Antibody Purification Made Easy – CaptureSelect® Affinity Products and Services

Remko Clasen, Business Development
BAC BV – History

**Organizational milestones**

- Setup business supported by Unilever Ventures
- External private financing; formal spin-out out of Unilever
- Closure of series B financing round
- Closure of series C financing round
- Acquisition of BAC BV by Life Technologies

**Bioprocess**

- GEHC umbrella agreement
- Launched 9 bioprocess products
- Products used for production of clinical and commercial biologicals

**Service**

- First Custom Project
- Executed > 15 custom development projects
- Successfully performed, media developed, licenses closed

**LSR tools**

- Web-shop direct customer sales
- Launched a portfolio of 5 different product categories > 30 different products

**Commercial milestones**

- 2002
- 2003
- 2004
- 2005
- 2006
- 2007
- 2008
- 2009
- 2010
- 2011
- 2012
- 2013
CaptureSelect® Affinity Products

**LIGAND**

Single-domain [VHH] antibody fragments  
Highly selective  
Produced in yeast (*S. cerevisiae*)

**COUPLING**

Coupling to solid support

**PRINCIPLE**

Immobilized Ligand + Impurities  
Purification target  
Complex  
Impurities  
Purified sample

**PRODUCT**

CaptureSelect® products enable increased purity and yield, lower cost of goods and reduced time-to-market for end-users  
- R&D, >30 products (>250 customers)  
- Biopharmaceutical manufacturing and Process Development, 9 products (>150 customers) through partners
CaptureSelect® Technology Platform

1) Discovery of VHH binding fragments
   - Immunize
   - Construct expression libraries

2) Screen operating conditions
   - Test for binding and elution
   - Determine stability

3) Matrix testing
   - Top candidates are cloned into Yeast
   - Evaluate process conditions in small scale columns

4) Ligand production
   - Pick lead candidate
   - Produce in-house at large scale

5) Affinity product
   - R&D purposes
   - Clinical & commercial manufacturing
CaptureSelect® Services and Products

Custom Ligand Projects
- Service for development of product specific affinity solutions
- Products tailored to customer’s products and process needs
  - >15 successful programs run

Bioprocess Products
Products for clinical and full scale manufacturing
- 9 products developed with partners, 1 in marketed process
Custom Project Needs

- Increase yield / purity
- Circumvent IP
- Reduce the number of steps in a process
- Offer platform approach for purification/detection
- No generic purification strategy
- Enabling technology
- Selective removal of unwanted compounds
CaptureSelect® Custom Ligand Service Project from Target to Product

*WP = work package
FVIII Custom Development Project

- Initiated in 2006 with Bayer Healthcare to develop a manufacturing process for next generation Kogenate® (recombinant factor VIII)

- Following completion of the program Bayer was not ready for implementation of the newly developed resin

- Entered into collaboration with GEHC to further develop VIIISelect chromatography media
  - now widely used by customers producing recombinant factor VIII
    > Biogen, CSL, Octapharma, Minapharm, CMC, Gallus
WP1: Immunization & library construction

- Immunize llama with Kogenate®
  - Follow immune response
- Construct ligand expression libraries for WP2

Lama Immune Response against Target

Target reactive VHH Expression Libraries
FVIII Custom Development Project

WP2: Screening operating conditions

- Library screening at monoclonal level on application conditions
- Based on customer product/process needs

- Binding to Target (no binding to contaminants, HCPs)
- Broad / Narrow Specificity (e.g. species specific)
- Binding & Elution Conditions (mild)
- Ligand Stability (acidic / caustic cleaning)

Capture-ELISA

Biacore / Octet (binding kinetics)
FVIII Custom Development Project

WP3: Matrix testing

- Cloning into yeast expression system
- Small scale affinity chromatography under process conditions

Column: Tricorn 5/100, 2 cm bed height
Ligand density: 2.5 mg/ml
Flow rate: 150 cm/h
FVIII Custom Development Project

WP4: Lead selection

- Production of lead candidates at larger scale (10L fermentation) to produce sufficient material for testing
- Evaluate ligand candidates
  - Rank
  - Pick lead candidate
- Deliverable
  - Research purification resin
  - Analytical tools
    > POROS analytical column
    > Conjugated ligands
FVIII Custom Development Project

WP5: Develop and Scale Affinity Purification Product

- Ligand upscaling
  - Naarden, The Netherlands

- Yeast based production
  - No animal derived components
  - ISO9001
  - Audited by end customers

- Resin upscaling

- RSF
  - Document with regulatory support needed for filing

- Deliverable
  - cGMP compatible affinity resin
Case Study: Purification of rh-FVIII

Application of a novel affinity adsorbent for the capture and purification of recombinant Factor VIII compounds

Justin T. McCue*, Keith Selvitelli, Joshua Walker

Biogen Idec Corporation, Bioprocess Development, 14 Cambridge Center, Cambridge, MA 02142, USA

Effective impurity clearance:

- HCP clearance: ~ 3 logs
- DNA clearance: ~ 5 logs
- Robust viral clearance (model viruses):
  - enveloped (XMuLV): ~ 4 logs
  - non-enveloped (MMV): > 5 logs

VIIISelect currently incorporated in rhFVIII production process
Targeting Free Light Chains

- FLC specific affinity ligands useful for:
  - Detection and quantitation of free LC’s in antibody preps / culture supernatants
    - screening on properly expressed Ab formats
    - monitoring stability during DSP
    - analyzing % FLC in intermediate / final products (QA/QC)
  - Scavenging of free LC impurities

- Preferred selectivity through epitope presented on CL domain (identical for all kappa or lambda Fab fragments)
  - epitope should be masked by CH1 in intact Fab fragments / IgG &
  - epitope should still be accessible in FLC dimers
Targeting Free Light Chains: Biacore

- Proof of concept demonstrated in Biacore:
  - Biotin conjugated ligands immobilized on SA sensor chips
  - Analytes:
    - intact human IgG Fab (polyclonal; Jackson ImmunoResearch): 10 ug/ml in HBS-EP
    - Bence Jones kappa proteins (FLC-kappa; Biodesign): 10 ug/ml in HBS-EP

Unique selectivity’s for Human Fab/IgG analysis
3/4 Mab Contaminant scavenging (bi-specific Mab platform)

- Source: mammalian
- Selective binding to free CL-kappa
- Regeneration: pH 2

WP-1 - Immunization - Library

WP-2 - Lib Screen

WP-3 - Matrix screen

WP-4 - Lead selection

- 4 Ligand candidates selected and sampled to Roche (WK51-12)
  - Affinity Resins
  - Biotin conjugates
Bioprocess Products
CaptureSelect® Affinity Products
A platform for purification of all biologics

- **Selectivity**
  - high purity in single step / feed stock independent

- **Mild elution conditions**
  - retaining biological activity of target

- **Reduction of process steps**
  - higher yields, reduced costs

- **Efficient clearance of HCP, DNA, virus**
  - high selectivity in capture step

Increase purity / yield, lower Cost of Goods and reduce time-to-market
Bioprocess Products
Resins launched since 2006 in collaboration with GEHC

Antibodies and antibody fragments
- IgSelect
- KappaSelect
- LambdaFabSelect

Recombinant and Plasma Proteins
- VIIISelect
- Alpha-1 Antitrypsin Select
- VIISelect
- GCSF Select
- IXSelect

Vaccines and Viruses
- AVB Sepharose High Performance

All launched products are suitable for clinical grade and final manufacturing of therapeutic products and do have a GEHC Regulatory Support File.
Human Fab Fragments

Capto L
Binding to human VL-κ1, 3 and 4
no binding to VL-κ2 or any VL-λ
- no platform for all Fabs

KappaSelect / LambdaFabSelect
- all human isotypes and subclasses
- whole IgG, M, A, D and E
- human Fab fragments
- platform for all Fabs
Fab Purification through targeting CL domain (KappaSelect / LambdaFabSelect)

Efficient single-step purification of human Fab-κ fragments from \textit{E.coli} feed stock (spiked)

**Column**: 400 µl KappaSelect (Tricorn) 2 cm bed height  
**Binding and Wash**: PBS pH 7.4  
**Sample**: 5 ml periplasmatic fraction spiked with 0.4 mg Fab’s/ml  
**Elution buffer**: 0.1 M glycine pH 2.0 (Neutralized 1M Tris pH 8.0)  
**Linear flow rate**: 150 cm/h

![Red line: OD280 nm, Green line: % buffer B, Gray line: pH](image)

- [Image of gel electrophoresis](image)
  - M: BenchMark pre-stained Protein ladder
  - 1. Fabs (human polyclonal Fab kappa, Bethyl (P80-315))
  - 2. Periplasmatic fraction of \textit{E.coli}
  - 3. Periplasmatic fraction spiked with 0.4 mg Fab/ml
  - 4. Flow through fraction KappaSelect column
  - 5. Elution fraction KappaSelect column

![Diagram of Fab purification](image)
Targeting LC-κ/λ: Fab analytics (HPLC)

POROS® CaptureSelect® resins exhibit high speed and efficient chromatography, irrespective of type of Kappa light chain.

POROS® CaptureSelect® LC Kappa columns exhibit good linear range (8X) and linear correlation coefficients ($R^2$ range: 1 to 0.9991)

POROS® CaptureSelect® Affinity Columns deliver precise, rapid results
Research Products: Unique Selectivities
CaptureSelect® Research Products

**Antibody Toolbox®**
Unique set of products for antibody and antibody Fragment purification

**Proteomics Toolbox®**
Single-step protein depletion products used for biomarker discovery, clinical diagnostics and sample preparation

**Protein Purification**
Biopharmaceutical purification
- CaptureSelect® C-tag
  - Purification platform for C-tagged proteins

**Ligand Conjugates**
CaptureSelect® ligands chemically conjugated to biotin for use in analytical assays
Human IgM and IgA purification

CaptureSelect® IgM affinity matrix

1 Human serum  2 Flow-through  3 Elution  M Marker

Binds human IgM, μ chain
Binds rat and mouse IgM
No binding to:
- human IgG, IgA
- bovine IgM

CaptureSelect® IgA affinity matrix

1 Human serum  2 Flow-through  3 Elution  M Marker

M 1 2 3

Binds human IgA, α chain
No binding to:
- human IgG, IgM
- IgA from other species

Ligands and resin characteristics:
• High IgM / IgA purity in a one-step process
• Mild elution conditions between pH 3 and 4
CaptureSelect® IgG-CH1 Ligand
A platform for human antibodies and fragments thereof

Anti IgG-CH1
- all human IgG subclasses
- independent of light chain
- independent of Fv
- no binding to free light chains (FLC)

100 % coverage of any human Ig and Fab fragments thereof
Targeting IgG-CH1 domain of Fab

- Detection and purification of human Fab fragments often hampered by the presence of free light chains (FLC’s)
  - anti human LC affinity ligands, like Protein L, do not discriminate between intact Fab and FLC

No cross-binding observed for FLC’s (monomers and dimers) when targeting IgG-CH1 domain
Targeting IgG-CH1 domain: Fab purification

- Separation of FLC’s from intact human Fab fragments on IgG-CH1 affinity resin
  - batch wise / spin columns (Mobitec)

**Load (100 μl resin / load)**

1. **Intact human IgG Fab**
   (polyclonal; Jackson Immunoresearch)
   100 μg in 200 μl PBS

2. **FLC’s kappa**
   (Bence Jones kappa; Biodesign)
   100 μg in 200 μl PBS

3. **Intact Fab + FLC’s kappa**
   2x 100 μg in 200 μl PBS

**Load** | **Flow through** | **Elution**
--- | --- | ---
![Load](image1.png) | ![Flow through](image2.png) | ![Elution](image3.png)

Generic tool for any human IgG derived Fab fragment