

Vaccines *Europe*

Conference
3 Vaccine Scale-Up
and Manufacturing



The **Affinity** Experts

CaptureSelect® Affinity Ligands
- Future Tool in Vaccine Manufacturing -

Pim Hermans, Director Ligand Discovery, BAC BV

info@bac.nl

www.bac.nl

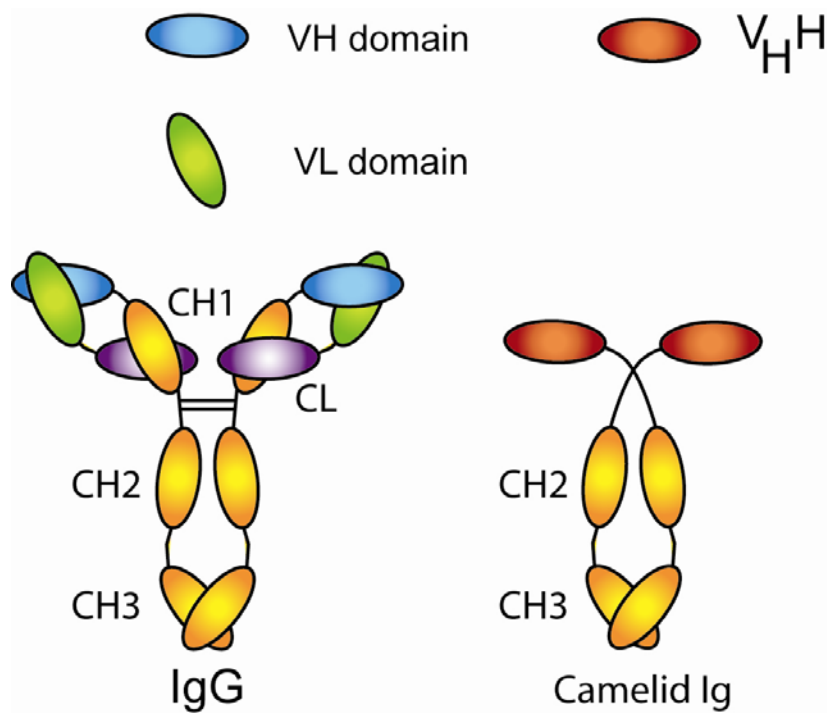


- ⌚ Biotherapeutics fast growing area

- ⌚ 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
- ~ Suitable for both scavenging and purification
- ~ No animal-derived components

CaptureSelect® – Key Advantages



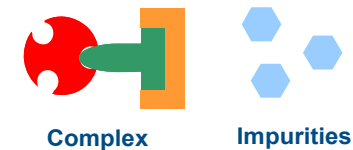
Selectivity

- high purity in single step / feed stock independent



Mild elution conditions

- retaining biological activity of target



Reduction of process steps

- higher yields, reduced costs

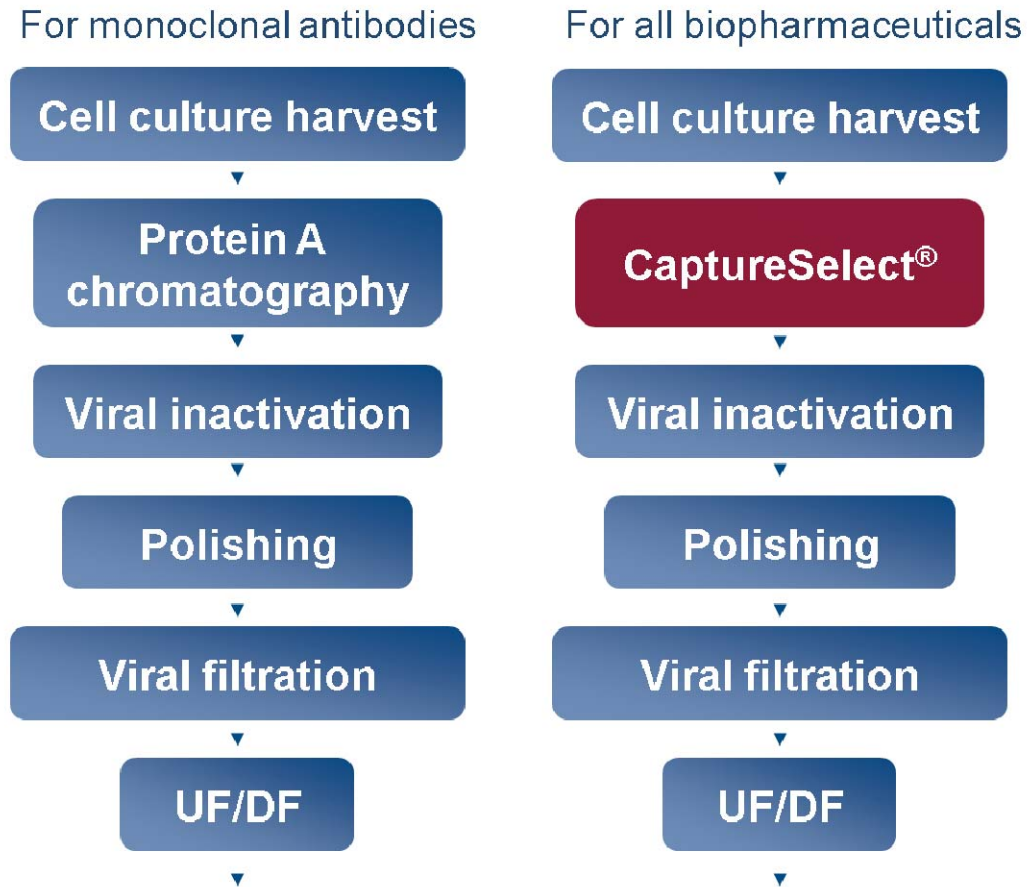
Efficient clearance of HCP, DNA, virus

- high selectivity in capture step



Increase purity/yield, lower Cost of Goods and reduce time-to-market

A Platform Approach to Purification

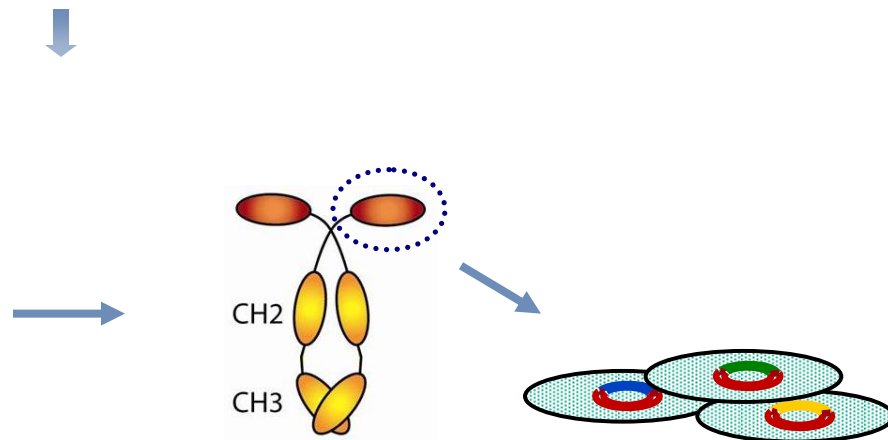
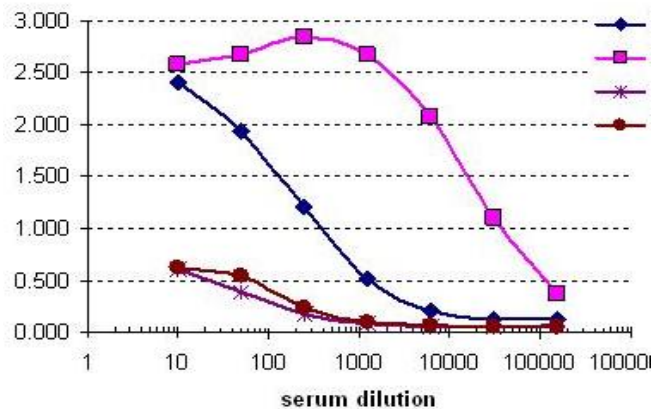


From Target to Lead Ligand (phase 1)

I TARGET LIBRARY

Immunization with target
Construction ligand expression libraries

~ 3 months



• Lama Immune Response against Target

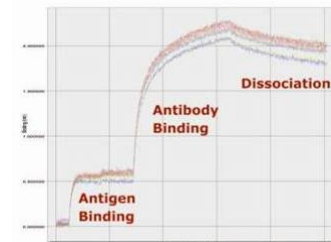
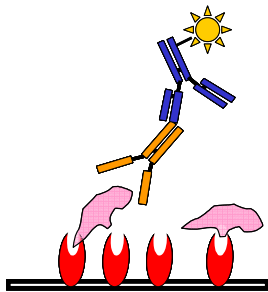
• Target reactive VHH Expression Libraries

From Target to Lead Ligand (phase 1)

II LIBRARY SCREENING

Library screening at monoclonal level on application conditions

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



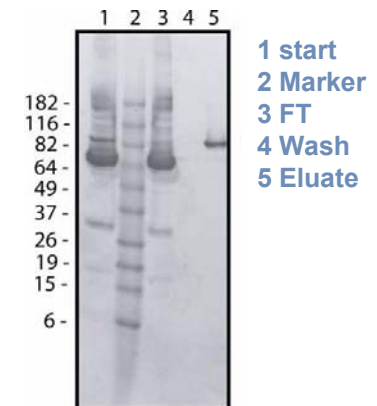
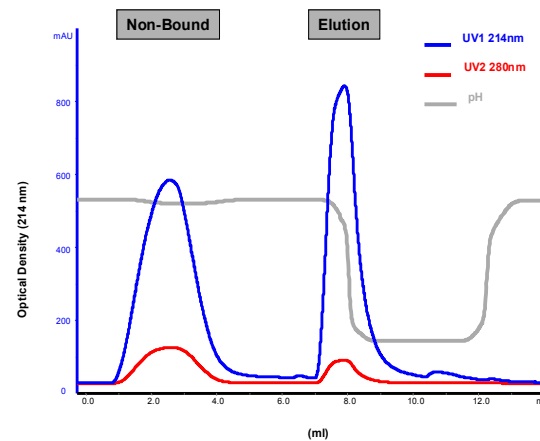
From Target to Lead Ligand (phase 1)

III LEAD IDENTIFICATION

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



Lead ligand

Production

Fermentation



Biomass Removal & Concentration

Microfiltration & Ultrafiltration



Purification

Chromatography



- ~ Microbial production of the ligands using Baker's yeast
 - Quick discovery and scale up, non animal derived sourcing
- ~ ISO9001
- ~ Audited by GEHC and end-customers

Phase-1

Library construction
Library Screening
Lead Identification



~ 8 months

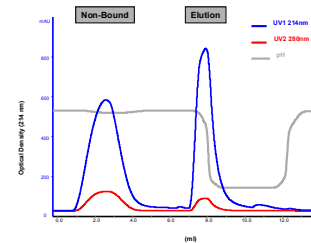
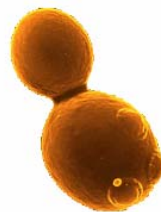
Phase-2

Product Development



Strategic Partnerships

> 20 Patents and licenses





Life Sciences Research

Proteomics
Pre-purification

Separation
Antibody Toolbox

BAC products incorporated in all major supplier kits for proteomics for sample treatment of plasma in drug discovery

Webshop for immobilized finished products

Antibody Toolbox™ Media
Leakage ELISA

- Support for bioprocess products



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

→ End-users



sanofi pasteur
The vaccines business of sanofi-aventis Group



octapharma



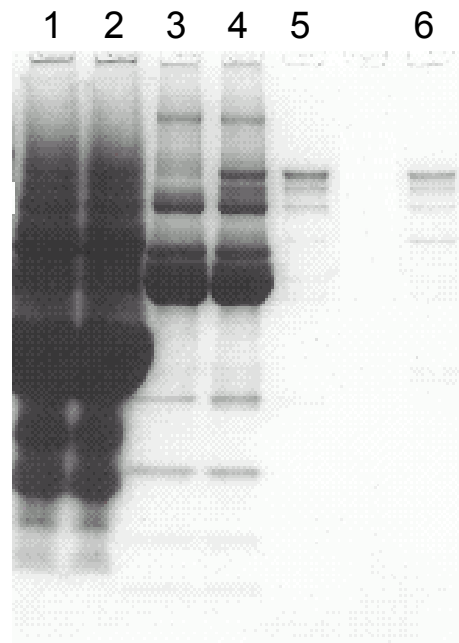
Bayer HealthCare
Biological Products Division

~ 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
- 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

Mild Elution Conditions



Improved recovery of alpha-1 antitrypsin (AAT) under neutral conditions



>95% elution efficiency for purification of plasma derived AAT using a 20 mM Tris, 2M MgCl₂ elution buffer at pH 7

Target Molecule	MgCl ₂ 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	++	++	+	+/-	++
α-1 anti trypsin	++	-	+	+/-	++
Transferrin/ Transferrin fusions	ND	++	ND	+/-	++
Fibrinogen	+	++	ND	-	++

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



Reduction of Process Steps



One-step purification of human Fab fragments

Ligand

- Ligand developed and produced by BAC B.V. Netherlands.
- Will also have affinity for IgA, IgE, IgG and IgM containing kappa light chain.
- Binds to the light chain constant domain (see fig1).

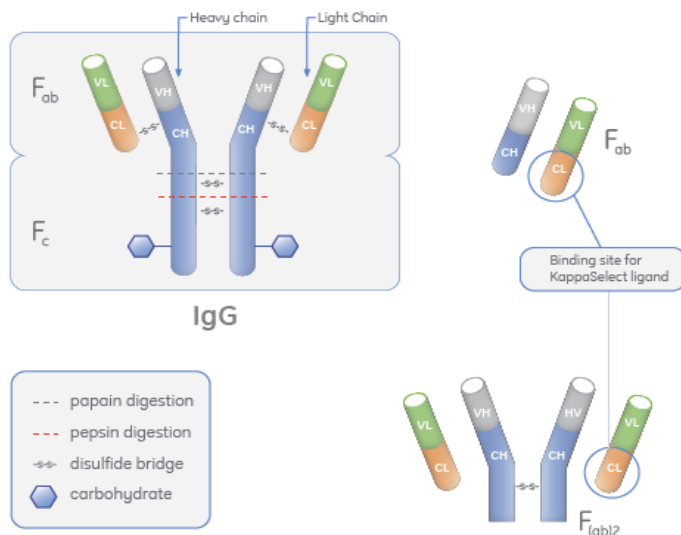
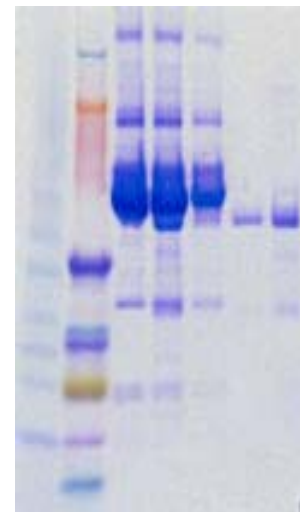


Fig 1. Antibody structure and binding site for KappaSelect ligand to Fab fragment.

M 1 2 3 4 5



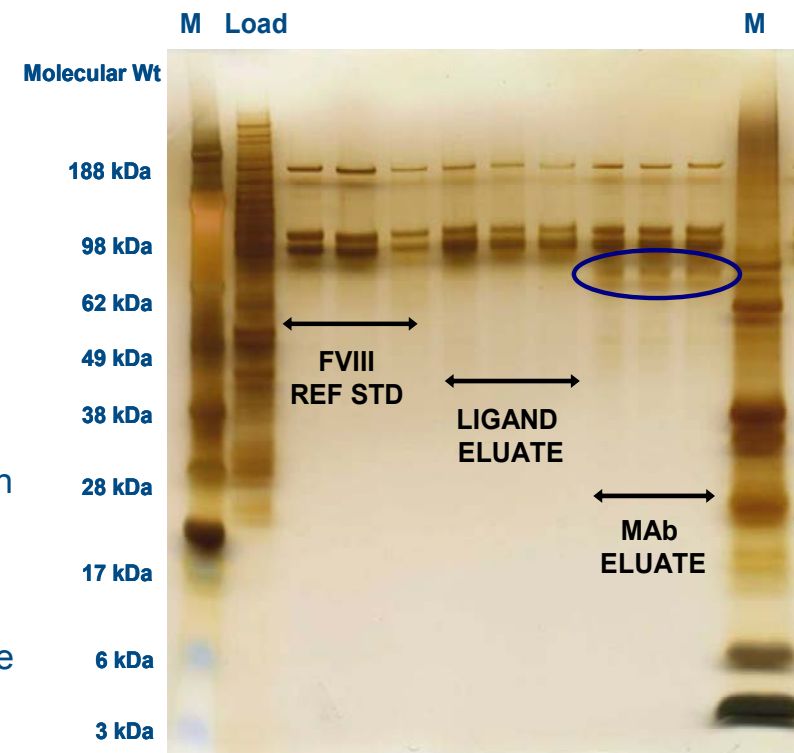
M Marker

- 1 Tissue culture medium
- 2 (1) + Fab-κ
- 3 Flow-through
- 4 Elution
- 5 Fab control

~ Purification of recombinant B domain deleted FVIII from feedstock

VIIISelect evaluation by Biogen-Idec

- ~ Highly selective step with > 75% product recoveries
- ~ Capacity ~20000 IU/ml gel
- ~ Effective impurity clearance
 - DNA clearance (~5 logs) and HCP clearance (~3 logs)
 - Robust viral clearance for both enveloped (XMuLV, ~4 logs) and non-enveloped (MMV, > 5 logs) model viruses
 - VIIISelect ligand leachate below detection level (detection limit = 1ng/ml)
- ~ Reusable over multiple cycles
 - No measurable loss in capacity or change in performance (measured up to 25 cycles)
- ~ VIIISelect currently incorporated in rhFVIII production process

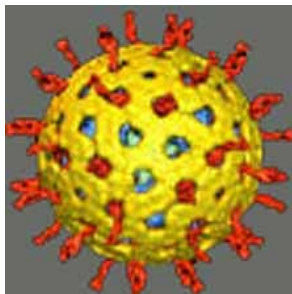


GE Healthcare

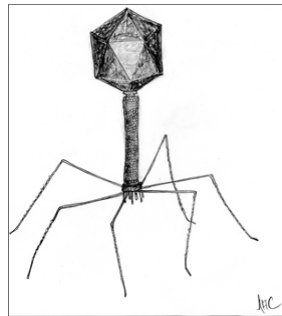
VIIISelect

Development of Affinity Ligands against Viral Targets

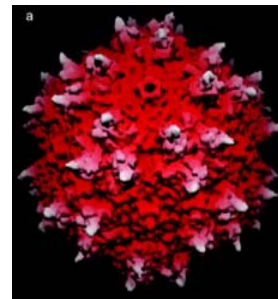
- VHH antibody fragments have been generated against surface exposed antigens of different virus types
- Besides reported effects on viral neutralization, VHH fragments can also serve as affinity ligands for the purification of whole virus particles and / or specific viral proteins (like HA antigen of influenza viruses)



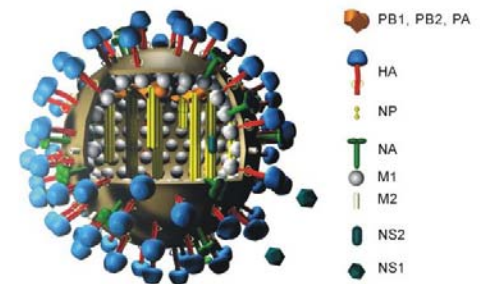
Rotavirus



Bacteriophages



AAV



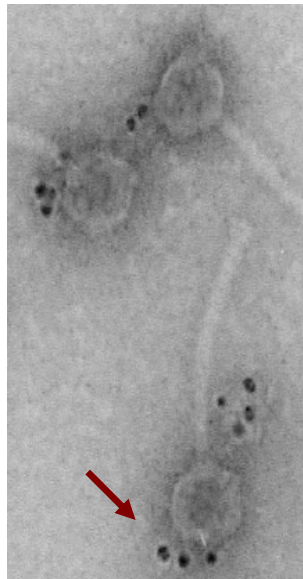
Influenza

⌘ Inhibition of Viral infectivity by anti-virus VHH fragments

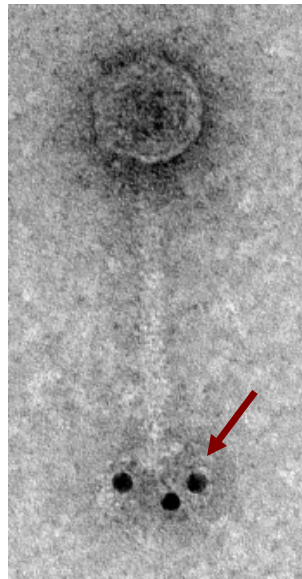
Lactococcal Bacteriophage p2



- Responsible for considerable losses in industrial production of cheese.

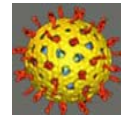


Non-neutralizing VHH



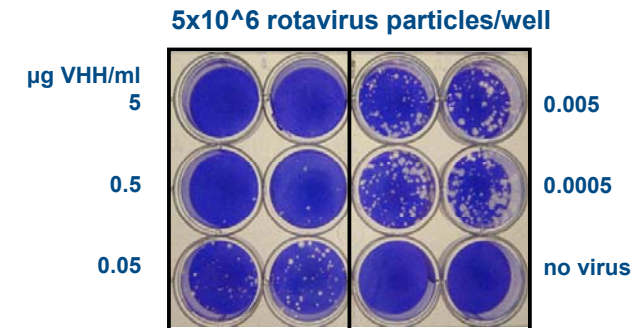
Neutralizing VHH

Human Rotavirus

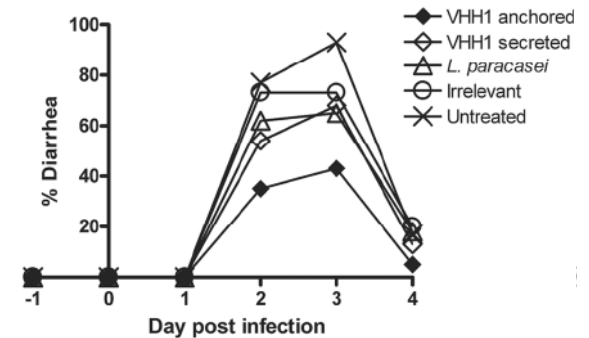


- Rotavirus: most important viral cause of gastroenteritis
 - Neutralization by anti-Rota VHHs

In Vitro



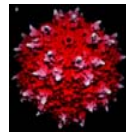
In Vivo



Affinity Purification of Adeno Associated Virus



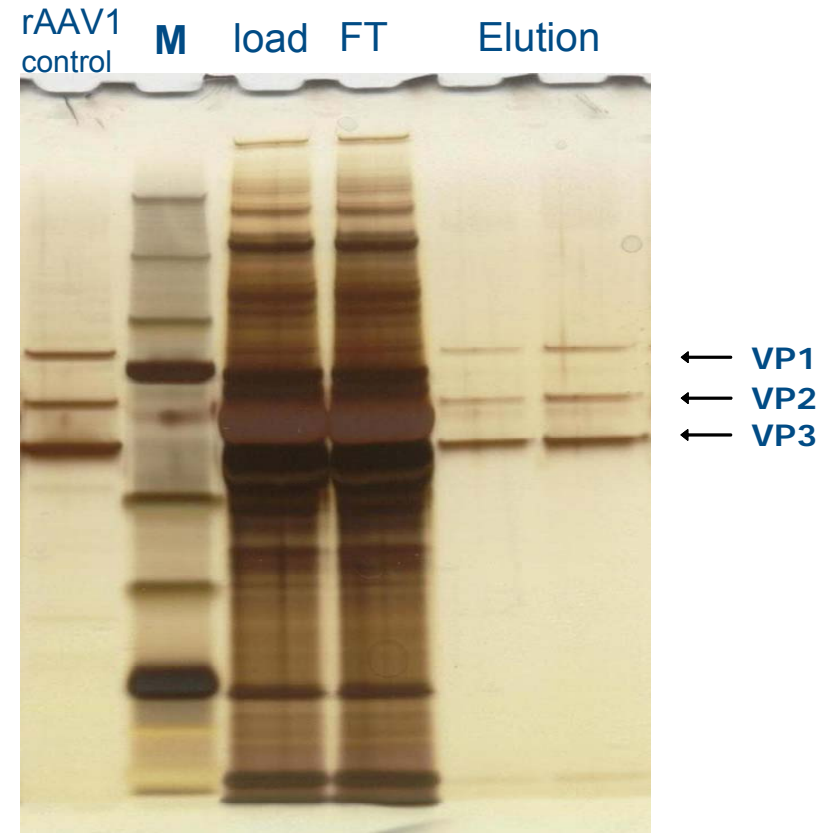
Custom ligand project



~ BAC successfully identified ligands for purification of AAV

~ 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"

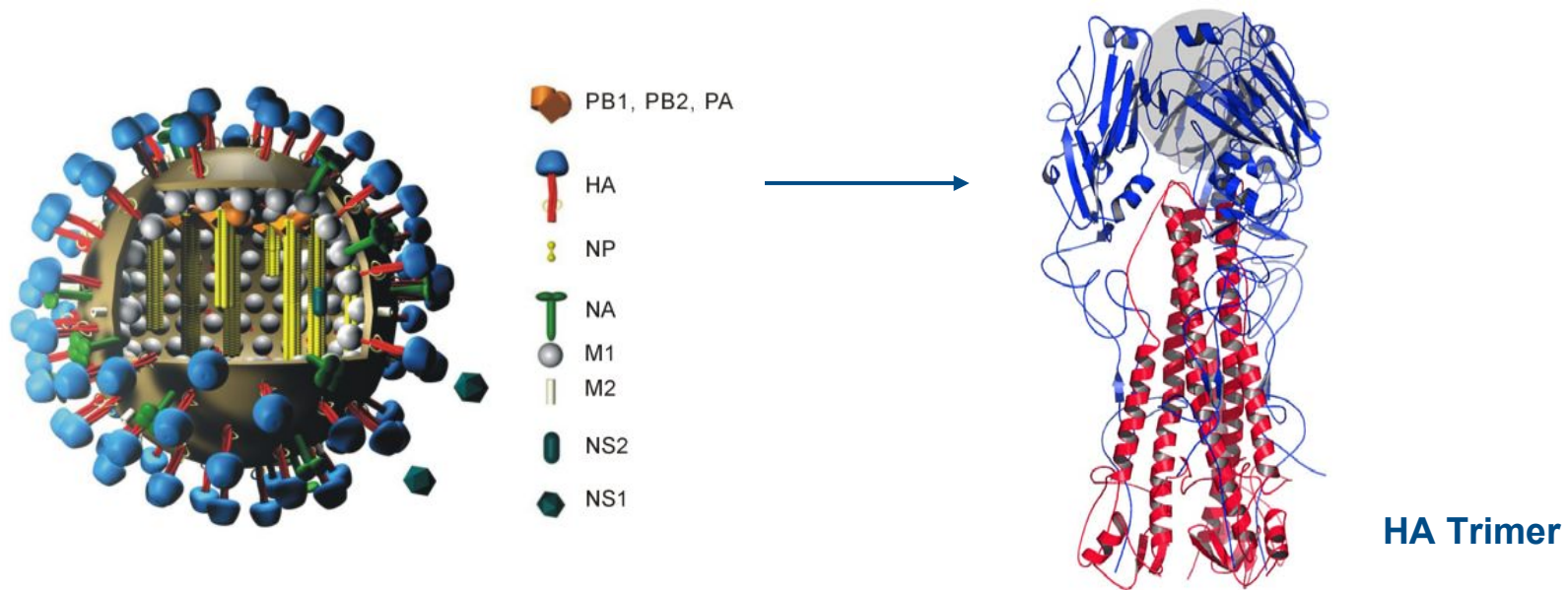


Vaccines: Purification of Influenza Viruses



~ Affinity purification of Influenza viruses by targeting a conserved region on the Hemagglutinin (HA) antigen of different A and B strains

- Hemagglutinin (HA), 566 aa, 60 kDa, 500 trimers per virion
- Immunity to HA correlates with resistance to infection disease
 - it is the main target for the host's immune system
- 15 HA subtypes have been identified.

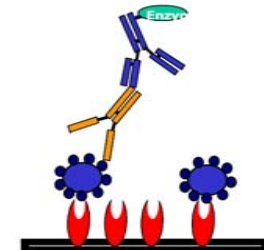


Vaccines: Purification of Influenza Viruses



VHH Ligands identified showing binding to HA antigen of a broad range of Influenza A strains in Capture-ELISA

Binding to purified HA antigen and whole virus particles



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	+++	++	++	+	++	-	-	-
A5	+++	+	+	+/-	++	-	-	-
A6	+++	++	++	+	++	-	-	-
A7	++	-	-	-	+/-	-	-	-
A9	+++	++	++	+	++	-	-	-
A10	+	+/-	-	+/-	+/-	-	-	-

Vaccines: Purification of Influenza Viruses



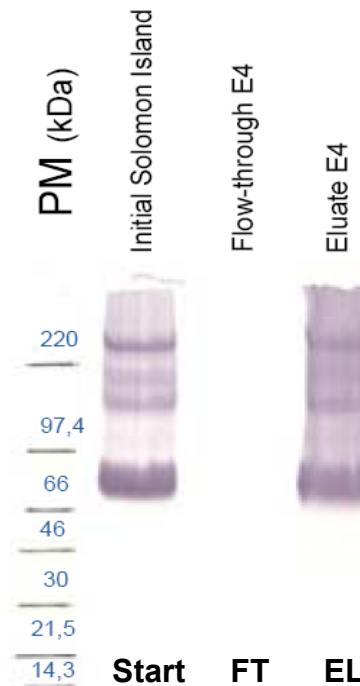
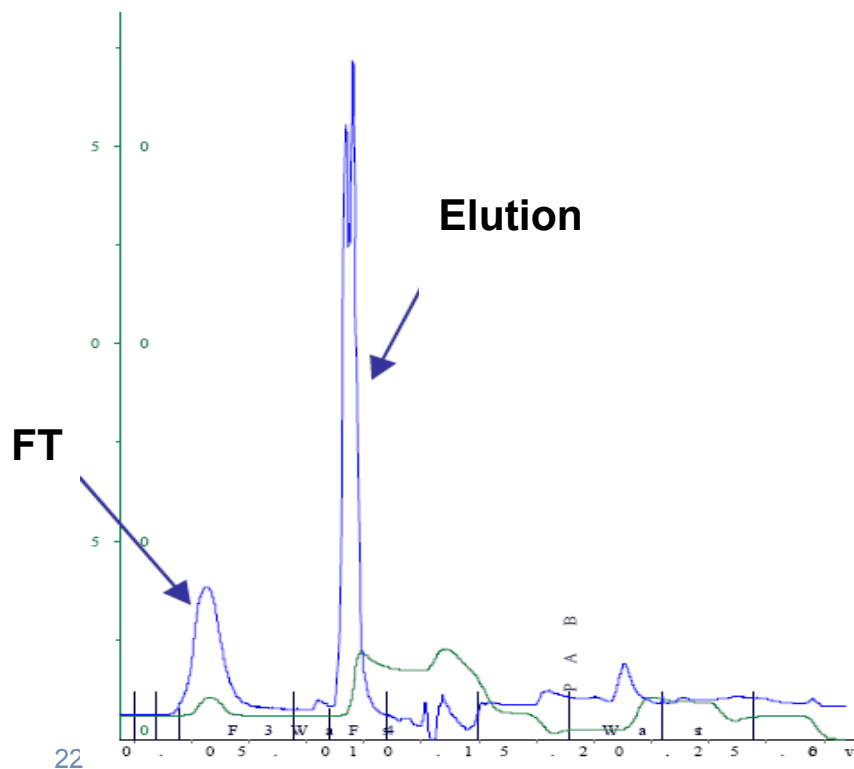
~ Affinity purification of Influenza A viruses (whole particles) from clarified concentrated harvest on resin prototype

A: H1N1 Solomon Island

western blot

Broad reactivity demonstrated with:

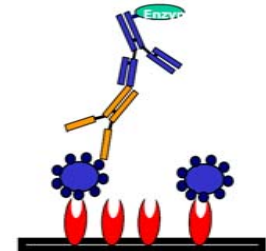
- A:H1N1 Solomon Island**
- A:H1N1 Brisbane**
- A:H3N2 Wisconsin**
- A:H3N2 Uruguay**



Vaccines: Purification of Influenza Viruses



VHH Ligands identified showing binding to HA antigen of a broad range of Influenza B strains in Capture-ELISA



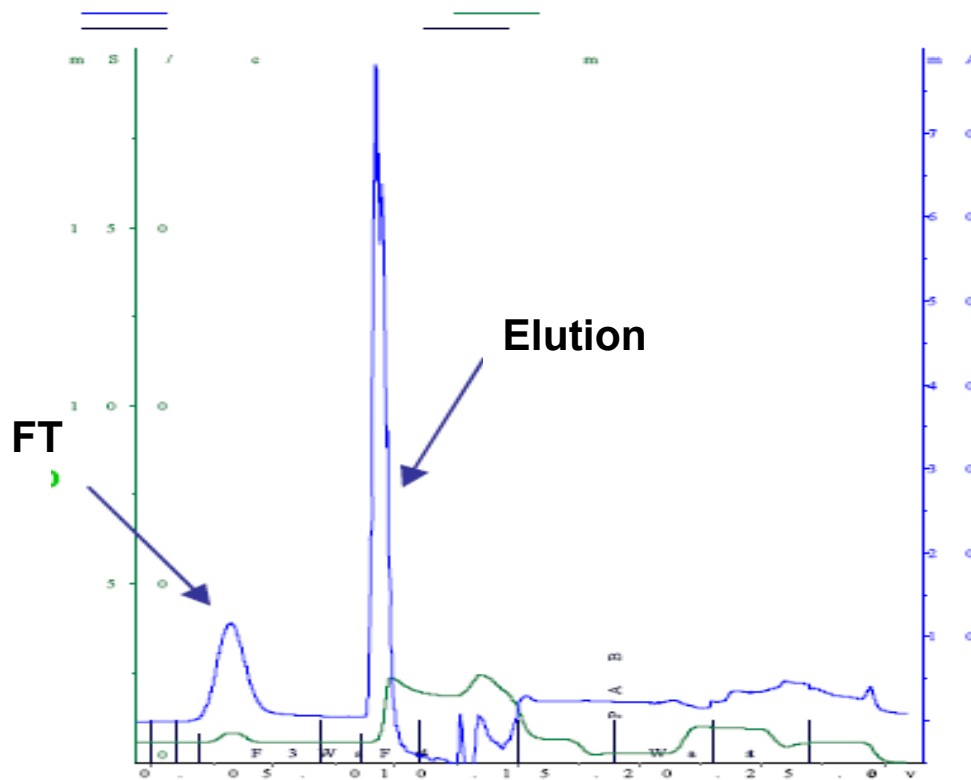
Anti-Flu Ligand	A: H1N1 Solomon virus	A: H1N1 Brisbane virus	A: H1N1 Brisbane HA	H3N2 Wiscon virus	B Malaysia virus	B Florida virus	B Brisbane HA
B1	-	-	-	-	+	+/-	+++
B3	-	-	-	-	-	+/-	++
B4	-	-	-	-	+/-	-	+/-
B6	-	-	-	-	-	+/-	+
B7	-	-	-	-	+	+	+++
B8	-	-	-	-	+	+	+++

Vaccines: Purification of Influenza Viruses

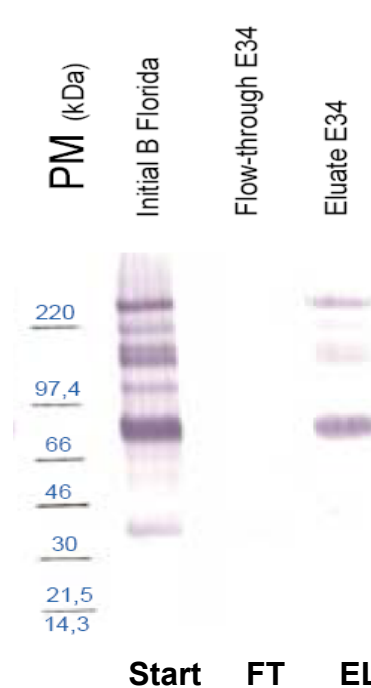


~ Affinity purification of Influenza B viruses (whole particles) from clarified concentrated harvest on resin prototype

B Florida



western blot

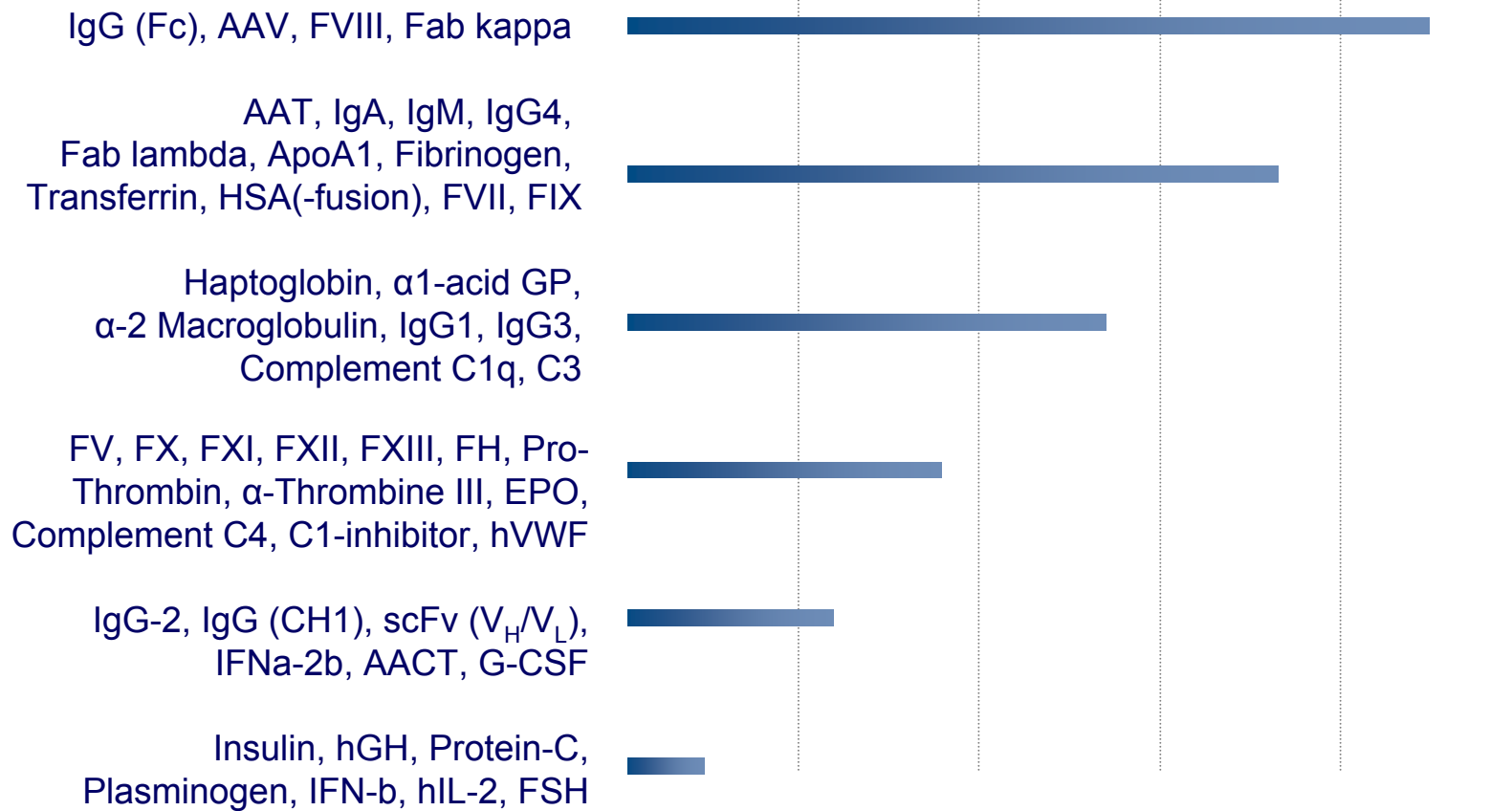
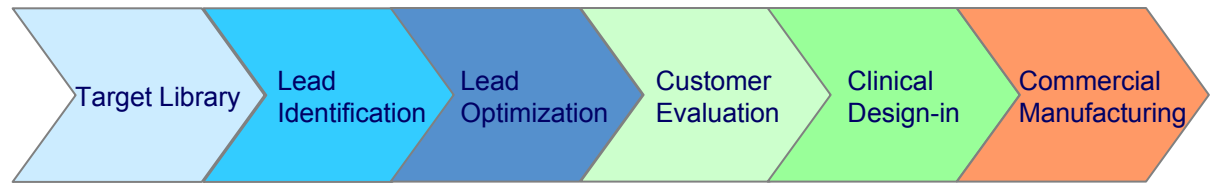


Broad reactivity demonstrated with:

- B Florida**
- B Malaysia**
- B Brisbane**



BAC's Pipeline



Acknowledgements



Biogen-Idec



– Justin T. McCue

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– Olivier Pitiot



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