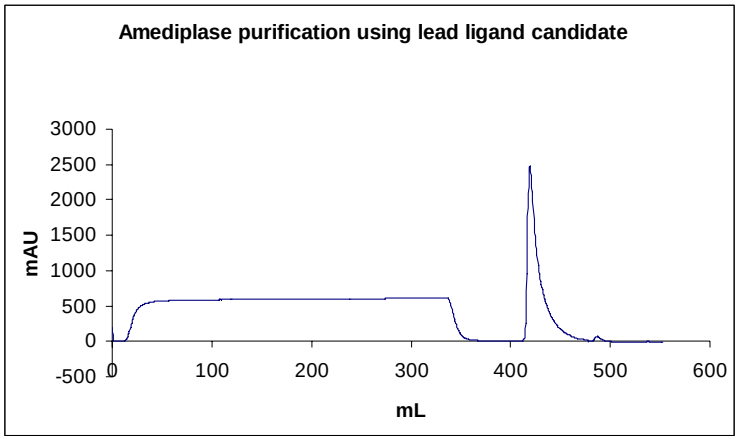


Lane	Fraction
1	Load
2	B1 Flow through
3	B1 Elution
4	C1 Flow through
5	C1 Elution
6	D1 Flow through
7	D1 Elution
8	B7 Flow through
9	B7 Elution
10	B8 Flow through
11	B8 Elution
12	F8 Flow through
13	F8 Elution
14	MW Marker
15	Amediplase standard

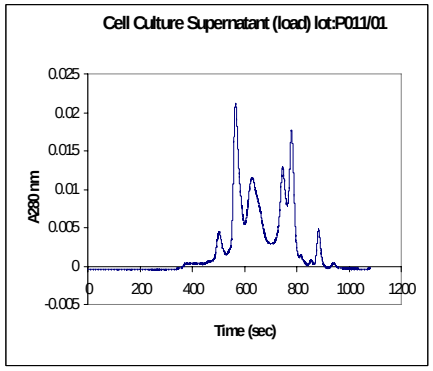
the MENARINI group

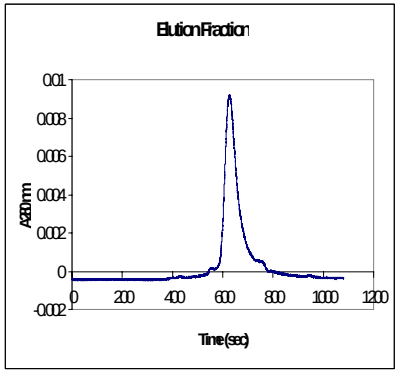
PROMETIC BIOSCIENCES

Identification of an affinity ligand for purification of a tPA-Urokinase fusion protein



- Column dimensions: 10mL column - 4.5cm bed height, 1.6cm diameter
- Adsorbent: Ligand #23/B2 Analogue 1 attached to Purabead™ 6HF; ligand density 17 μmol/g.
- Flow rate: 300cm/hr
- Equilibration / wash buffer: 25mM sodium phosphate, pH 6.5, 100mM NaCl
- Loading with concentrated cell culture supernatant, pH 6.5
- Elution buffer: 25mM sodium phosphate, pH 6.5, 0.5M NaCl, 40% v/v ethylene glycol
- Sanitization: 0.5M NaOH





- Capacity: 11.4 mg/ml
- Purity: 99%
- Recovery: 92%
- Loading flow rate: 300 cm/h
- Alkali stable (0.5 M NaOH)

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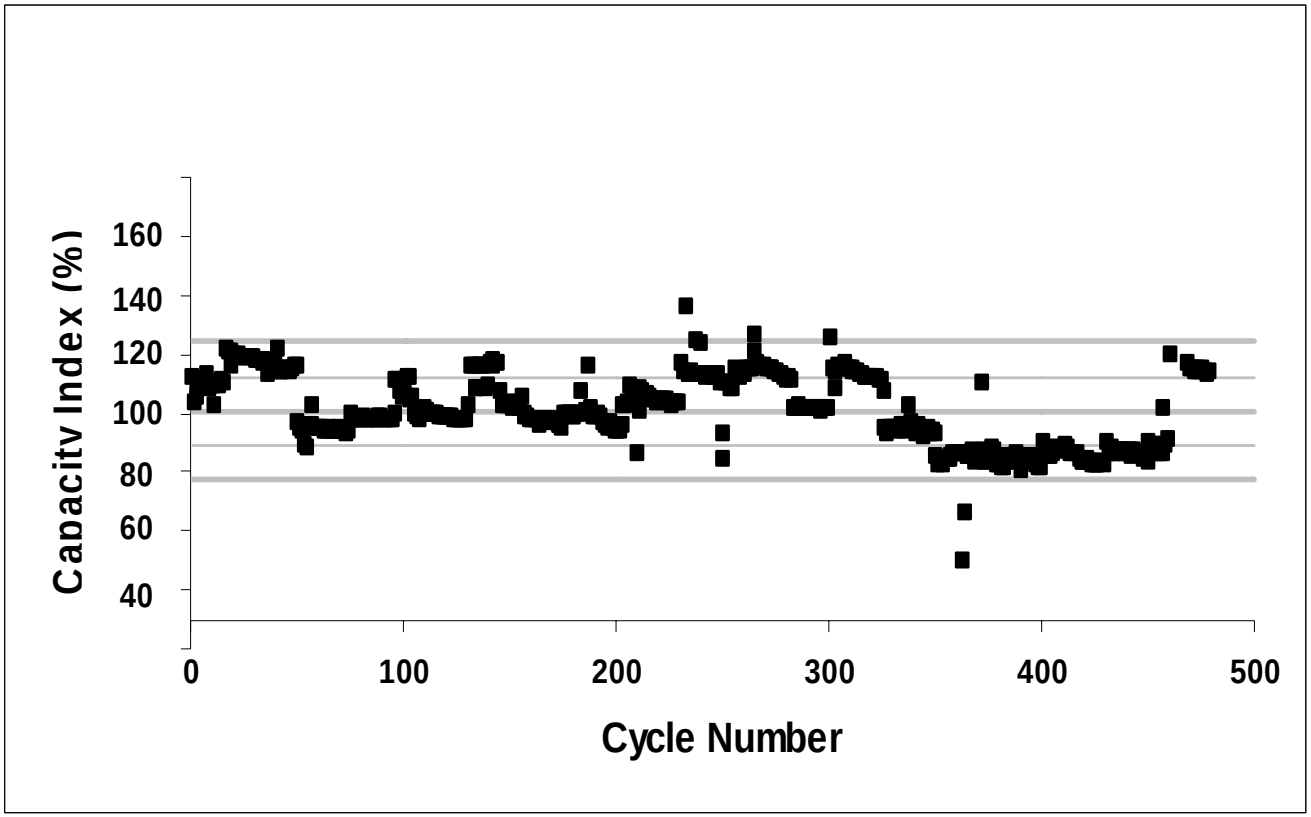
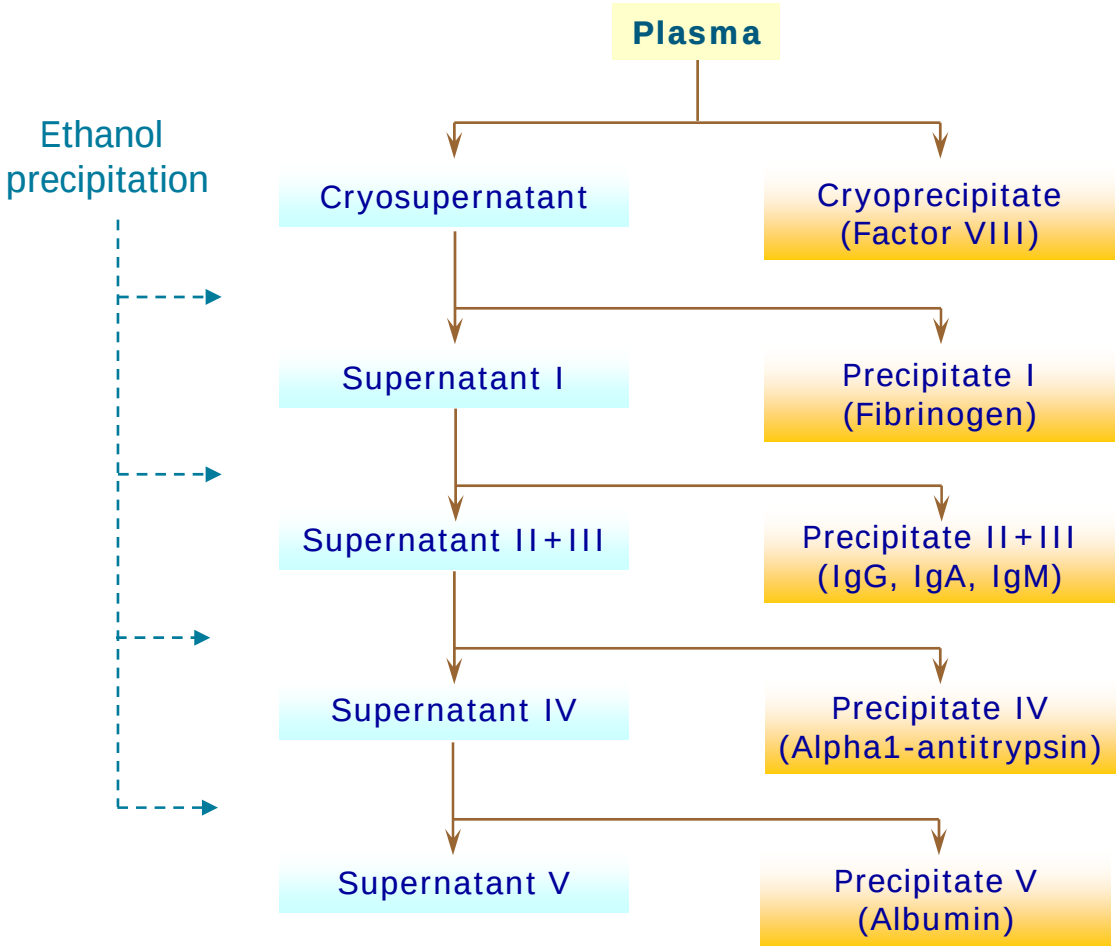
Advantages of Target Selective chromatography



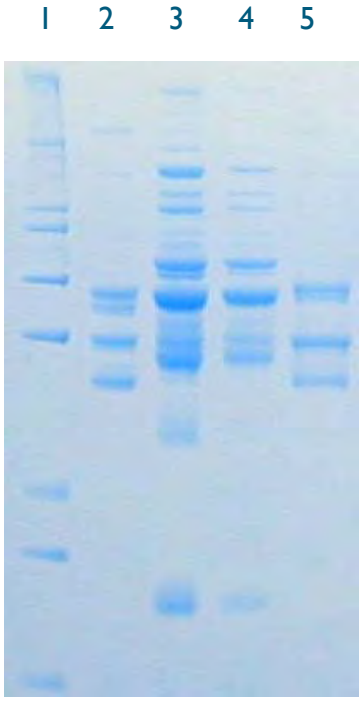
PROMETIC BIOSCIENCES

Basic plasma protein fractionation scheme using the Cohn process (1946)



PROMETIC BIOSCIENCES

Affinity Purification of Fibrinogen



Lane	Loading
1	MW Marker
2	Fibrinogen Standard
3	Plasma (HSA reduced)
4	Flow through
5	Fibrinogen Elution

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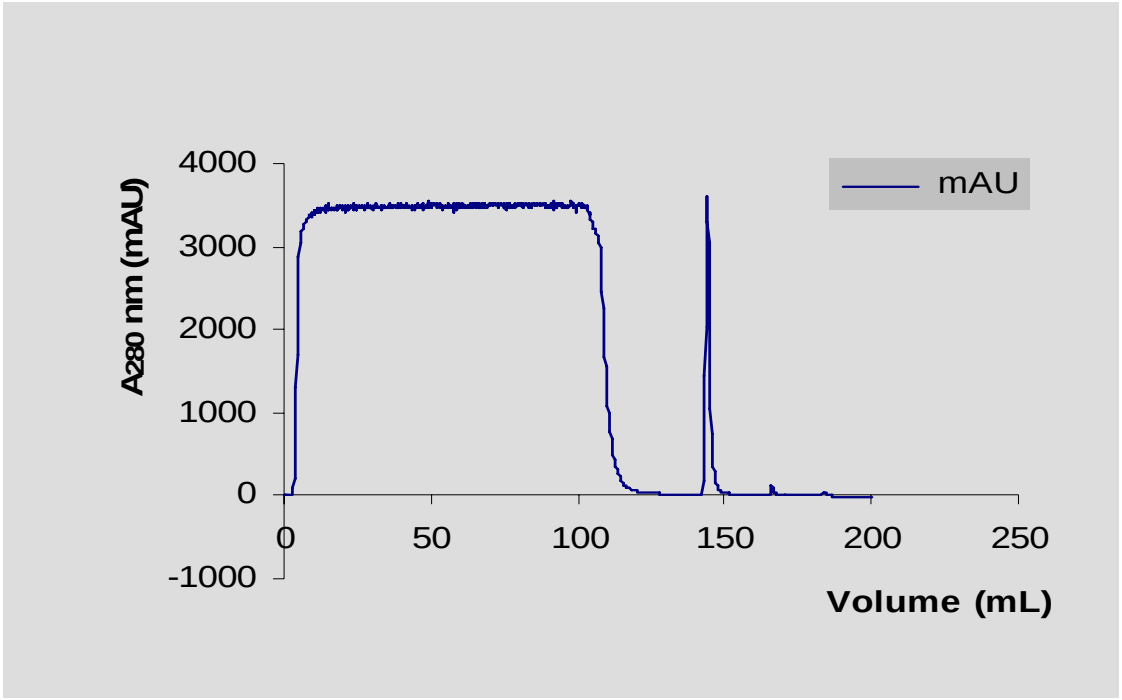
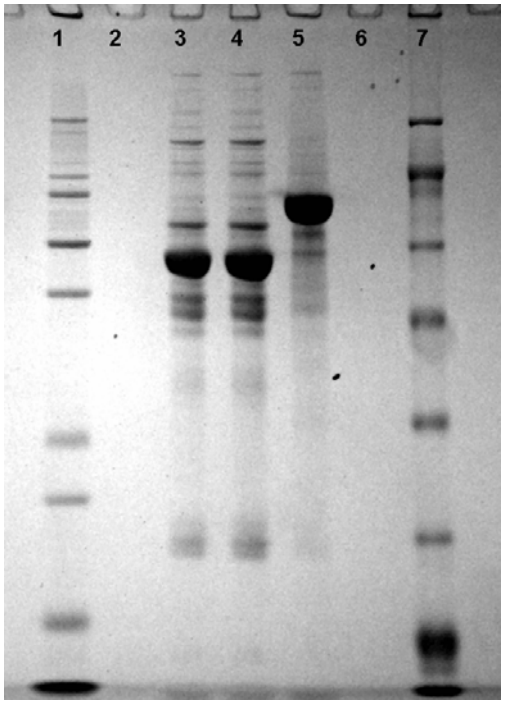
Capture Purification – Plasminogen





4 mL column
Linear flow rate: 50cm/hour
Equilibration buffer: 50mM Sodium Phosphate pH 7.5 (5CV)
Load: 100 mL filtered human plasma
Post load wash buffer: 50mM Sodium Phosphate pH 7.5 (10CV)
Elution buffer: pH 7.5 (5CV)

Lane 1: MW Marker
Lane 2
Lane 3: Human plasma load
Lane 4: Flow through fraction
Lane 5: Elution fraction.
Lane 6:
Lane 7: MW Marker

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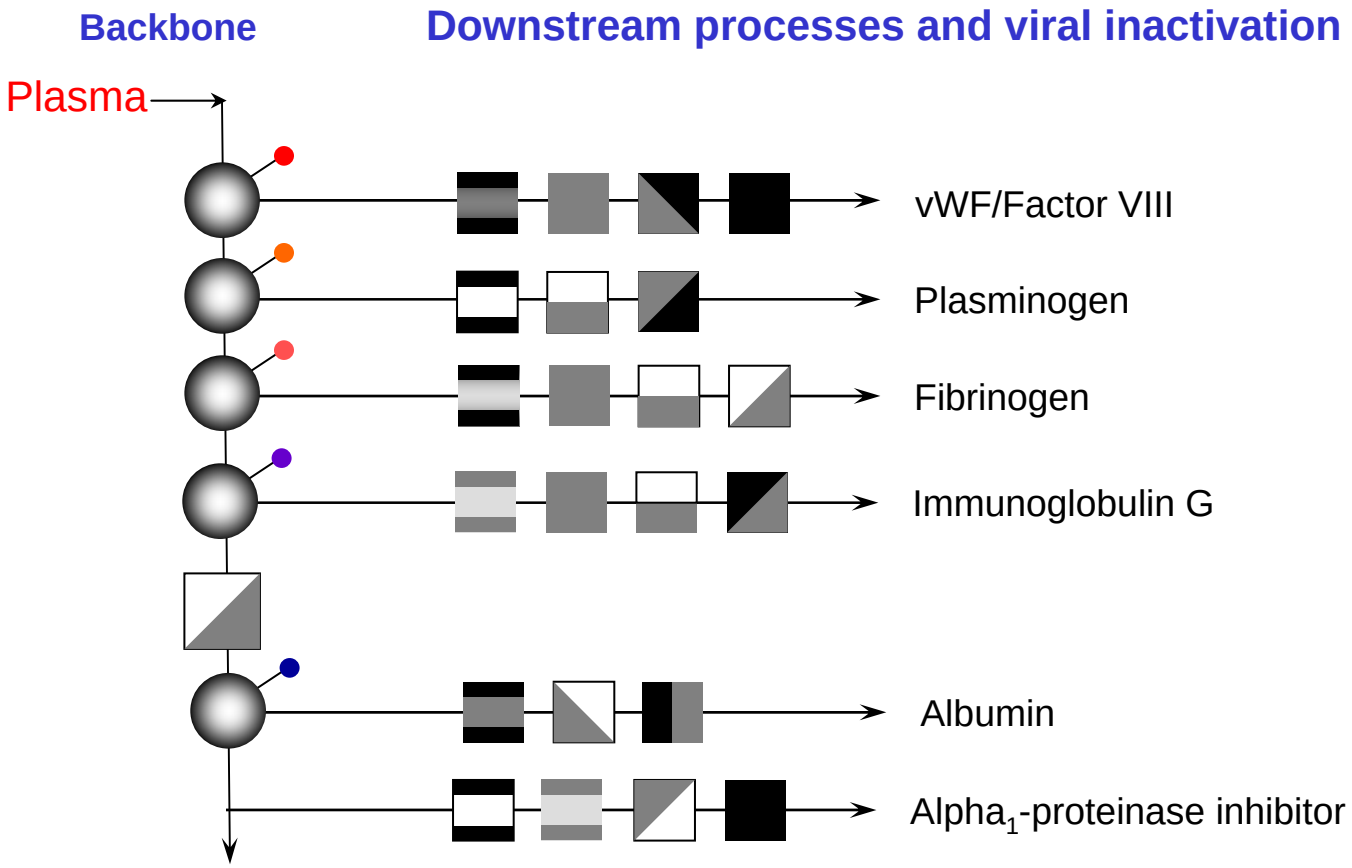
ProMetic-ARC New Fractionation Process



American Red Cross

ProMetic BioSciences

Mimetic™ ligands developed

Target Biomolecule

- Albumin
- IgG
- Insulin
- Factor VII
- Factor VIII
- Alpha-1-Antitrypsin
- Fibrinogen
- Plasminogen
- tPA
- tPA-Urokinase
- Alkaline Phosphatase
- Endotoxin
- Prions

ProMetic BioSciences

Benefits of ProMetic Approach

- Screening performed with actual feed-stock.
- Screening performed with synthetic (Mimetic Ligands™) firmly attached to a high-flow chromatographic matrix.
- Adsorbents used for screening can be scaled-up and used in manufacture with little or no modifications.
- Early availability of adsorbent samples for in-house evaluations by sponsor/end-user.
- Bulk quantities of “cGMP” grade adsorbent produced as an integral part of the development programme.
- Synthetic ligands – inexpensive; no materials of animal origin.
- Ligand screening, adsorbent development and adsorbent manufacture performed by same company.
- Short development times; modular approach.
- Proven technology.

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Manufacturing Validation & Scale-up

- ISO 9001:2000
- “cGMP” standard manufacture
- Class 100,000, 10,000 & 100 areas
- Drug Master files/regulatory support files
- Single batch sizes to 250 litres

(Applies to entire UK operations)

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ProMetic

more of what you want
less of what you don't