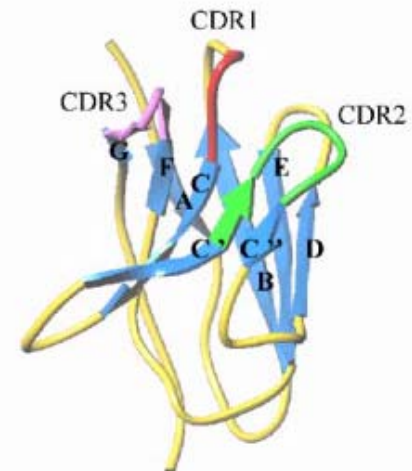


Efficiency Improvements in the Discovery and Development of Novel Antibody Format and Protein-Based Biotherapeutics Using CaptureSelect® Affinity Ligands and Yeast Display Discovery Tools

*Hendrik Adams, Scientist
BAC BV
The Netherlands*



Corporate Profile

- Unilever spin-off : Majority share holder Unilever, Minority VC
- Combining Unilever's IP, know-how and experience in custom manufacturing
 - Assets include R&D department and manufacturing site
- Affinity ligand company: making proprietary products
 - Based on CaptureSelect® platform technology
- CaptureSelect® :
 - are being sold into LifeScience Research through all major distributors
 - Web-shop for own immobilized LifeScience Research products
 - Standard products for IgG, Fab, rFVIII and AAV purification for Bioprocess immobilized and distributed by GE-Healthcare
 - Pipeline of New Bioprocess products in R&D phase
 - Active Custom Ligand projects with Pharmaceutical companies



Markets

Life Sciences Research

Proteomics
pre-purification
(depletion & enrichment)

Separation
human IgG
affinity tags
viruses

Biopharma Manufacturing

Multi-Customer Ligands
human IgG
antibody fragments
viruses

Custom ligands
recombinant proteins
scavenging
blood proteins

Healthcare

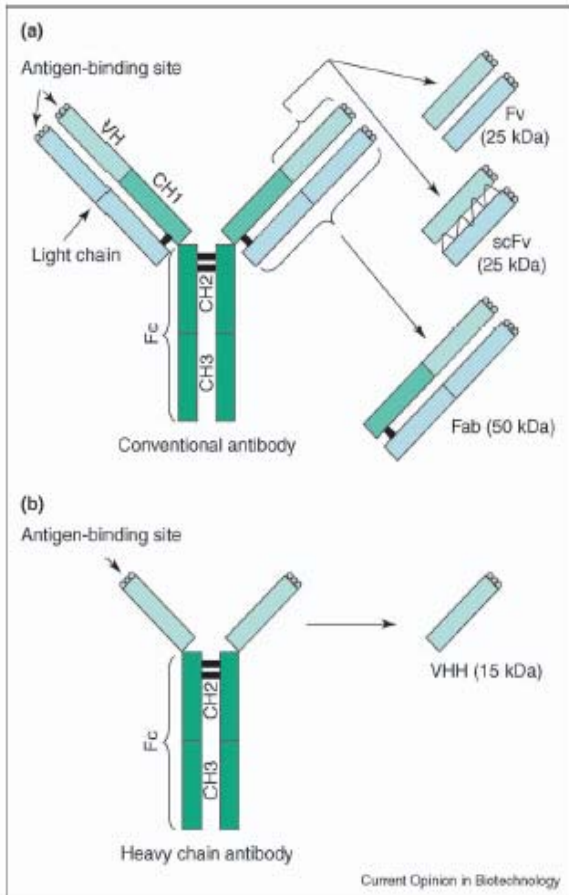
Therapeutic Apheresis

Renal & Hemo Dialysis

Initiative:
•Foods



CaptureSelect affinity ligands



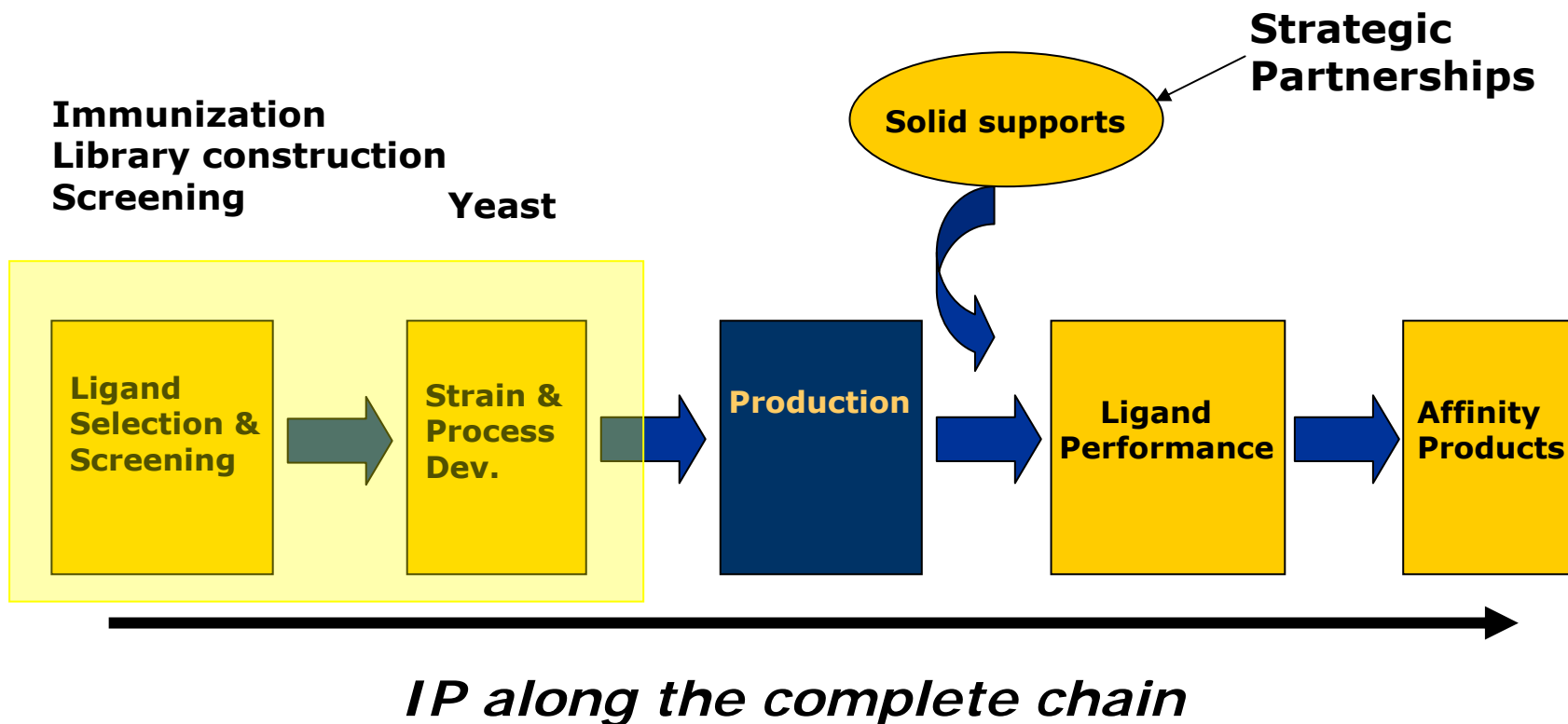
• CaptureSelect Affinity Ligands use the Uniqueness of VHH Antibody Fragments

• Advantages:

- Specificity - Broad / Narrow
- Stability - Cleaning Agents
- Screening - Operating Conditions
- Affinity - nmol Kd



*Product Development and Supply
-in-house capabilities-*

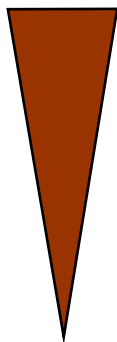


Case study: Ligand Discovery recFVIII

VHH Library

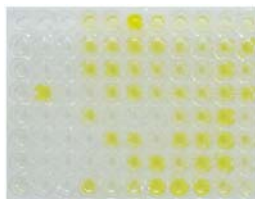
- Llama immunisation with Factor VIII
- Serum Check
- Library construction
- Colony picking

Library Screening

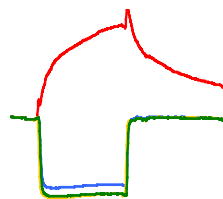


- Binding to Target
- Binding under defined binding conditions
- Elute under defined elution conditions

ELISA

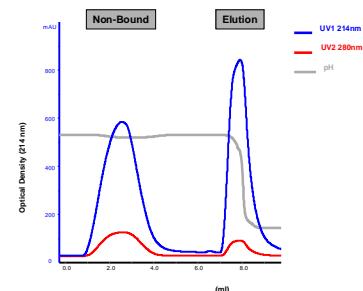


Biacore



Small Scale AC

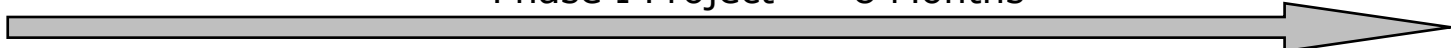
- Test ligand in chromatography under defined conditions



- Evaluation of lead ligands

Phase I Project

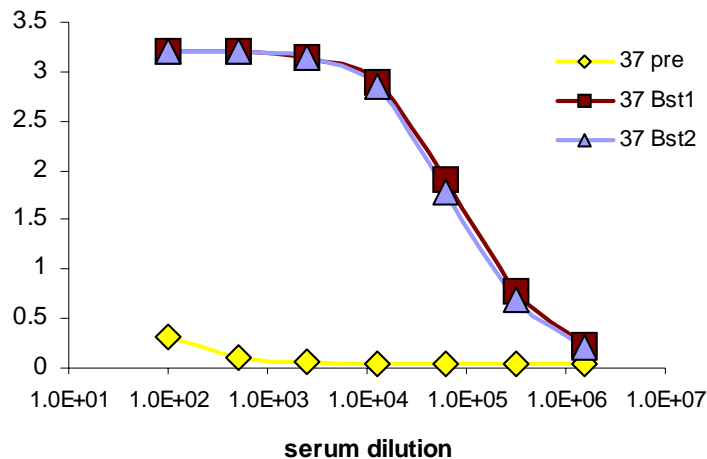
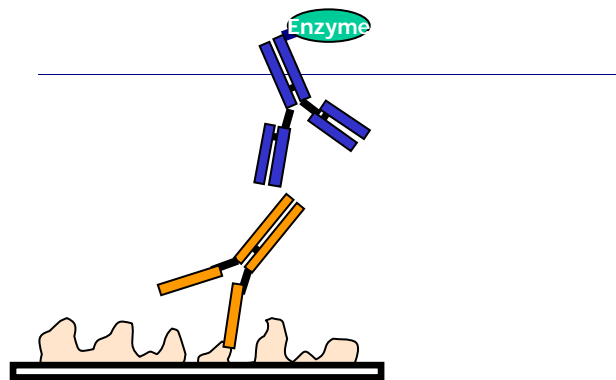
8 Months



Llama Immunisation and Serum Check

www.bac.nl

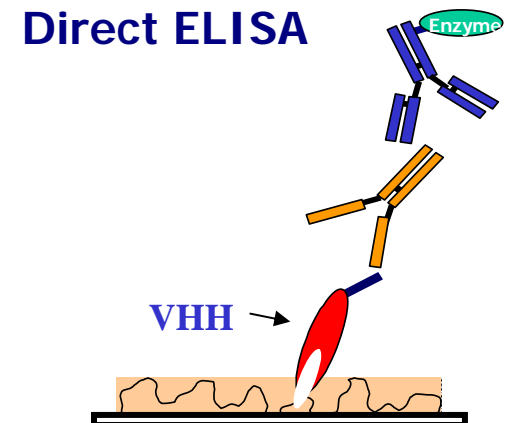
- Llama's immunised with purified recFVIII
- Immune Response: Total Serum in ELISA



- VHH expression libraries constructed after 2nd boost

Individual clone analysis by direct ELISA

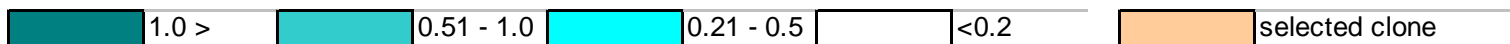
- Single clones isolated from VHH expression libraries for ELISA screening
- Objective was to screen for binding and elution at neutral pH by using different additives
- Three elution buffers (E1-E3) were chosen for screening purposes
- Screening for elution was performed by direct ELISA and refined by using a capture ELISA



Individual clone analysis by direct ELISA - Results

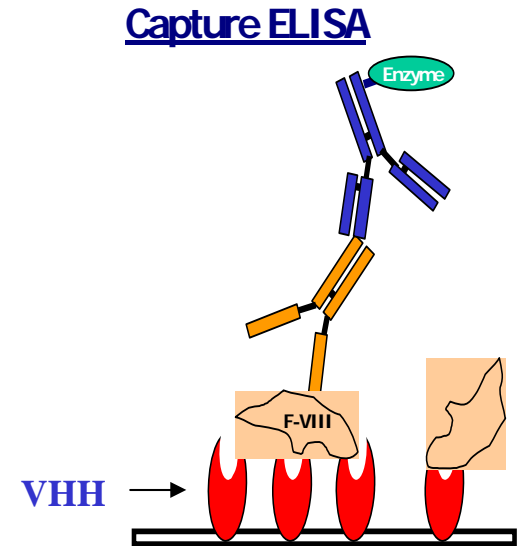
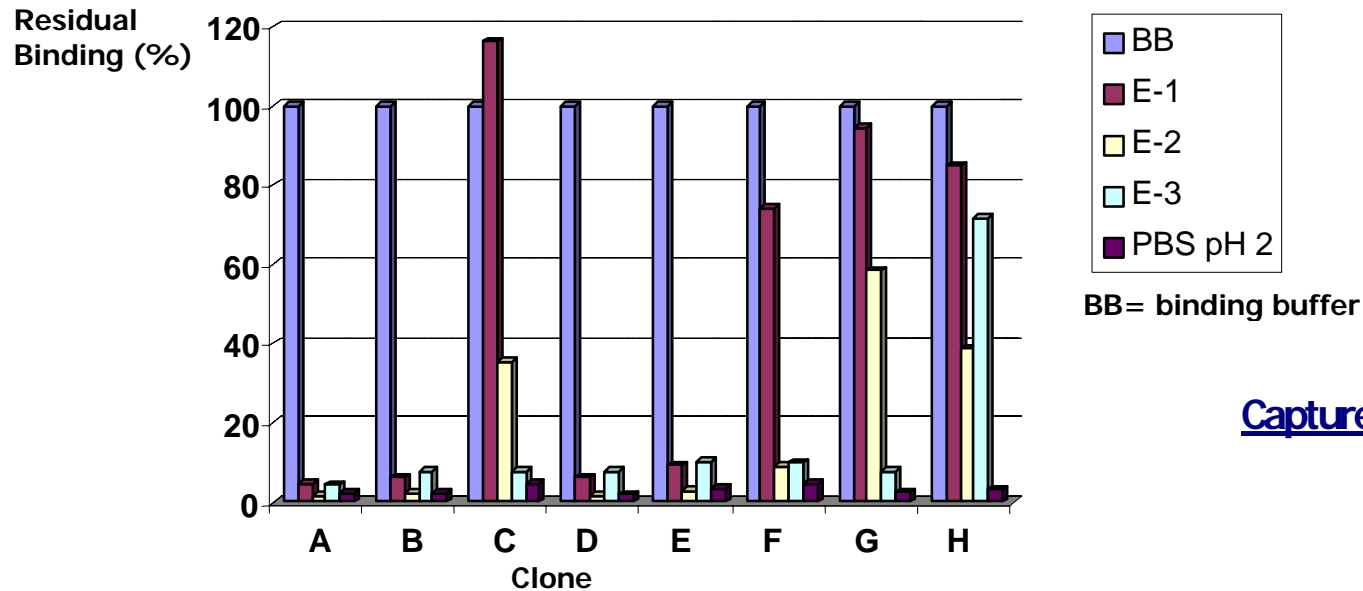
Clone	BB	E1	E2	E3	Clone	BB	E1	E2	E3	Clone	BB	E1	E2	E3
1	1.122	0.059	0.060	0.097	9	1.662	1.812	1.455	1.692	17	1.006	1.174	1.123	0.132
2	1.885	0.104	0.067	0.400	10	0.084	0.105	0.117	0.064	18	1.214	0.063	0.056	0.140
3	1.725	0.083	0.063	0.330	11	1.450	0.091	0.058	0.292	19	0.598	0.073	0.071	0.055
4	0.057	0.053	0.054	0.060	12	0.115	0.121	0.102	0.090	20	2.047	1.723	1.716	0.679
5	1.544	0.103	0.068	0.359	13	0.151	0.065	0.072	0.067	21	1.835	1.678	1.742	0.140
6	1.381	1.433	1.558	1.250	14	0.890	0.871	0.555	1.022	22	0.941	0.374	0.704	0.146
7	1.480	0.096	0.078	0.401	15	0.367	0.075	0.070	0.057	23	0.077	0.075	0.061	0.048
8	1.530	1.544	1.515	0.251	16	1.210	0.095	0.070	0.385	24	1.637	1.575	1.784	0.236
25	1.346	1.781	1.498	1.535	33	1.760	1.549	1.376	1.323	41	1.473	1.032	1.336	1.726
26	1.801	1.525	1.388	1.396	34	0.068	0.053	0.057	0.048	42	0.532	0.074	0.082	0.060
27	0.063	0.061	0.057	0.045	35	1.617	1.574	1.541	1.227	43	1.788	1.809	1.852	1.232
28	1.529	1.256	1.475	0.862	36	1.549	1.510	1.467	0.339	44	0.065	0.065	0.065	0.051
29	0.241	0.136	0.137	0.152	37	1.063	1.241	1.314	0.351	45	1.084	0.572	0.839	0.930
30	1.199	0.945	0.341	0.126	38	1.226	1.242	1.492	1.171	46	1.279	0.076	0.075	0.174
31	1.523	1.149	0.112	0.349	39	0.056	0.058	0.056	0.048	47	0.280	0.061	0.062	0.058
32	0.602	0.567	0.422	0.572	40	1.458	1.704	1.599	1.321	48	0.072	0.050	0.038	0.059
49	0.329	0.205	0.221	0.199	57	0.075	0.104	0.073	0.067	65	0.064	0.057	0.055	0.048
50	0.351	0.281	0.230	0.198	58	0.057	0.057	0.061	0.055	66	0.053	0.057	0.058	0.039
51	0.713	0.147	0.090	0.334	59	1.592	1.673	1.763	1.538	67	0.055	0.058	0.053	0.049
52	0.065	0.063	0.055	0.054	60	0.110	0.218	0.079	0.111	68	1.602	1.366	1.494	1.559
53	1.791	1.735	1.802	1.577	61	1.595	1.665	1.022	1.534	blanc	0.052	0.057	0.087	0.047
54	0.063	0.061	0.064	0.057	62	1.193	0.627	0.064	0.388					
55	0.065	0.058	0.059	0.057	63	1.570	1.385	1.327	1.041					
56	1.491	1.261	1.721	1.030	64	1.637	1.757	1.597	1.381					

OD450 signal:



Elution test of selected ligands by capture ELISA

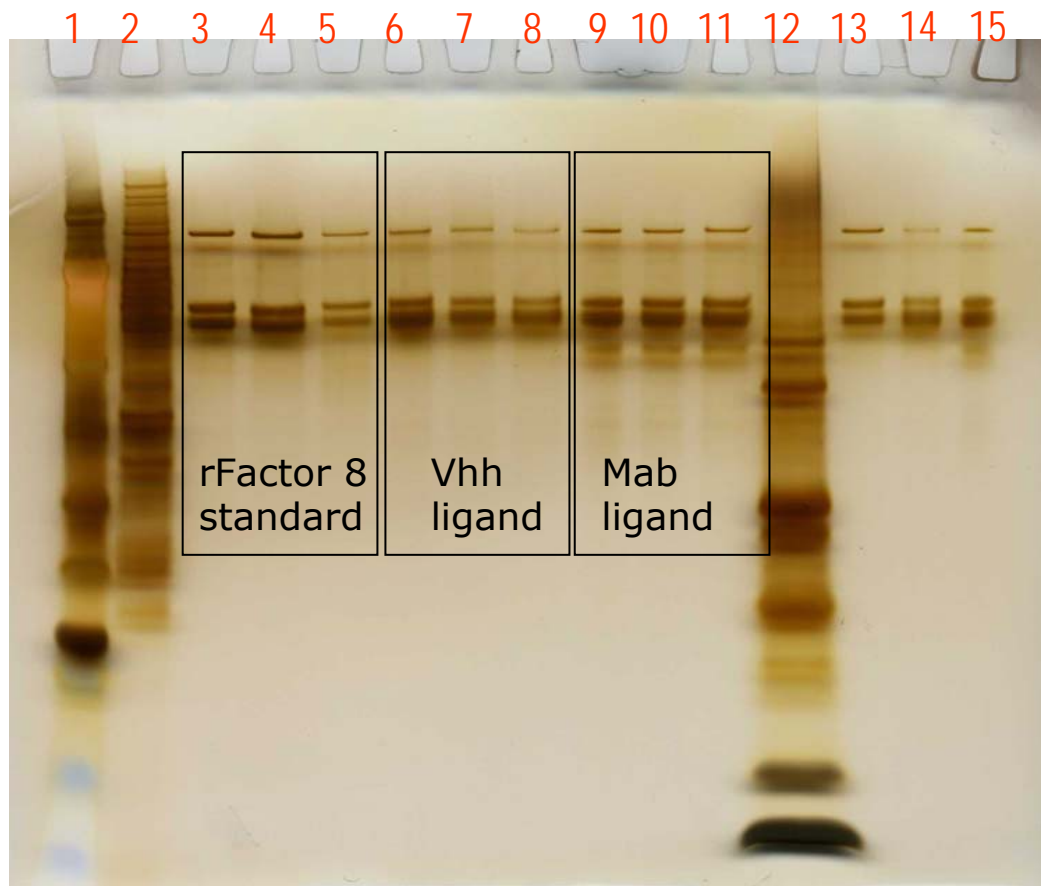
Screening of elution conditions in capture ELISA



Purification of rFactor VIII with Lead Ligand



Bayer HealthCare
Biological Products Division



Lanes

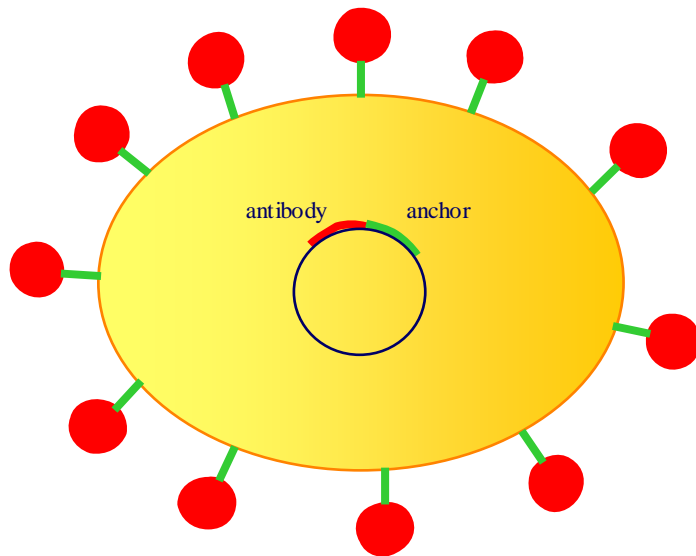
1. SeaBlue Plus 2 Marker
2. Load ~ 216ng
3. F-VIII Ref. std. ~ 200ng
4. F-VIII Ref. std. ~ 140ng
5. F-VIII Ref. std. ~ 88ng
6. VHH column Eluate ~ 200ng
7. VHH column Eluate ~ 144ng
8. VHH column Eluate ~ 80ng
9. Mab affinity column Eluate ~ 216ng
10. Mab affinity column Eluate ~ 140ng
11. Mab affinity column Eluate ~ 87ng
12. Mark 12 Marker
13. F-VIII Ref. std. ~ 100ng
14. VHH affinity column std. ~ 100ng
15. Mab affinity column Eluate ~ 108ng



SDS page silver stain of Factor VIII affinity purification

Application of Yeast display technology

- Immune Libraries contain 10 to 30% Target binders
- When this is $< 5\%$, application of Yeast display of VHH



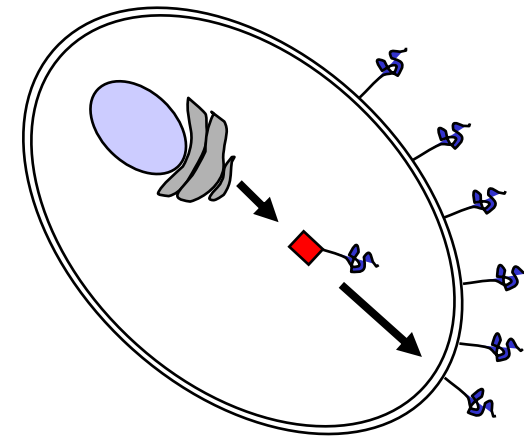
BAC's Yeast display technology

Expression construct with alpha-agglutinin to enable protein display



●Principle:

- Protein fused to Yeast cell-wall protein
- Fusion is transported and directly anchored into the Yeast cell wall
(different from Aga1/Aga2 system, Wittrup)
- Enables selection for proteins with enhanced functionality
 - FACS
 - Magnetic beads (MACS)



●Advantages over other *in vitro* evolution methods:

- Eukaryotic expression and processing system
- Quality control mechanisms of the yeast secretory pathway
- Quantitative library screening through FACS / MACS

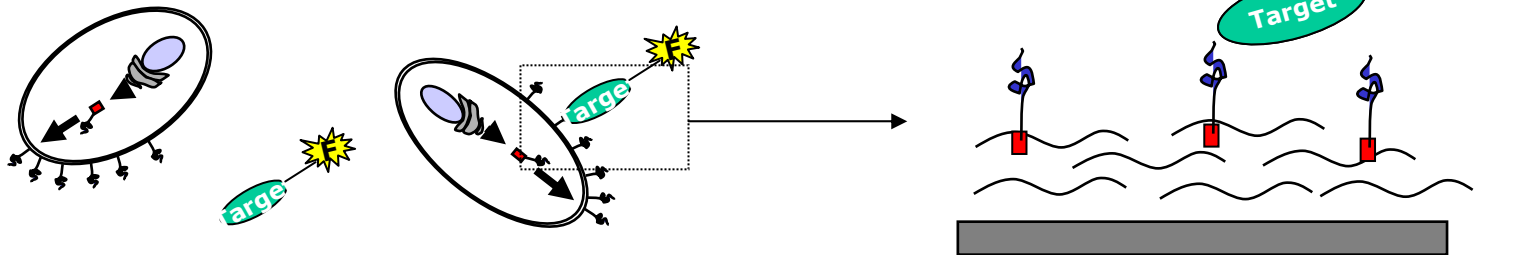


BAC's Yeast display technology

Ideal toolbox for selection & screening of binding scaffold libraries

- Protein binding scaffolds
 - single domains (Camelid, sharks, human)
 - scFV fragments
 - non-antibody based (e.g. peptides)

- Display Applications
 - selection of Target binders from libraries
 - selection of scaffolds with enhanced functionality:
 - affinity maturation
 - protein expression

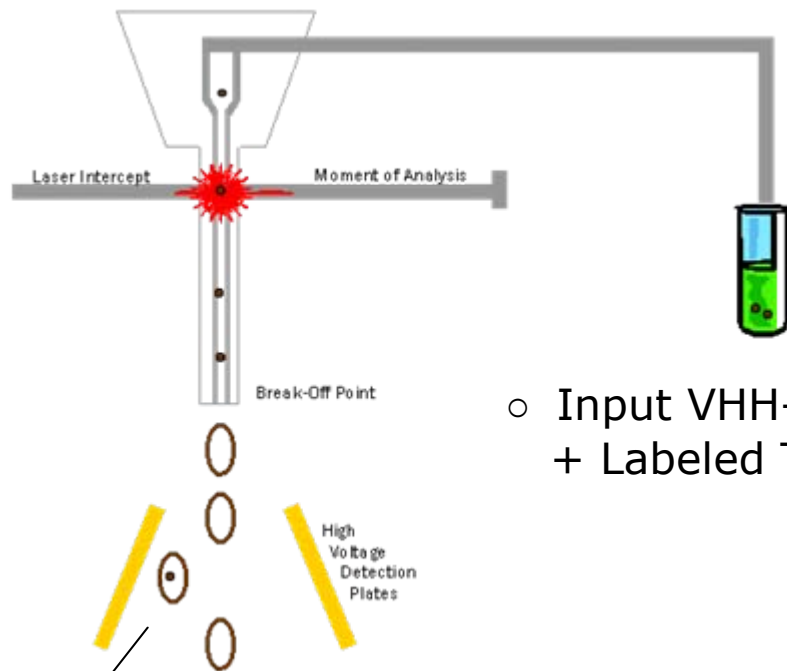


- Scaffold library + labeled Target

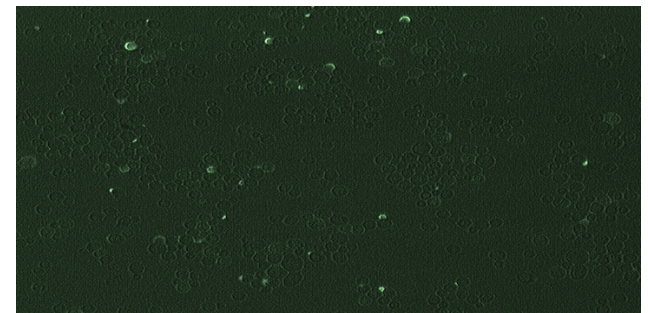


Selection on Target Binding: Yeast display + FACS

Selection of Target binding VHH domains using Yeast display + FACS

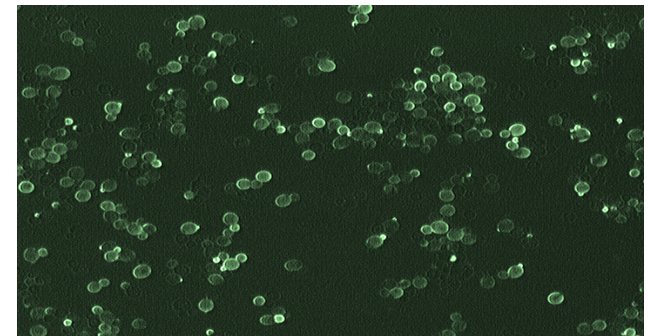


○ **Input VHH-library**



○ Input VHH-library + Labeled Target

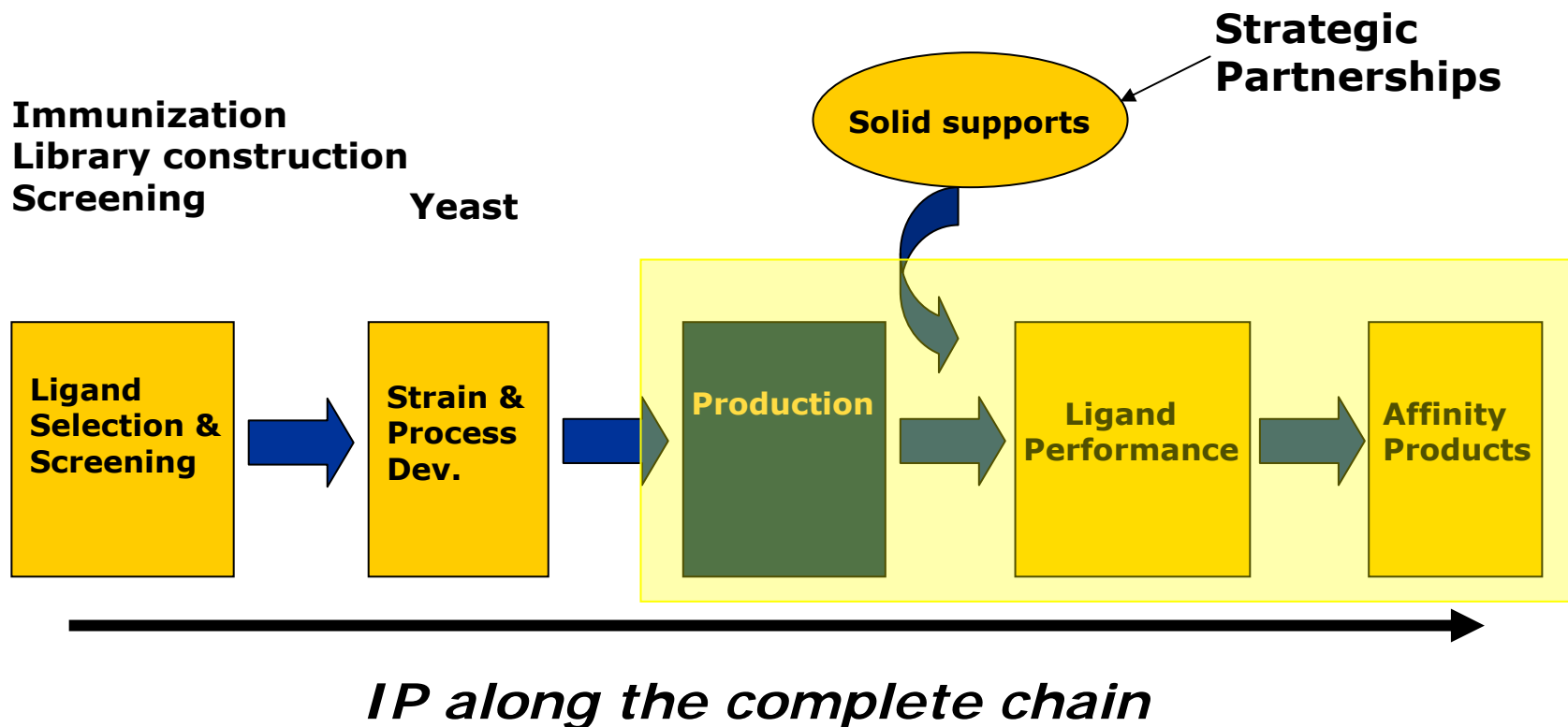
● **Target enriched VHH-library**



● **Target enriched VHH-library**
(or single cell collection of binders)



*Product Development and Supply
-in-house capabilities-*



Ligand Manufacturing

- Microbial production using *Saccharomyces cerevisiae*
 - Ligand gene is integrated in the yeast genome
 - No *E. coli* sequences
 - Completely free of animal derived components
- Production, upstream and downstream
 - Performed in our own facility
 - 15m³ scale yielding kg's of ligand per batch
 - Production process:
 - upstream: fed-batch
 - primary recovery: micro and ultrafiltration
 - final recovery: 2 chromatography steps in controlled clean room facility



**Life Sciences
Research**

Proteomics
pre-purification
(depletion &
enrichment)

Separation
human IgG
affinity tags
viruses

Range of Proteomics ligands available and sold to all major suppliers of proteomics kits

- HSA, IgG non-exclusive
- Additional products pending collaboration



Antibody purification Toolbox

- **Human IgG Fc ligand (Protein A)**

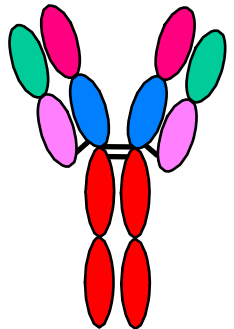
- Purification of monoclonal/polyclonal IgG from any source

- **Multiple species IgG ligand (Protein G)**

- Purification of IgG's from multiple species

- **Human kappa/lamba light chain ligands (Protein L)**

- Antibody fragment purification
- IgG, IgM, IgA purification



Products for biopharmaceutical industry

Biopharma Manufacturing

Multi-Customer Ligands

human IgG
antibody fragments
viruses

Custom ligands

recombinant
proteins
scavenging
blood proteins

Current products:

- Mono/polyclonal IgG ligand
- AAV
- recFactor VIII
- Antibody Fragments

—————> GEHC products



Ongoing collaborations with
Biopharma, e.g. Sanofi, Baxter

sanofi pasteur

The vaccines business of sanofi-aventis Group

Baxter

Worldwide



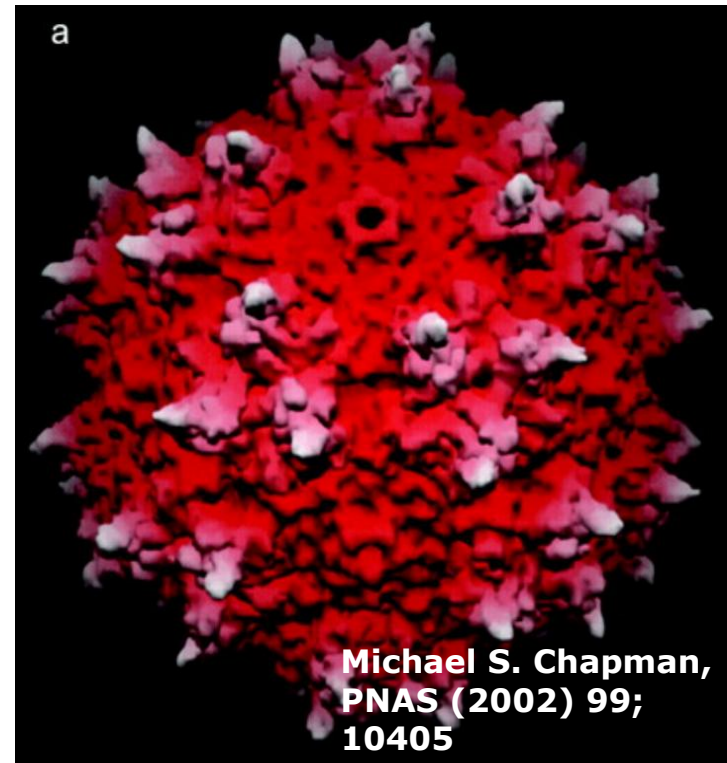
AAV purification

DSP for AAV1 :

- Density gradient centrifugation
- Ion-exchange chromatography
- HIC chromatography

DSP for AAV2 :

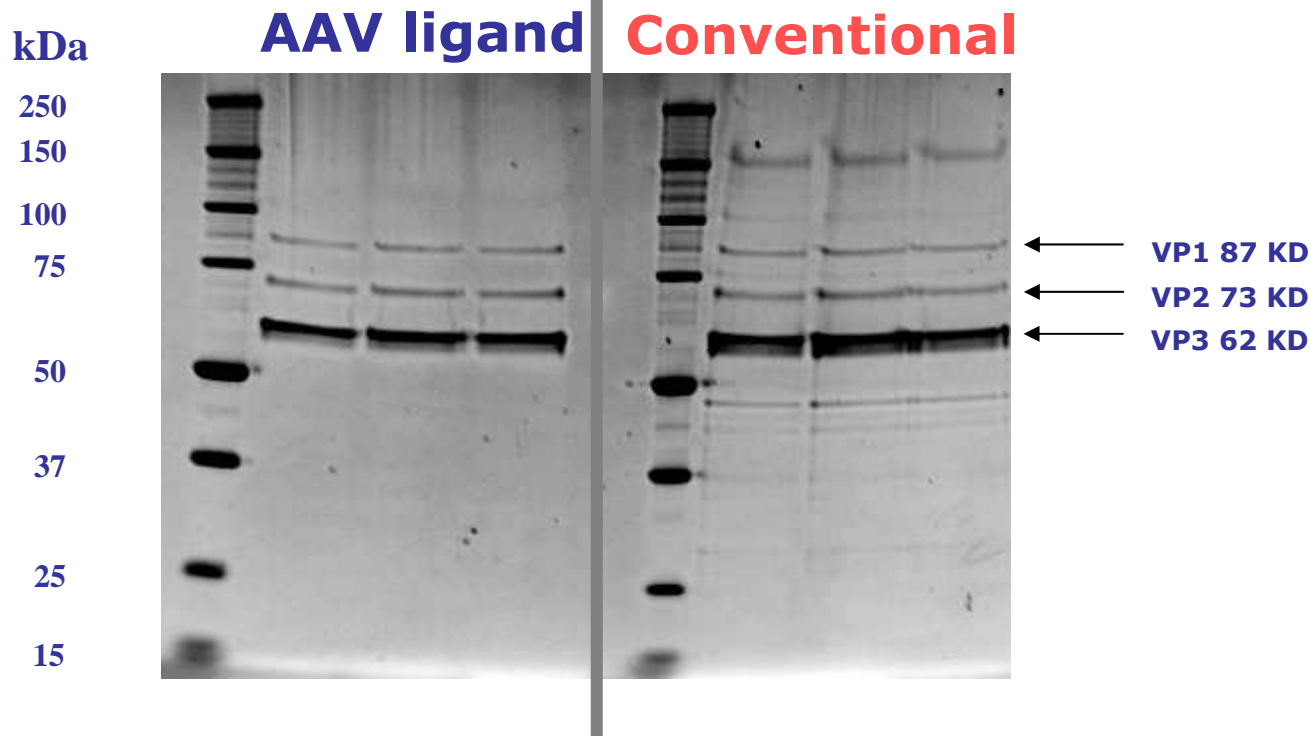
- Density gradient centrifugation
- Heparin column chromatography
- Ion-exchange chromatography
- A20-affinity chromatography



AAV2



AAV1 purification



- Equilibration:
5 CV:
100mM Tris/HCL pH8.5

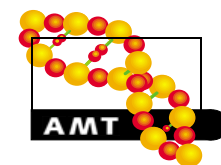
- Sample Loading:
250 CV: AAV1 in
10mM Tris/HCL,
150mM NaCL,
1mM MgCl₂; pH8.5

- Wash
10 CV:
100mM Tris pH 8.5

- Elution
30 CV:
PBS pH 3.5

-Neutr
1/10 volume
1M Tris pH 8.5

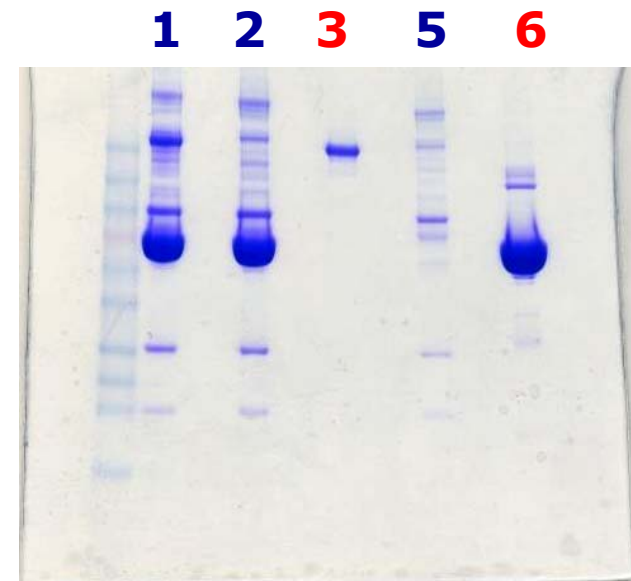
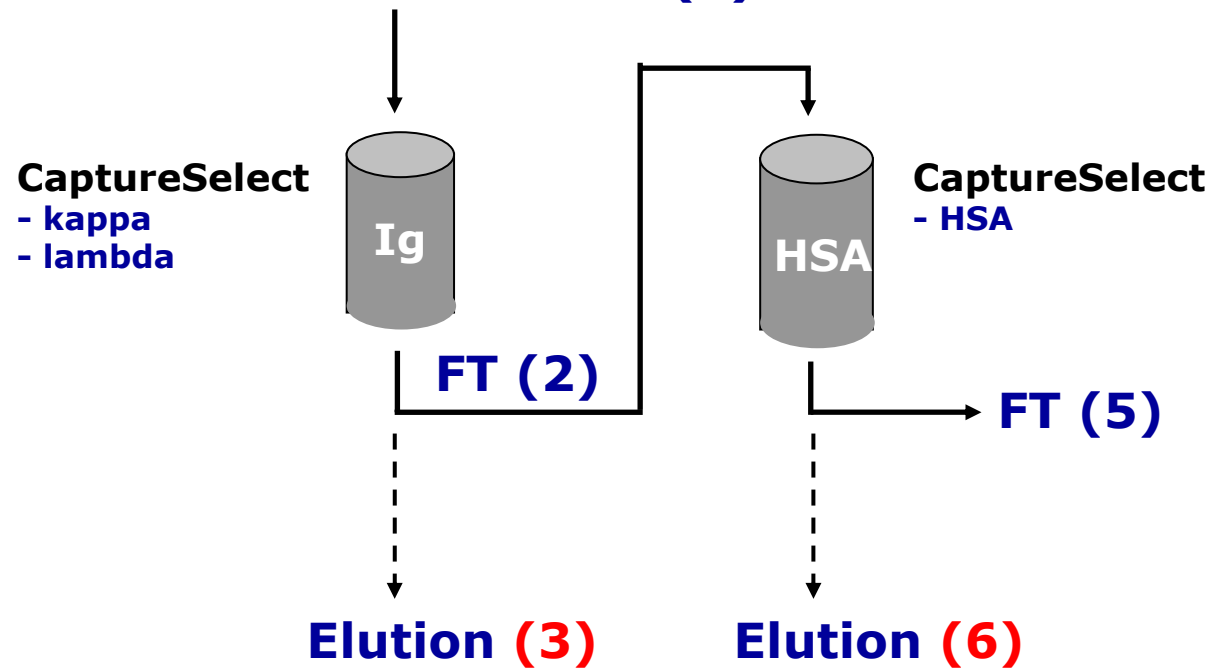
- Recovery > 60% (30%)
- Capacity > 10¹² GC /ml matrix



Multi Targets: Human Plasma Library

Antigen: human plasma depleted from Immunoglobulins and Albumin

Human Plasma (1)

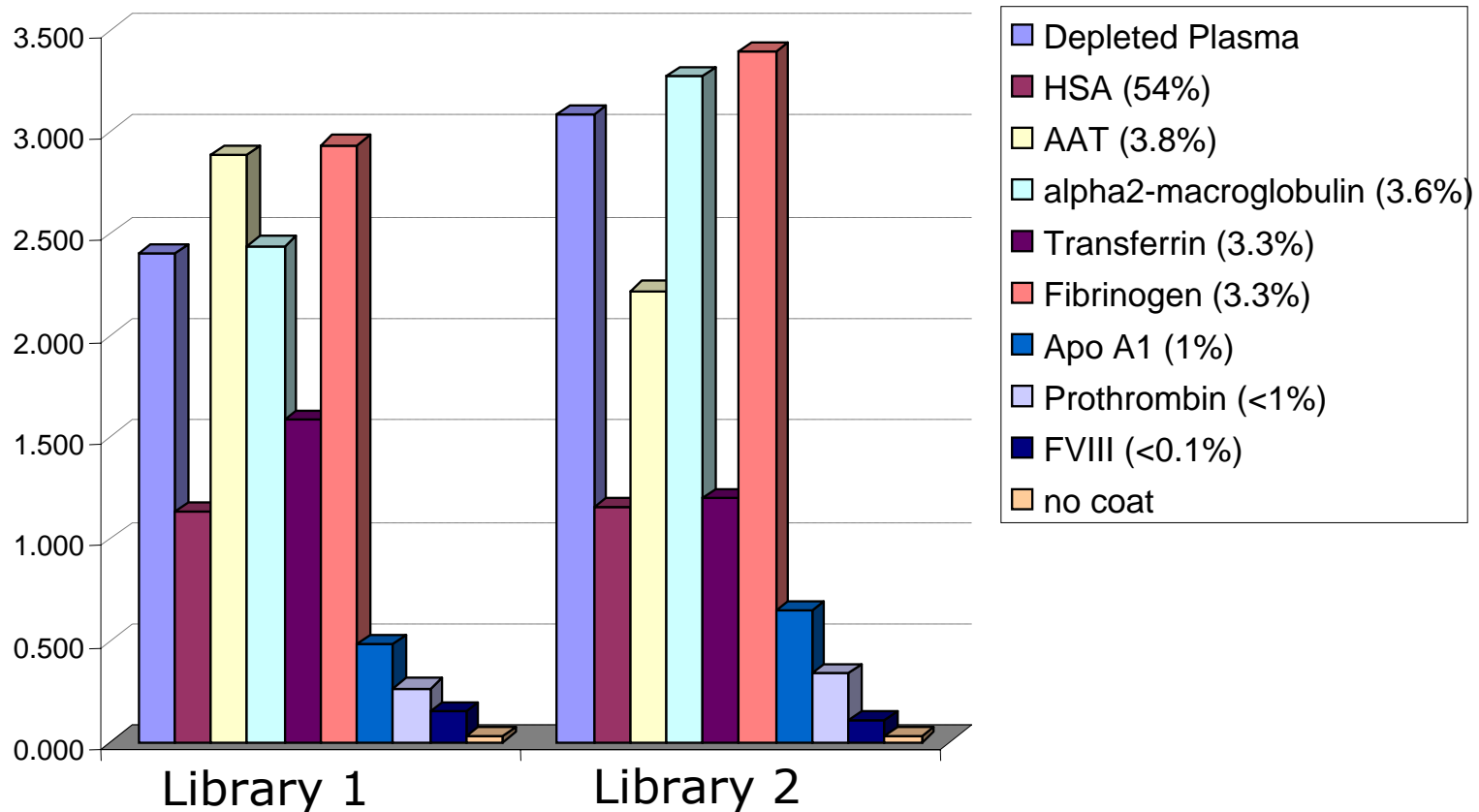


FT Sample 5: Plasma depleted from albumin, IgG, IgM, IgA
(= ±70% of total protein content in plasma)



Multi Targets: Human Plasma Library




Immune Response: broad reactivity of heavy chain antibodies towards human plasma proteins in ELISA



Multi Targets: Human Plasma Library

Human plasma VHH libraries: Multiple Libraries




Initial screening against:

-  AAT (3.8%)
-  Apo-A1 (2 %)
-  Prothrombin (< 1%)



For each Target Single clones identified showing specific binding in ELISA !

VHH Library reactivity:

Target	Library 1	Library 2
 AAT	+++	++
 Apo-A1	+/-	+
 Prothrombin	-	+



Conclusions

- **Captureselect affinity ligands offer Unique Properties for Affinity Purification of Antibodies and Protein Scaffolds**
 - Tunable Specificities
 - Subclass, format, idiotype, glycoform, ...*
 - Optimal Affinities
 - Capture, scavenging*
 - Screened for desired stabilities/elution conditions
 - Non-animal sourced
 - Select “ideal” solid support per application area
- **BAC is Fully Integrated**
 - Discovery
 - Optimization
 - Manufacturing Capabilities to Multiple Kg’s
- **BAC’s Yeast Display Library is Available for Non-Exclusive Licensing**

