

# Protein Purification & Recovery

## TOOLS & TECHNOLOGIES





## CaptureSelect® Affinity Ligands

A Purification Platform for Biotherapeutic Proteins, Fusions and Antibody Constructs

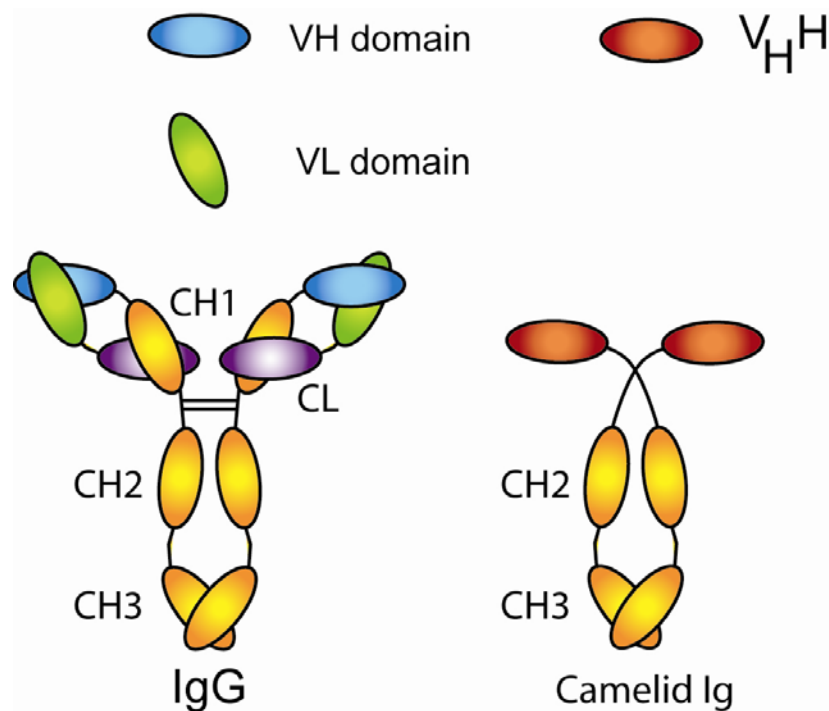
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- ⌚ Biotherapeutics fast growing area
  
- ⌚ Challenges in manufacturing have moved from upstream to down-stream
  - Purity is key for regulatory approval
  - Complex media
  - Not correctly processed forms, aggregates, instability
  - No general purification strategies: multi-step decreases yield
  - Time lost during process development
  - Manufacturing with high yield becomes key for biosimilars
  
- ⌚ BAC has a unique technology, multiple products and a rapidly expanding pipeline, which addresses the above needs

~ CaptureSelect® Affinity Ligands use the Uniqueness of VHH antibody fragments



## Advantages VHH:

- ~ 12-15 kDa fragment (~1/10th mAb)
- ~ Tunable specificity: Broad / Narrow
- ~ Screening Operating Conditions
  - Binding / Elution
  - CIP stability
- ~ Suitable for both scavenging and purification
- ~ No animal-derived components
  - Production in Yeast
- ~ Ability to work on any Solid Support

# CaptureSelect® – Key Advantages



## 1 Selectivity

- high purity in single step / feed stock independent



## 2 Mild elution conditions

- retaining biological activity of target



## 3 Reduction of process steps

- higher yields, reduced costs

## 4 Efficient clearance of HCP, DNA, virus

- high selectivity in capture step



## 5 A platform for downstream processing of biopharmaceuticals

- generic process: e.g. 1st capture – polishing - viral filtration – UF/DF (Prot-A)

Increase purity/yield, lower cost of goods and reduce time-to-market

# 1 Selectivity (antibodies)

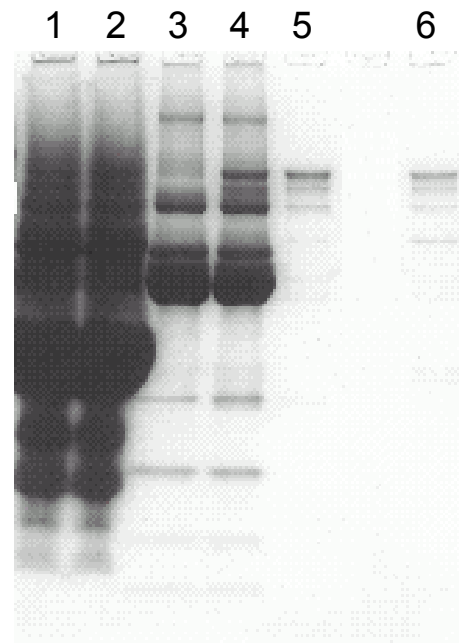


## ~ Purification of human IgG1-4 from different feed streams

- Cell culture (rec Mabs)
- Transgenic

### IgSelect Properties

- Human IgG-1 to 4
- Human specific
- Mild elution (pH 4.5)
- Acid / Caustic stable
- 20 – 25 mg/ml DBC



- 1 Cow milk
- 2 Cow milk spiked with Human IgG
- 3 Tissue culture medium
- 4 Tissue culture medium spiked with Human IgG
- 5 Elution fraction from Cow milk
- 6 Elution fraction from Tissue culture medium

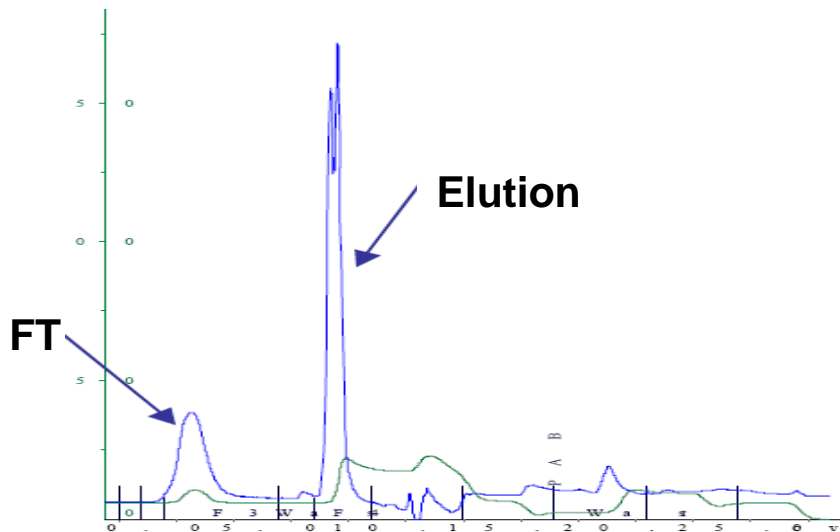


# 1 Selectivity (vaccines)

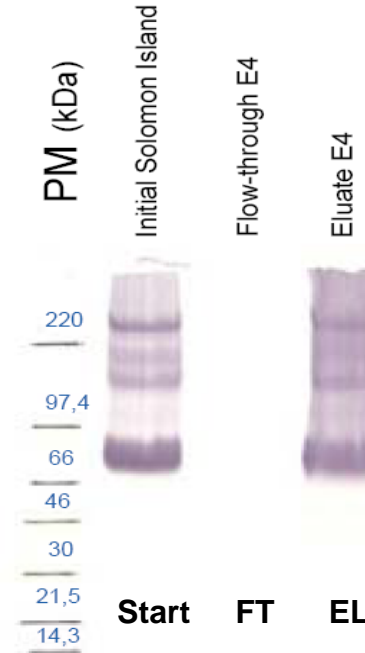


~ Affinity purification of Influenza A viruses (whole particles) from clarified concentrated harvest on resin prototype

## A: H1N1 Solomon Island

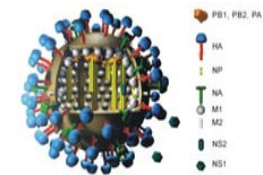


## western blot



Broad reactivity demonstrated with:

- A:H1N1 Solomon Island
- A:H1N1 Brisbane
- A:H3N2 Wisconsin
- A:H3N2 Uruguay

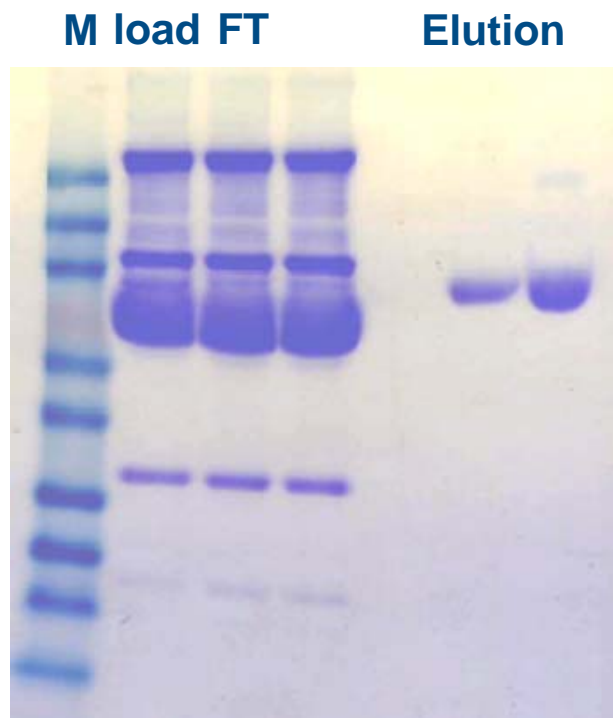


Anti-Flu Ligand	A: H1N1 Solomon virus	A: H1N1 Brisbane virus	A: H1N1 Brisbane HA	H3N2 Wiscon virus	H3N2* Wiscon HA	B Malaysia virus	B Florida virus	B Brisbane HA
A1	+++	++	++	+	++	-	-	-
A2	+++	++	++	+	++	-	-	-
A3	+++	++	++	+	++	-	-	-
A4	+++	++	++	+	++	-	-	-

## 2 Mild Elution Conditions



Efficient recovery of  $\alpha$ 1-antitrypsin (AAT) under neutral conditions



Target Molecule	MgCl <sub>2</sub> pH 7	50% (v/v) Propylene glycol pH 7	Arginine pH 6-8	Glycine pH 4.5	Glycine pH 3
Human IgG	-	ND	ND	++	++
Factor VIII	-	++	+	+/-	++
Human Albumin/ Albumin fusions	++	++	+	+/-	++
$\alpha$ -1 anti trypsin	++	-	+	+/-	++
Transferrin/ Transferrin fusions	ND	++	ND	+/-	++
Fibrinogen	+	++	ND	-	++

Elution Buffer:  
20 mM Tris, 2M MgCl<sub>2</sub>, pH 7

++ Elution 85-100% ; + Elution >75%; +/- Elution >60%;  
- no efficient elution; ND not determined

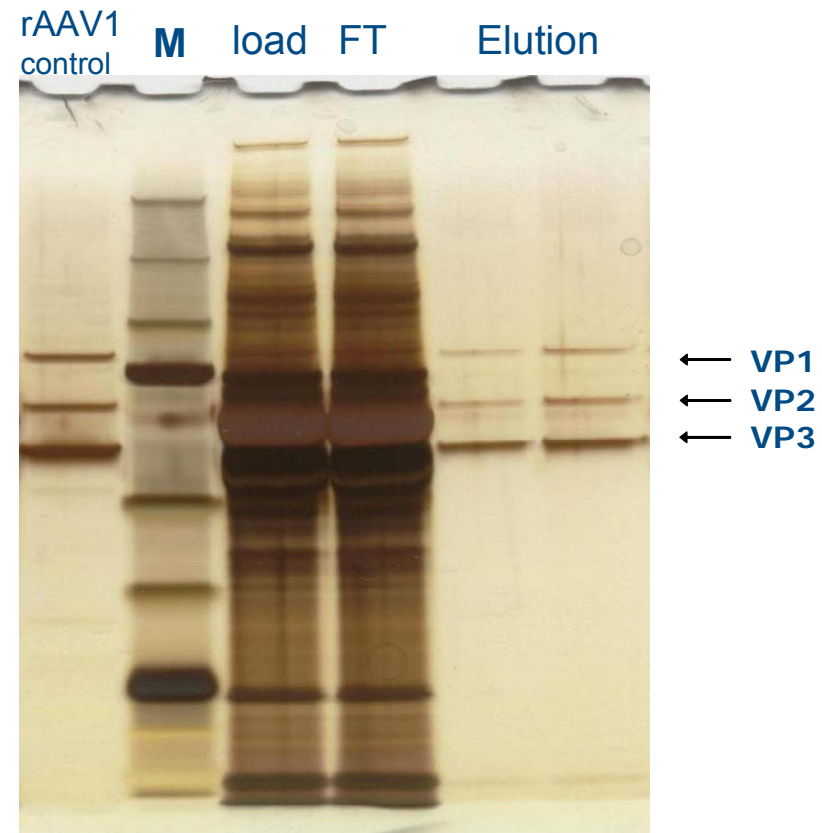


### 3 Reduction of Process Steps



#### ~ Affinity Purification of Adeno Associated Virus

- ~ BAC successfully identified ligands for purification of AAV particles
- ~ BAC was able to change a complex multi-step process into a high yield, high purity, 2-step purification run
- ~ Now widely distributed by GE under the name “AVB Sepharose High Performance”



## 4 Efficient clearance of HCP, DNA, virus



### Purification of recombinant B domain deleted FVIII from feedstock

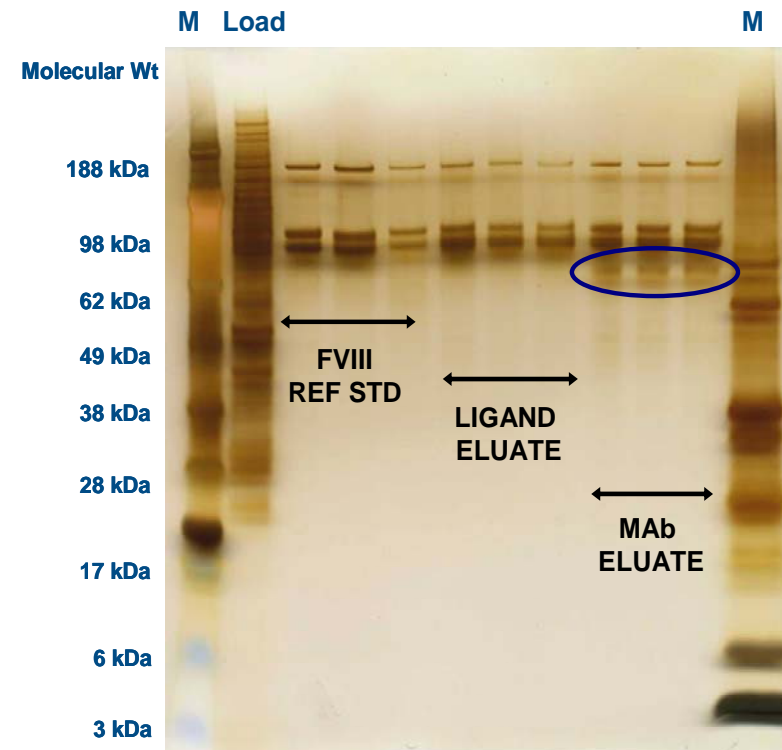
biogen idec

(Justin T. McCue, J. of Chrom A, 2009)

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**

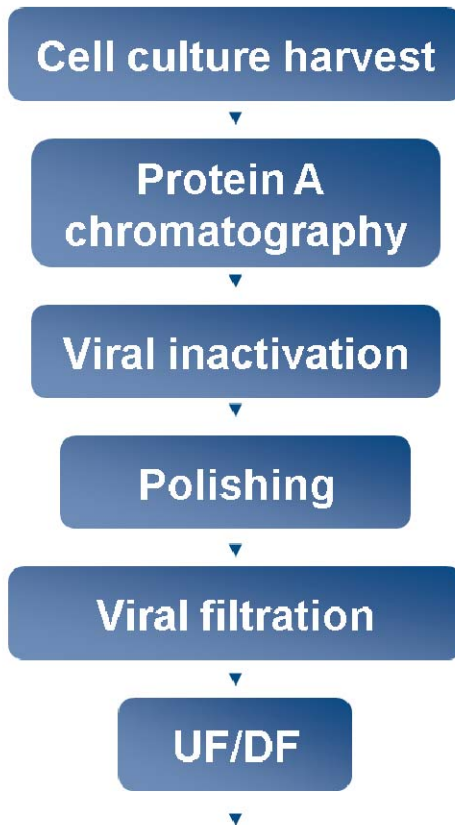
VIIISelect currently incorporated  
in rhFVIII production process



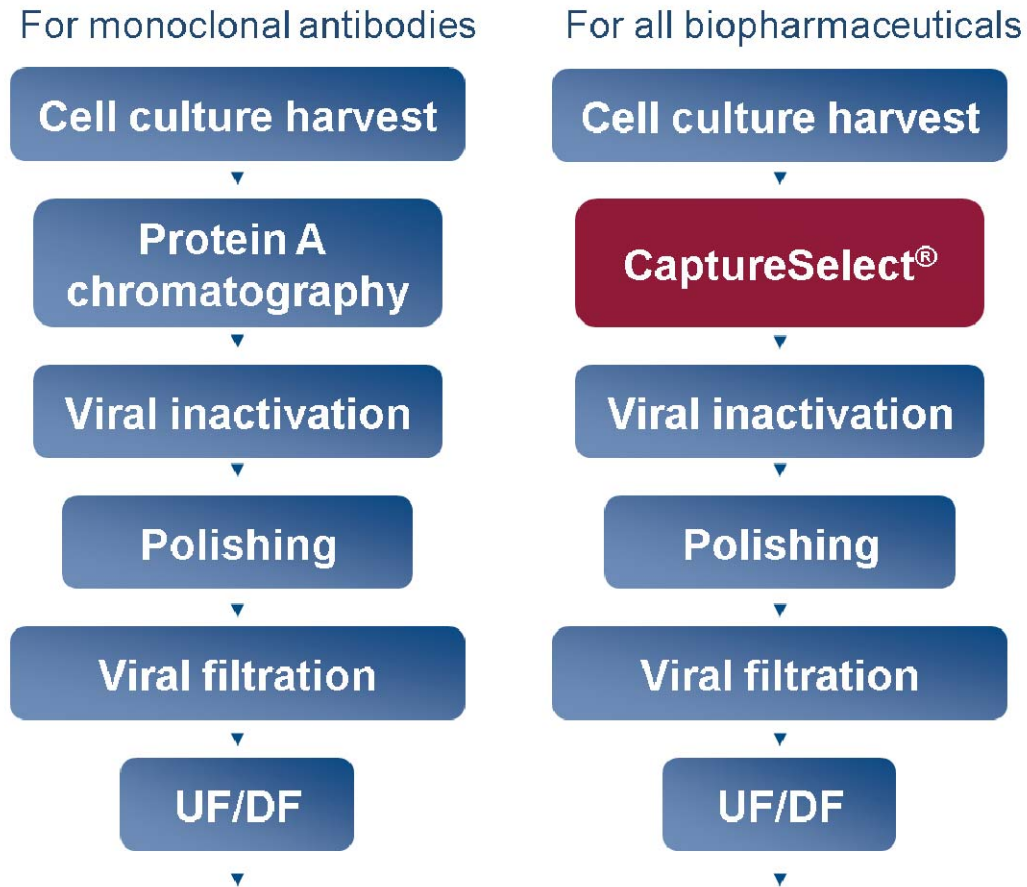
## 5 A Platform Approach to Purification



For monoclonal antibodies



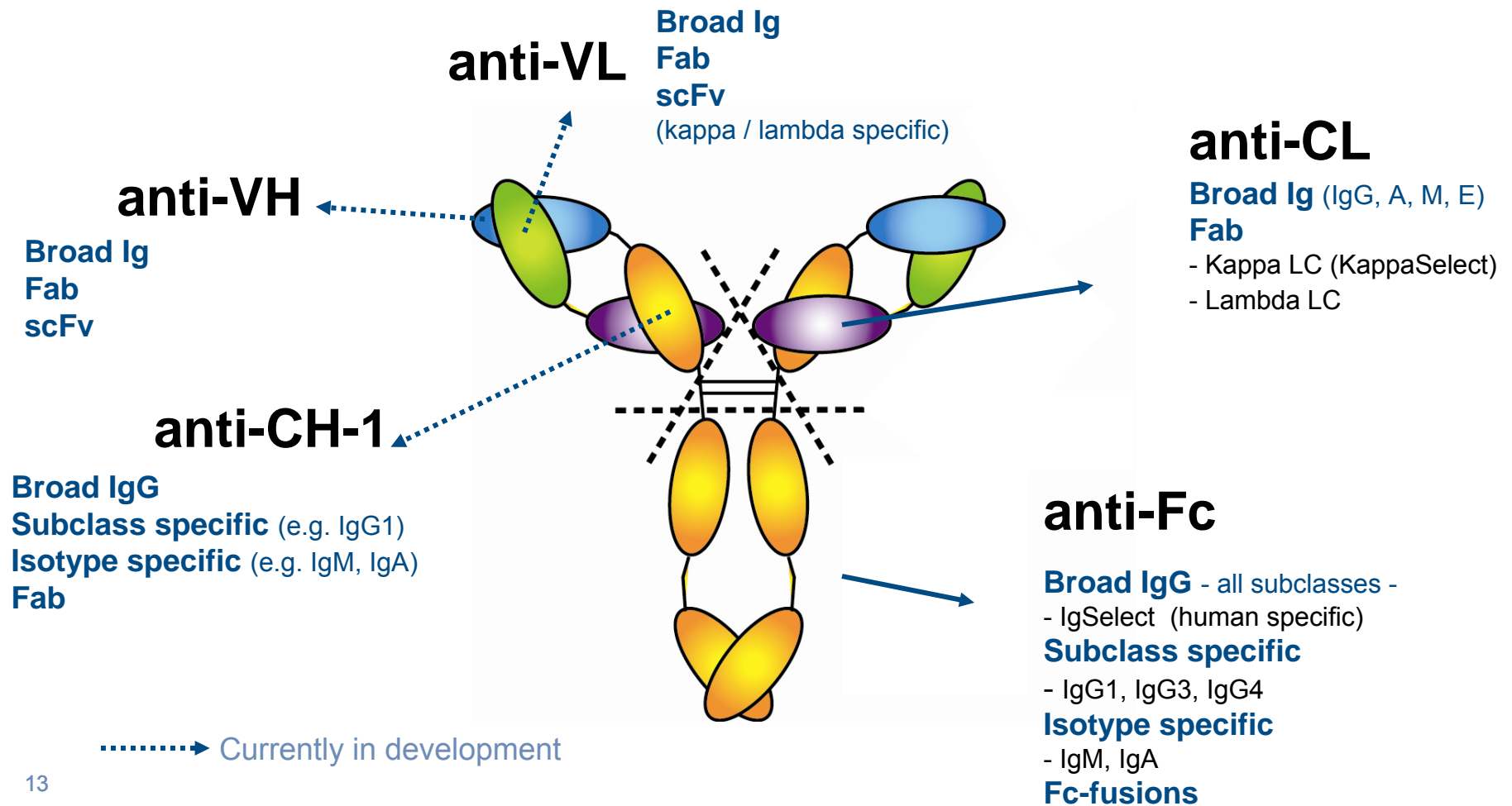
## 5 A Platform Approach to Purification



# Next Generation Antibody Purification



Unique specificities by targeting different antibody domains



## Biopharma Manufacturing

### Multi-Customer Ligands

- IgG
- Fab's
- AAV
- recFactor VIII

### Custom ligands

- Recombinant proteins
- Vaccines
- Plasma proteins



GE Healthcare

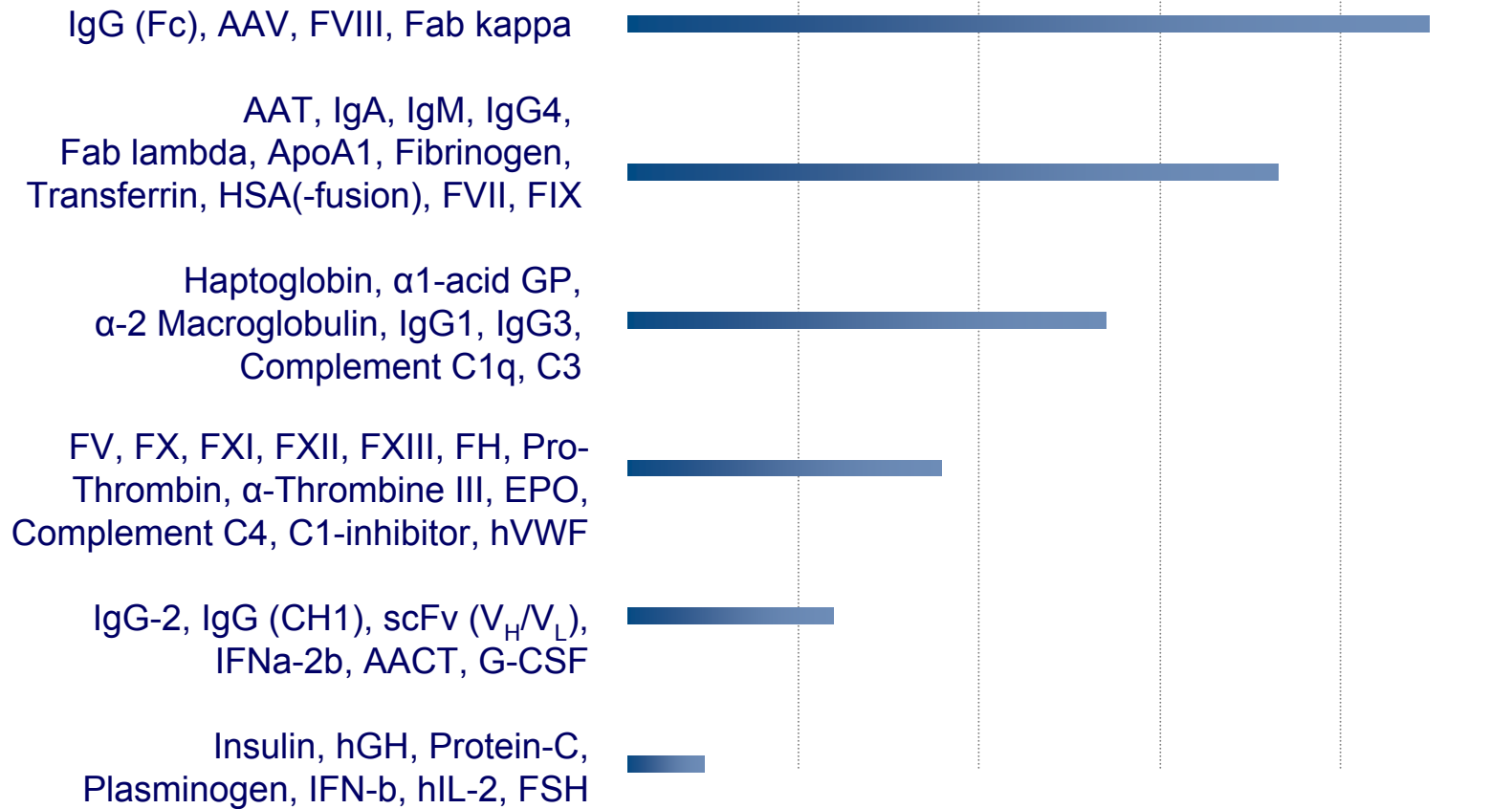
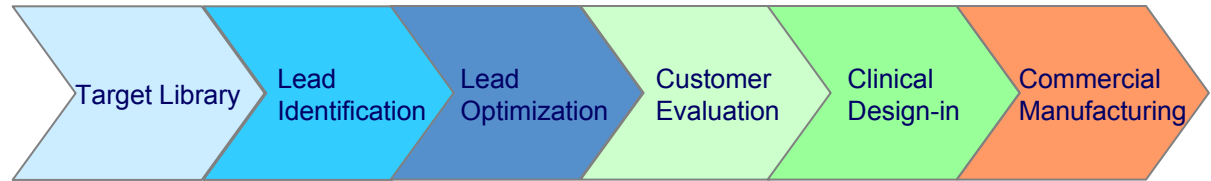
- IgSelect
- AVB Sepharose HP
- VIIISelect
- KappaSelect
- AAT
- *FIX / FVII*

→ End-users



Bayer HealthCare  
Biological Products Division

# BAC's Pipeline



- BAC's CaptureSelect technology has been used successfully:
- in obtaining suitable affinity ligands for the purification of antibodies, antibody fragments, viruses, plasma - and recombinant proteins for both R&D and large scale Bioprocess applications
  - in screening for highly specific elution conditions, facilitating further purification or to increase product stability during down-stream processing
  - being incorporated in multiple processes for clinical trial manufacturing



The **Affinity** Experts

Thanks for your attention !

Visit us at:

**Booth 38**

<http://www.bac.nl>

<http://www.captureselect.com>