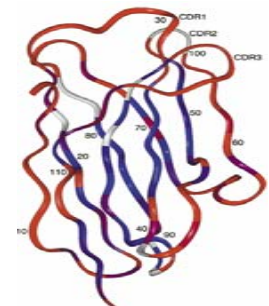




Affinity chromatography media for plasma products and recombinant plasma proteins

PPB07, Elba
Laurens Sierkstra
BAC BV, The Netherlands

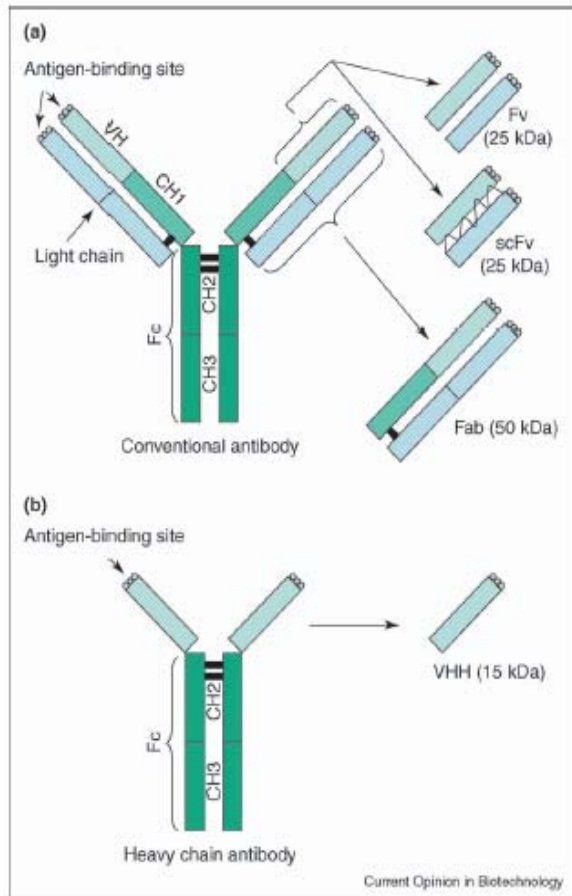


BAC develops and manufactures affinity ligands for purification:

- Life science research
 - proteomics: BAC's products in all major suppliers kits
 - albumin, Ig removal
 - protein separation: own immobilized products
 - antibody toolbox: IgG, IgM, IgA, antibody fragments
- Bioprocessing
 - multi-customer products
 - AAV, Fab's, IgSelect (GEHC poster 722)
 - custom designed ligands
 - e.g. sanofi pasteur on vaccines



CaptureSelect affinity ligands



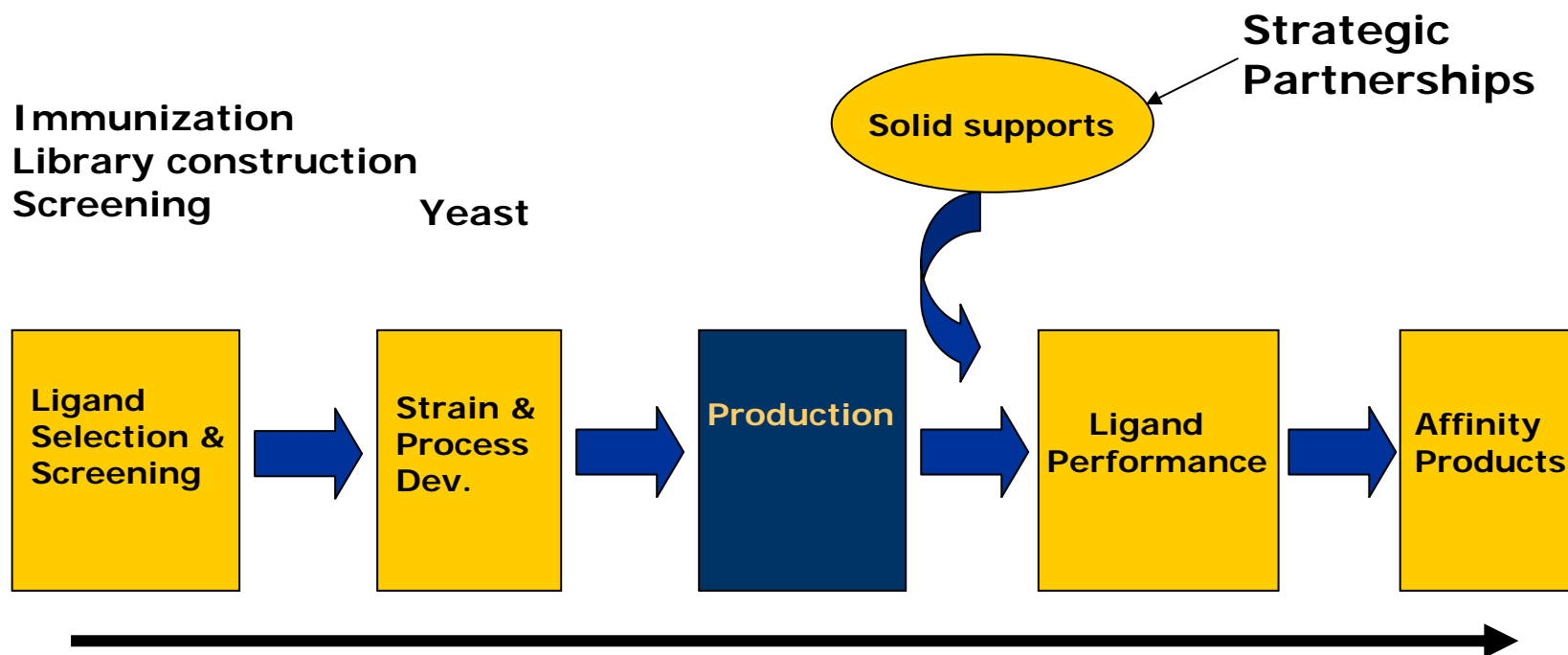
• CaptureSelect Affinity Ligands use the Uniqueness of VHH Antibody Fragments

• Advantages:

- Specificity - Broad / Narrow
- Stability - Cleaning Agents
- Screening - Operating Conditions
- Affinity - nmol Kd
- Capture and/or Polishing (Scavenging)
- Quick Discovery and Scale Up
- Non-Animal Derived
- Ability to Work on any Solid Support



Product Development and Supply



Ligand Discovery

VHH Library

- Immunization
- Library construction
- Colony picking

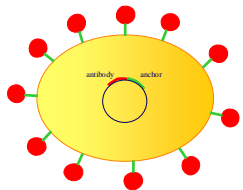
Target Binders

10 - 30 %

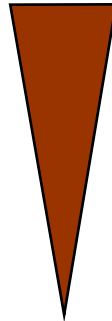
If < 5%



Yeast Display

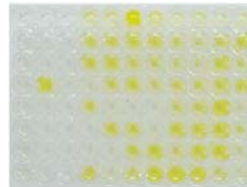


Library Screening

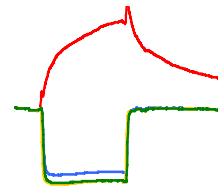


- Binding to Target
- Broad / Narrow Specificity
- Elution Conditions
- Stability

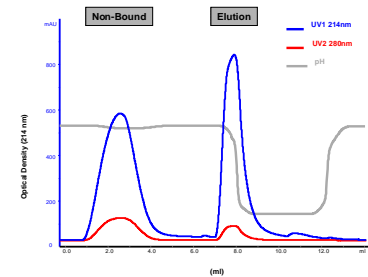
ELISA



Biacore



Small Scale AC



Ligand Leads



Phase I Project

8 Months



Ligand Manufacturing

- 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
- Microbial production using *Saccharomyces cerevisiae*
 - Ligand gene is integrated in the yeast genome
 - No *E.coli* sequences
 - Completely free of animal derived components
- MCB, WCB, EPC procedures in place
 - Including guideline testing



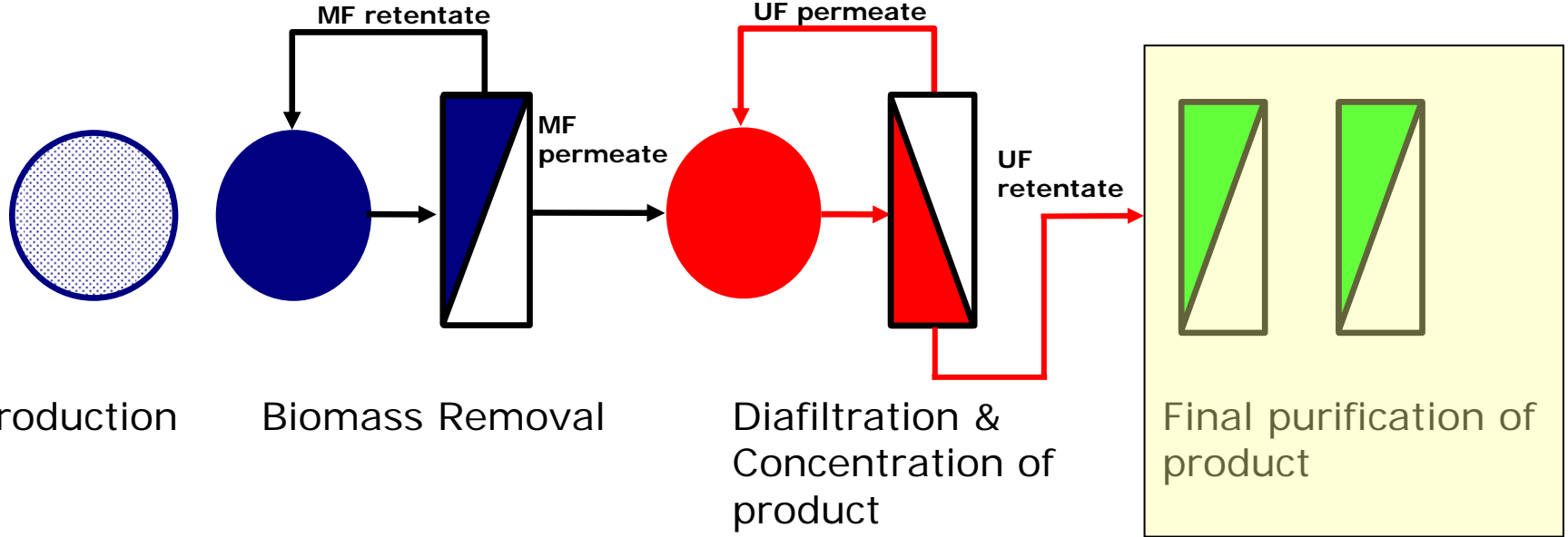
Production facilities

Fermentation

Microfiltration

Ultrafiltration

Chromatography



Production

Biomass Removal

Diafiltration &
Concentration of
product

Final purification of
product

Contained purification suite

10,000 liters

100 liters



Plasma products

BAC has used the technology to isolate and manufacture affinity ligands against a number of blood plasma proteins as well as recombinant proteins, e.g.

- albumin
- IVIG
- transferrin
- fibrinogen
- recombinant factor VIII
- AAT



Ligand Discovery

VHH Library

- Immunization
- Library construction
- Colony picking

Mono Targets:

- rFactor VIII
- AAT

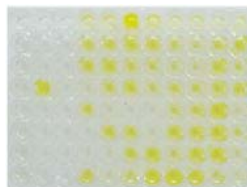
Multi Targets:

- Blood Plasma

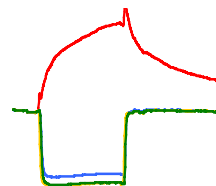
Library Screening

- Binding to Target
- Specificity
- **Elution Conditions**

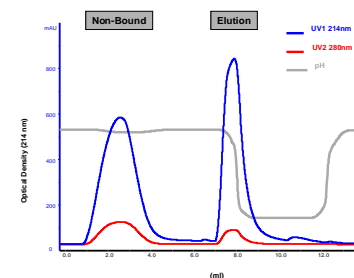
ELISA



Biacore



Small Scale AC



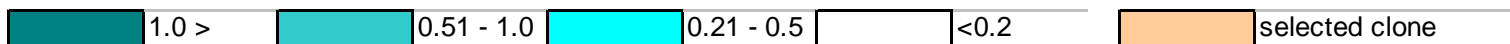
Ligand Leads



Individual clones: rFactor 8 direct ELISA

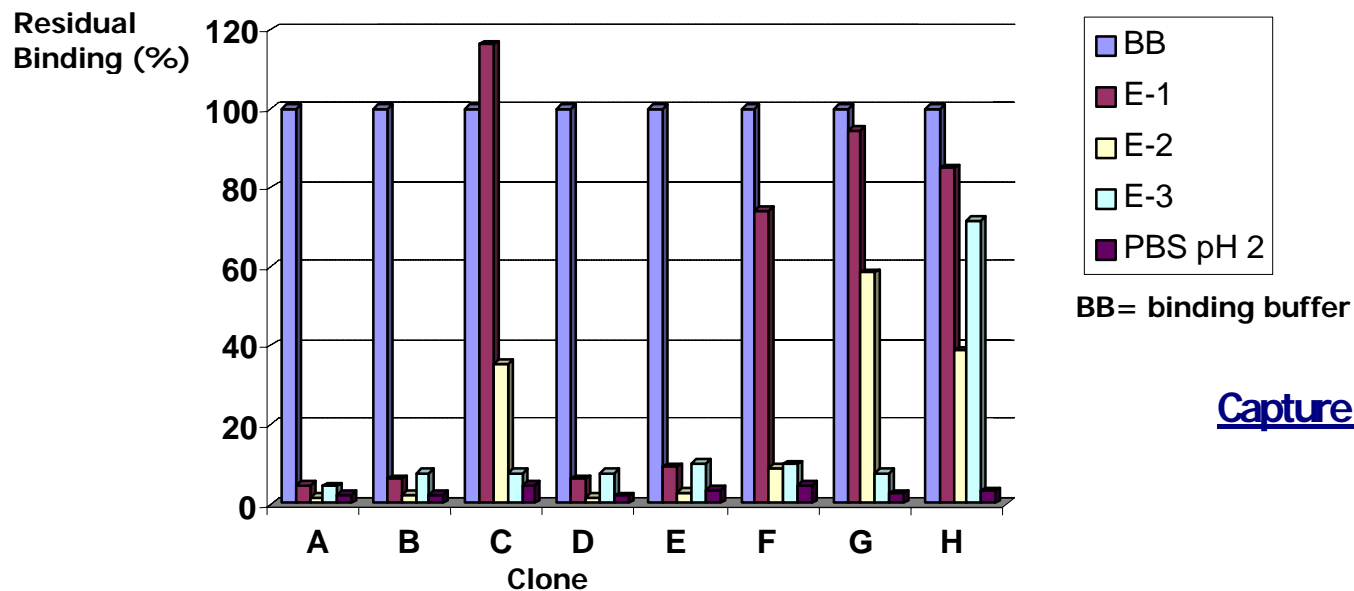
Clone	BB	E1	E2	E3	Clone	BB	E1	E2	E3	Clone	BB	E1	E2	E3
1	1.122	0.059	0.060	0.097	9	1.662	1.812	1.455	1.692	17	1.006	1.174	1.123	0.132
2	1.885	0.104	0.067	0.400	10	0.084	0.105	0.117	0.064	18	1.214	0.063	0.056	0.140
3	1.725	0.083	0.063	0.330	11	1.450	0.091	0.058	0.292	19	0.598	0.073	0.071	0.055
4	0.057	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:

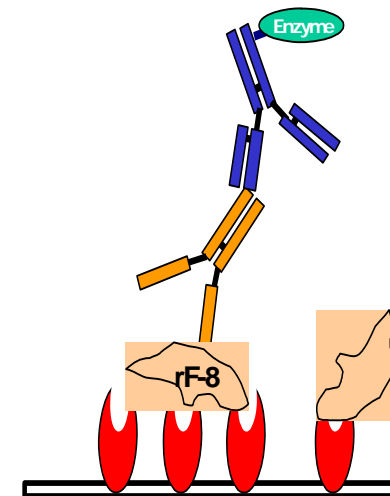


Elution profile: rFactor 8 ligands

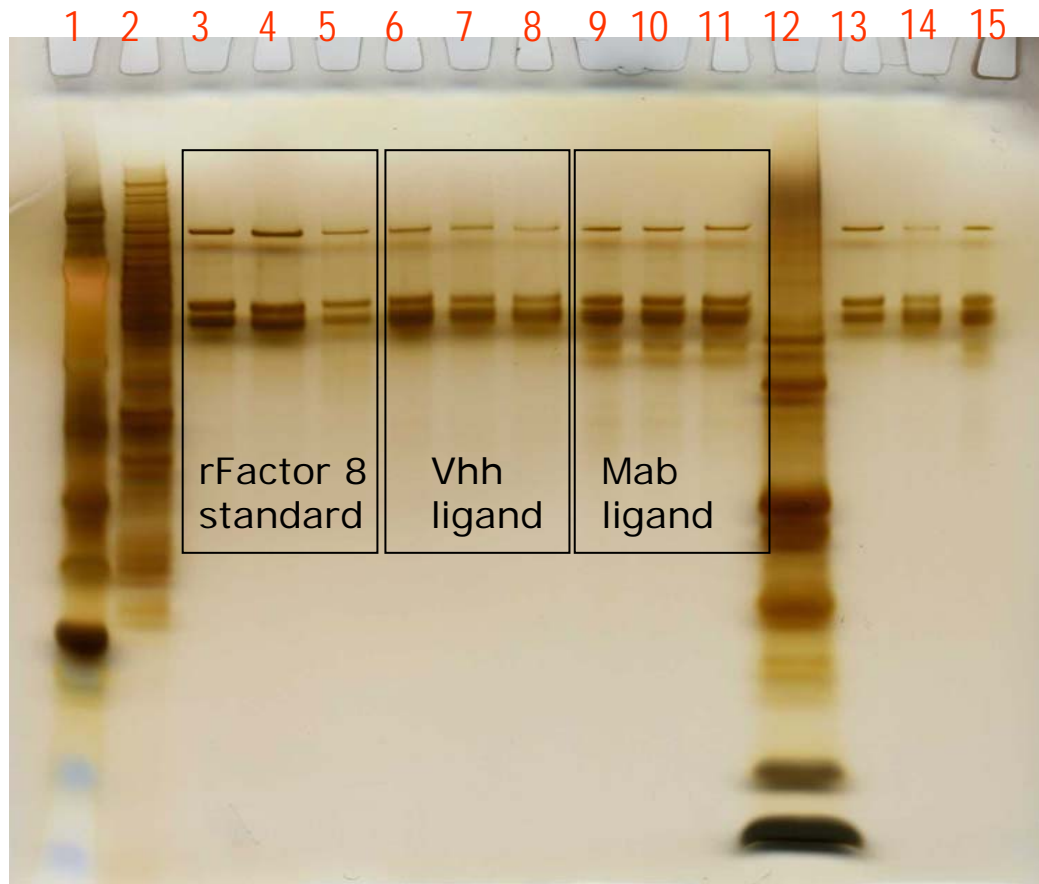
Screening of elution conditions in capture ELISA



Capture ELISA



Purification of rFactor VIII



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



Individual clones: AAT direct ELISA

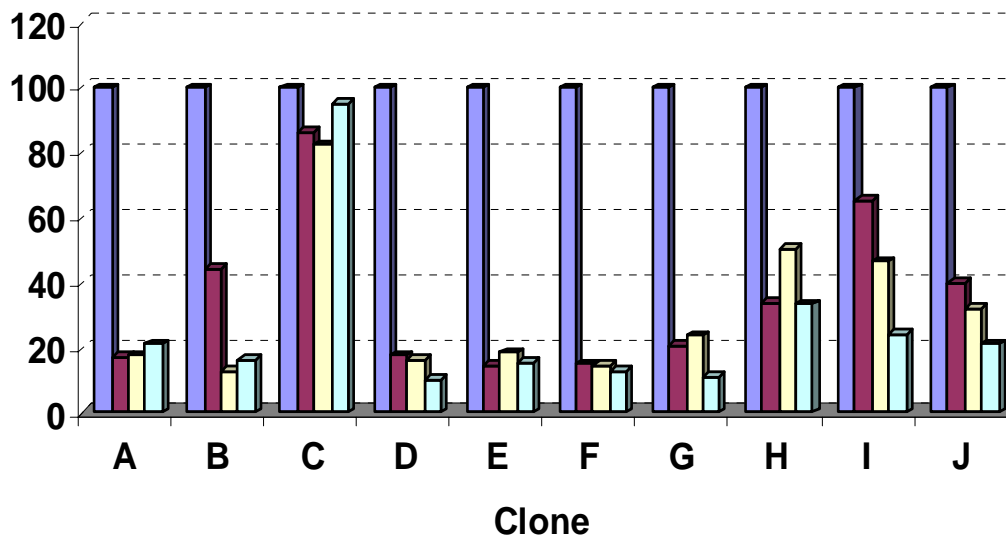
VHH clone	Binding buffer:			Elution Buffer 1			Elution Buffer 2			% EL-1 (10x)	% EL-2 (10x)
	10x	100x	1000x	10	100	1000	10	100	1000		
1F1	1.243	1.853	0.57	0.084	0.079	0.093	0.155	0.161	0.109	93	88
1H1	0.951	1.081	1.342	0.085	0.064	0.067	0.097	0.077	0.137	91	90
1D2	0.85	1.07	1.003	0.121	0.093	0.059	0.133	0.065	0.068	86	84
1E2	1.273	1.539	0.425	0.226	0.117	0.105	0.148	0.074	0.071	82	88
1D3	1.208	1.8	0.367	0.139	0.112	0.141	0.21	0.081	0.068	88	83
1D4	0.268	0.33	0.124	0.089	0.072	0.069	0.075	0.064	0.126	67	72
1E4	1.054	1.183	0.292	0.171	0.16	0.105	0.209	0.185	0.143	84	80
1D5	3.196	2.886	2.093	0.734	0.551	0.26	0.585	0.51	0.197	77	82
1F5	1.967	2.558	1.714	0.09	0.069	0.064	0.078	0.057	0.052	95	96
1G5	0.924	0.914	0.466	0.088	0.078	0.076	0.071	0.051	0.048	90	92
1H5	0.738	0.784	0.117	0.07	0.054	0.055	0.063	0.053	0.064	91	91
1A6	0.289	0.091	0.066	0.072	0.051	0.043	0.065	0.056	0.048	75	78
1E7	1.053	1.416	1.931	0.085	0.054	0.049	0.092	0.063	0.069	92	91
1A8	0.828	1.076	0.371	0.067	0.066	0.058	0.065	0.051	0.047	92	92
1C11	1.616	1.947	0.49	0.103	0.067	0.073	0.094	0.071	0.046	94	94
1G11	1.916	2.398	2.677	0.122	0.097	0.071	0.124	0.085	0.067	94	94
1A12	0.424	0.253	0.146	0.067	0.084	0.066	0.057	0.054	0.049	84	87
1B12	3.169	2.726	2.398	0.162	0.075	0.056	0.128	0.065	0.05	95	96
1E12	1.051	0.975	0.469	0.099	0.053	0.047	0.068	0.058	0.045	91	94
2E8	0.654	0.658	0.406	0.075	0.049	0.066	0.064	0.07	0.06	89	90
2H11	3.523	1.603	0.149	0.698	0.066	0.046	0.135	0.062	0.055	80	96
3E6	1.15	1.326	0.648	0.109	0.058	0.052	0.067	0.056	0.044	91	94
3E8	0.9	1.447	0.217	0.083	0.07	0.081	0.09	0.08	0.06	91	90
3B9	0.826	1.053	0.653	0.099	0.088	0.066	0.073	0.064	0.06	88	91
A2	3.007	0.126	0.071	2.082	0.063	0.051	1.409	0.075	0.053	31	53
F5	1.267	0.092	0.048	0.069	0.061	0.059	0.059	0.054	0.045	95	95
F7	2.407	1.938	0.22	0.091	0.064	0.055	0.062	0.053	0.049	96	97
1B3	3.623	3.501	1.592	1.855	1.925	0.275	0.736	0.695	0.115	49	80
1B8	3.657	3.483	0.338	3.39	2.12	0.129	0.66	0.374	0.062	7	82
1G6	0.41	0.068	0.062	0.156	0.057	0.063	0.103	0.05	0.049	62	75
2A8	3.1	0.109	0.062	1.179	0.065	0.056	0.145	0.054	0.06	62	95
E2	2.993	3.138	0.325	1.116	0.768	0.088	0.976	0.879	0.105	63	67
G2	3.595	3.514	1.522	0.491	0.322	0.104	0.131	0.1	0.073	86	96
G7	0.213	0.063	0.057	0.069	0.057	0.06	0.052	0.045	0.047	68	76
A11	1.155	1.072	0.235	0.071	0.06	0.067	0.06	0.054	0.049	94	95
blank	0.063	0.082	0.058	0.056	0.056	0.06	0.066	0.051	0.058		



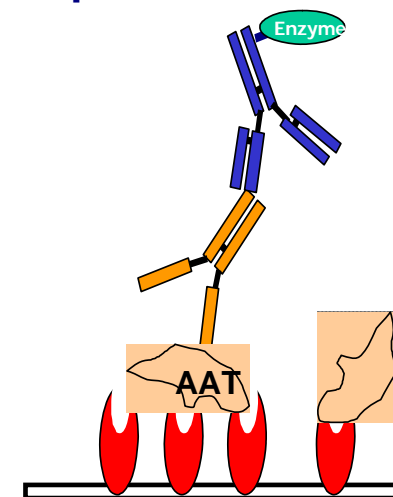
Elution profile: AAT ligands

Screening of elution conditions in capture ELISA

Residual
Binding (%)

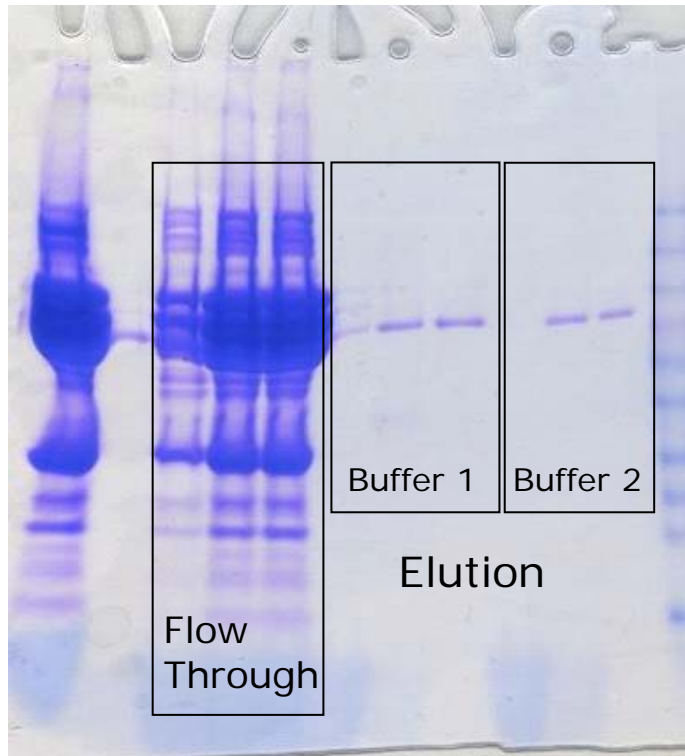


Capture ELISA

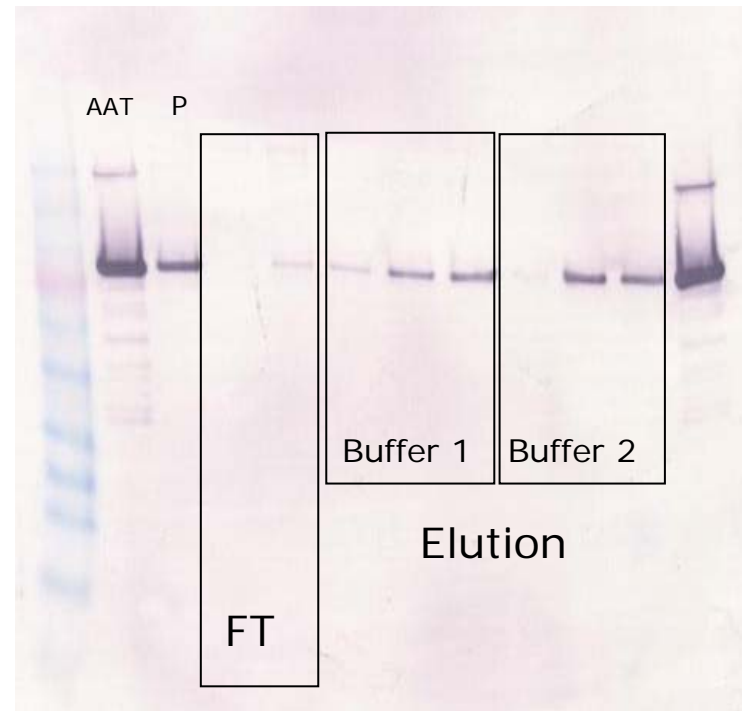


Purification of AAT

Cohn Fraction IV
-coomassie stained-



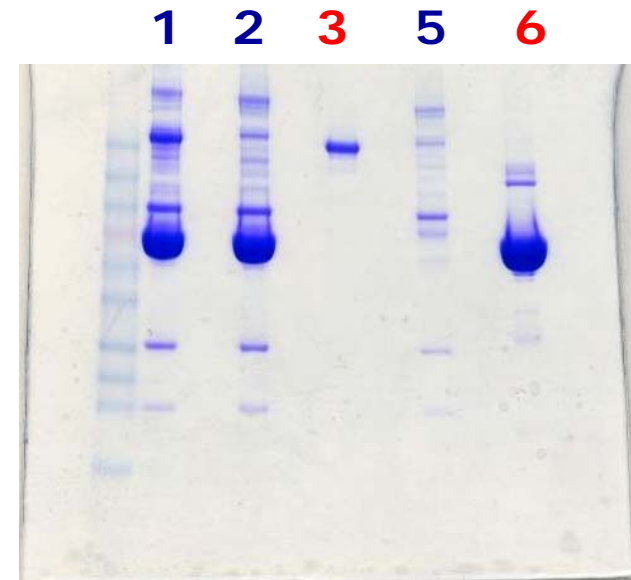
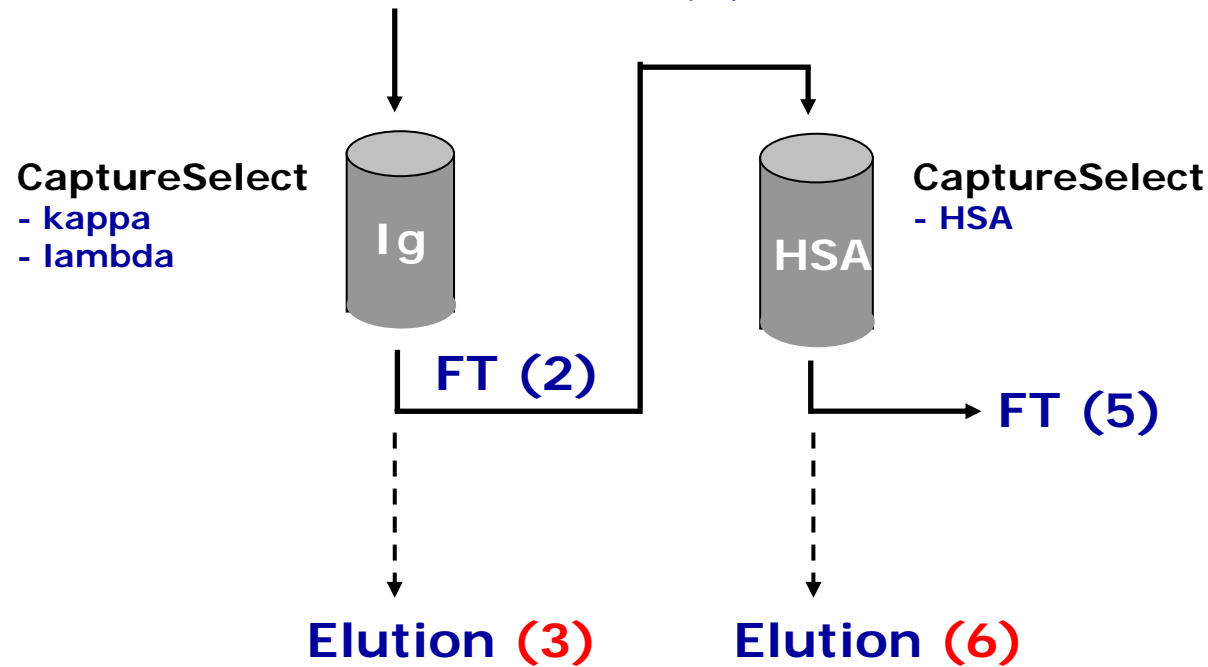
Plasma
-western blot-



Multi Targets: Human Plasma Library

Antigen: human plasma depleted from Immunoglobulins and Albumin

Human Plasma (1)

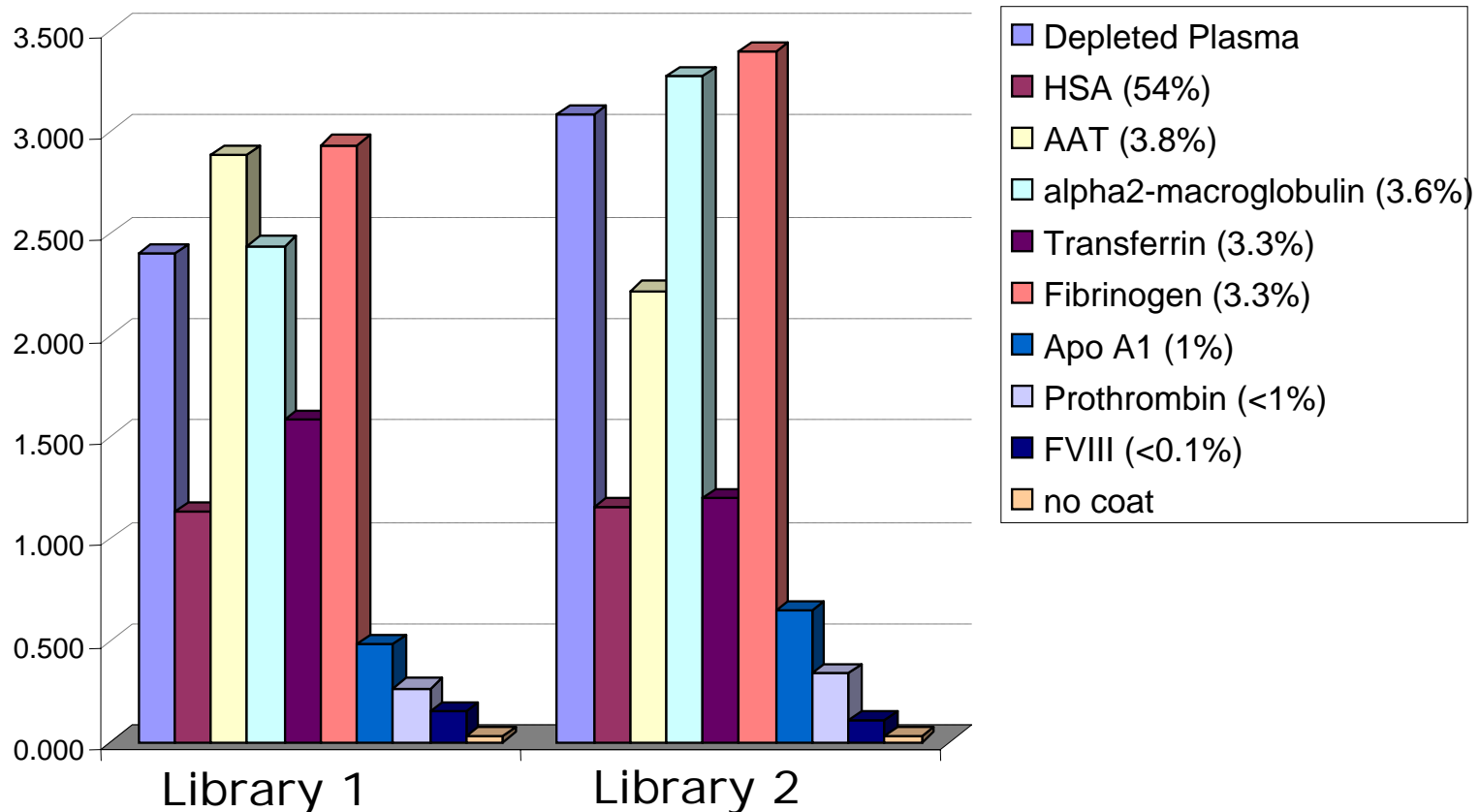


FT Sample 5: Plasma depleted from albumin, IgG, IgM, IgA
(= ±70% of total protein content in plasma)



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

BAC's CaptureSelect technology has been used successfully:

- in obtaining suitable affinity ligands for the purification of plasma proteins, either native or recombinant
- in screening for highly specific elution conditions, facilitating further purification or to increase product stability during down-stream processing
- in immobilizing ligands on standard media resulting in highly specific purification of plasma proteins
- used to generate multi-target libraries, reducing time to develop new affinity ligands against other targets considerably

