



*Development and Kinetic Characterization of CaptureSelect
Affinity Ligands Using Bio-Layer Interferometry*

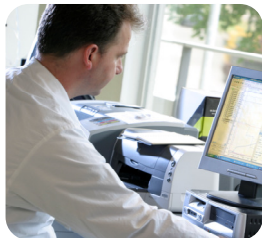
European Label-Free Assays for Bioprocessing, Protein Characterization and Octet Workshop

14 October 2011 Hannover

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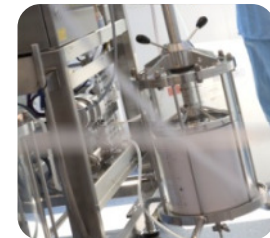
- Discovery, development and manufacturing of affinity ligands and affinity purification products
- Founded on unique technology, know-how and IP as Unilever spin-off in 2005
- Products increase purity and yield, lower cost of goods and reduce time-to-market for end-users



Ligand design service



CaptureSelect for research



CaptureSelect® for manufacturing

Questions Addressed



How to develop Tools for optimizing Library Screening Processes:

- Library screening under application conditions is key
 - Application = affinity chromatography



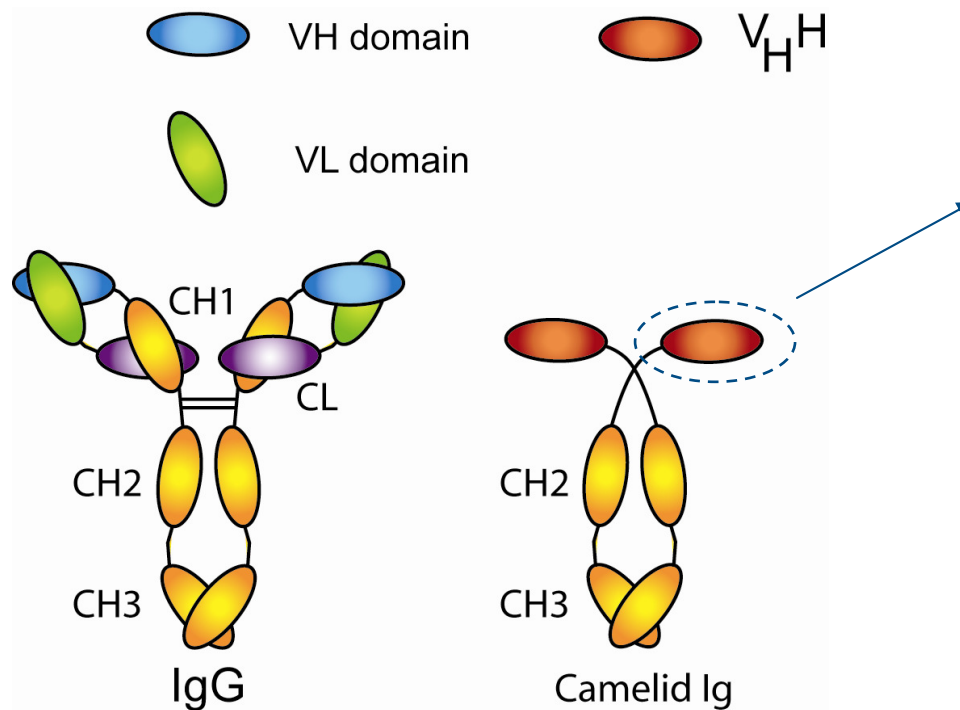
Label free and “in real time” binding assays can enable above screening criteria

Implementation of BLI : providing flexibility in setting up screening assays for the different key criteria

CaptureSelect® Affinity Ligands



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



Advantages VHH:

- ~ 12-15 kDa fragment
- ~ Tunable specificity
- ~ 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

- ➔ Affinity Purification: R&D, small scale → bioprocess scale manufacturing
- ➔ Affinity Analytics: HPLC-format, Capture agents for ELISA / BLI / SPR

Affinity Capture – Key Advantages



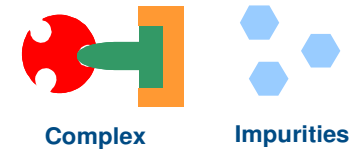
~ Selectivity

- 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



~ A platform for downstream processing of biopharmaceuticals

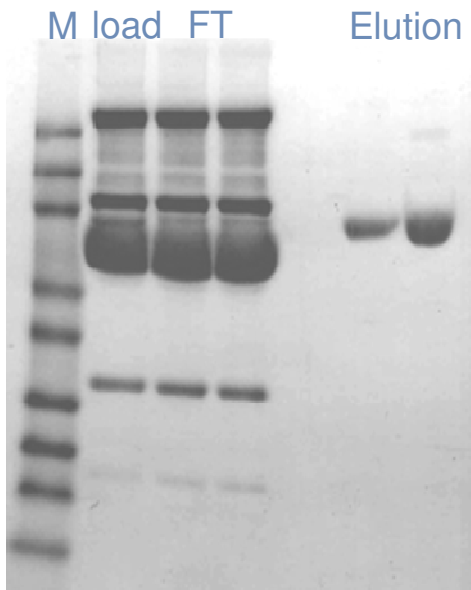
- generic process: e.g. 1st capture – polishing - viral filtration – UF/DF (Prot-A)

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

Broad Target Range: Proteins, Viruses and Antibodies

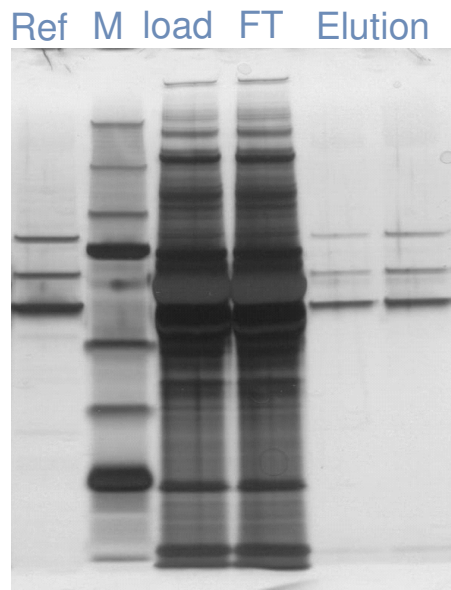


Alpha-1 Antitrypsin



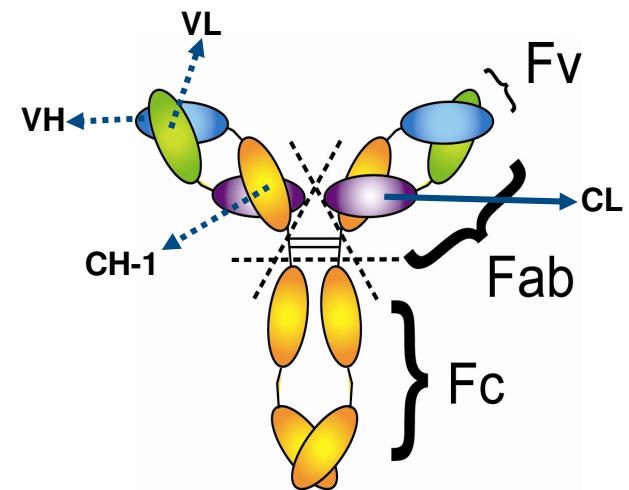
Mild elution conditions
 High Purity (single step)
 High Yield
 Product: α 1 Antitrypsin Select

Adeno-Associated Virus



High single step purity
 AVB-Sepharose

Antibody Formats



Unique Specificities
 IgSelect
 KappaSelect
 LambdaSelect

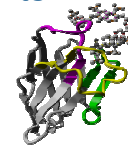


CaptureSelect®: Technology Platform



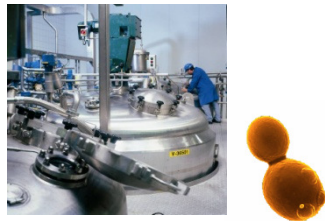
1) Discovery of VHH binding fragments

- Immunize
- Construct expression libraries



5) Affinity product

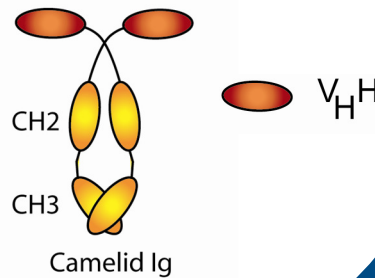
- R&D purposes
- Clinical & commercial manufacturing



4) Ligand production

- Pick lead candidate
- Produce in-house at large scale

CaptureSelect®



-
- Binding
 - Specificity
 - Elution
 - Ligand Stability

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



Integration of Octet in Ligand Discovery Process




BLI: label free screening of binding parameters relating to affinity chromatography

Ligand screening in Octet; Examples:

- specificity
- binding affinity
- elution profiles

Analytical Applications

- introducing CaptureSelect affinity ligands in Octet Platform

- 
- Binding
 - Specificity
 - Elution
 - Ligand Stability



The Octet System

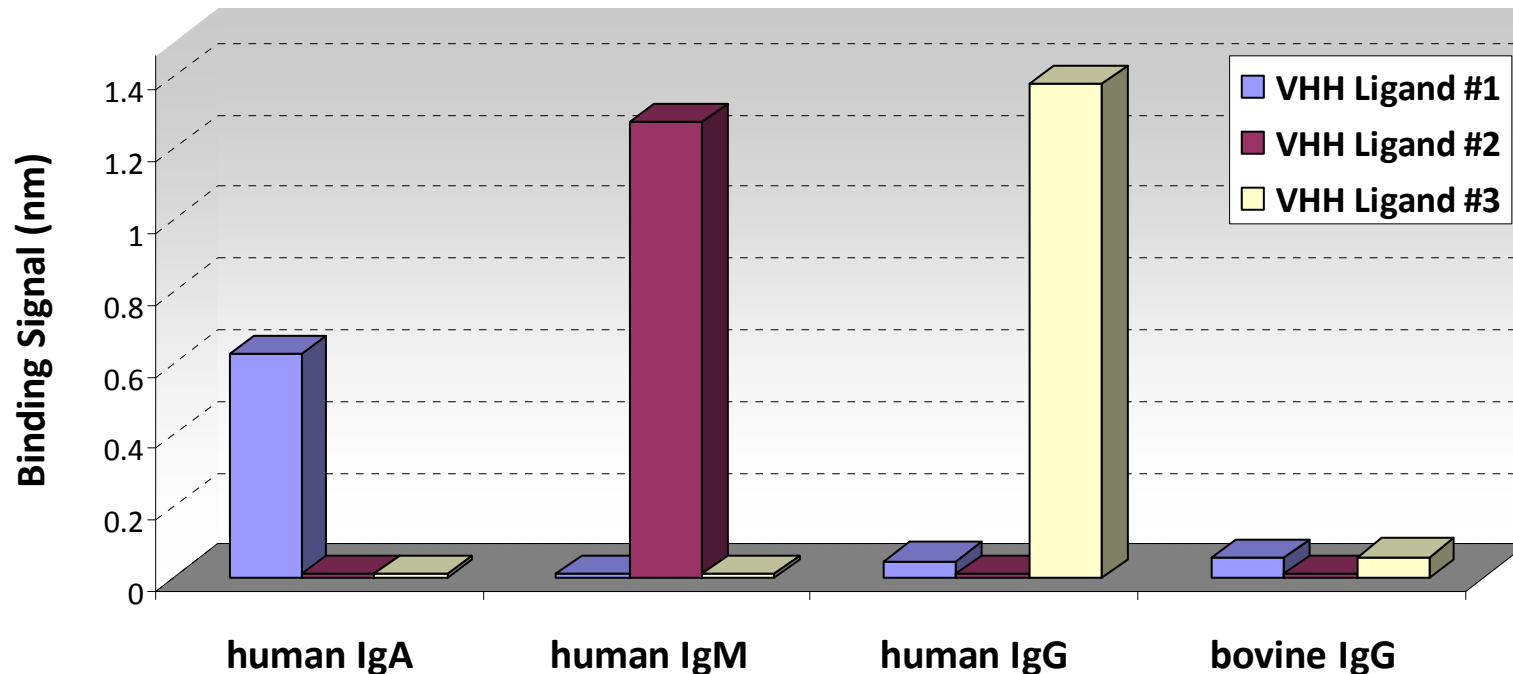


Lead Ligand

Ligand Specificity measurements in Octet



➤ Affinity ligands screened against different antibody isotypes



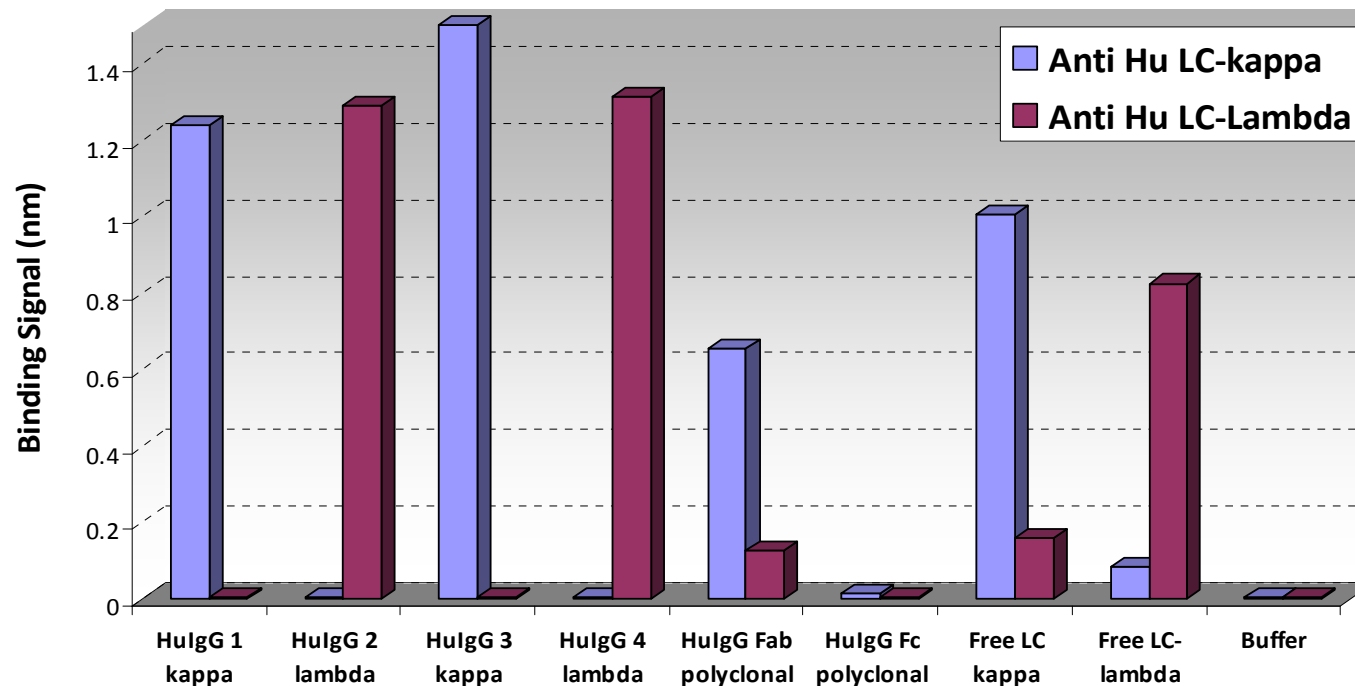
OctetQK

- 8 samples: 35 min
- 16 samples: 70 min

Capture ELISA

- comparable results
- 1- 96 samples: 6 hrs

➤ Affinity ligands directed against different human light chains



➤ Octet binding analysis for 2nd screen:

- correlating to final application format
- quick and easy

Ligand Affinity Screening in Octet



~ Ligand Affinity Ranking of clones derived from “Mutant Libraries”



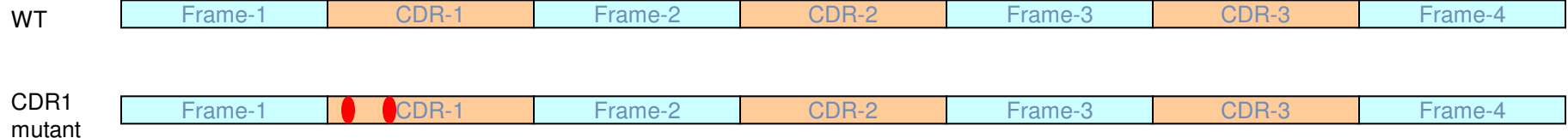
~ TAPE: Targeted Accelerated Protein Evolution (Pherecydes Pharma)

- a patented technology allowing to rapidly & simultaneously introduce defined densities of random mutations in any number of selected regions within a gene while conserving intact any number of defined coding domains in this same gene

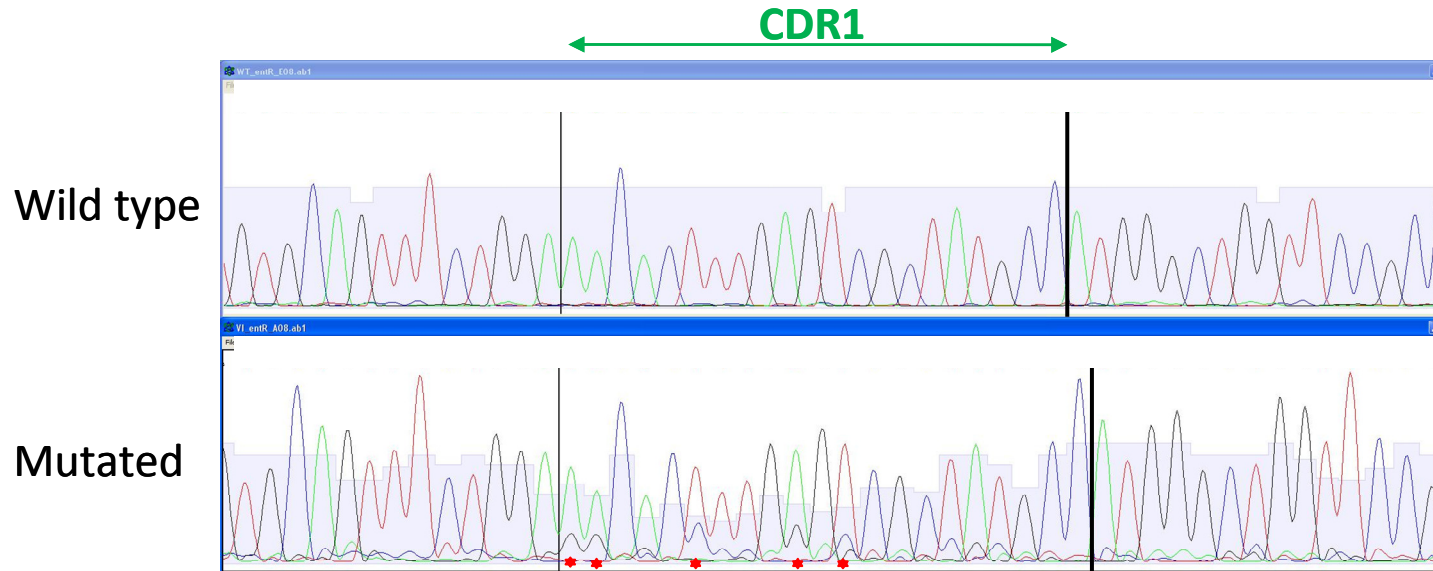
▶ for more information, please contact jerome.gabard@pherecydes-pharma.com

~ POP: TAPE technology for VHH format

- defined hot spot (mutation) regions: CDR1 or 2 or 3
- random VHH libraries created with sequence variability in either CDR1, 2 or 3



Ligand Affinity Screening in Octet



Kinetic parameters of Positive clones from Variant Library 1

VHH Ligand #	Target	VHH	ka	kd	KD
3	1.3090	1.5926	2.22E+05	1.05E-03	4.73E-09
7	1.3370	1.6383	1.70E+05	1.90E-03	1.12E-08
10	1.0301	1.5203	4.02E+05	1.69E-03	4.20E-09
12	1.1366	1.6546	2.09E+05	8.73E-04	4.18E-09
14	0.6630	1.4719	3.22E+05	1.87E-03	5.81E-09
15	0.9769	1.5180	3.19E+05	1.10E-03	3.45E-09
16	1.3213	1.5772	1.77E+05	6.15E-04	3.47E-09
26	1.4637	1.6056	1.57E+05	2.30E-03	1.46E-08
32	1.1185	1.5438	2.15E+05	1.09E-03	5.07E-09
36	0.9509	1.6804	2.90E+05	1.35E-03	4.66E-09
40	1.2077	1.5269	1.91E+05	1.60E-03	8.38E-09
42	1.3894	1.6505	2.71E+05	1.27E-03	4.69E-09
WT	1.3086	1.6953	1.81E+05	6.55E-04	3.62E-09

In grey: comparable value compared to WT
 In orange: decreased affinity compared to WT
 In green: increased affinity compared to WT

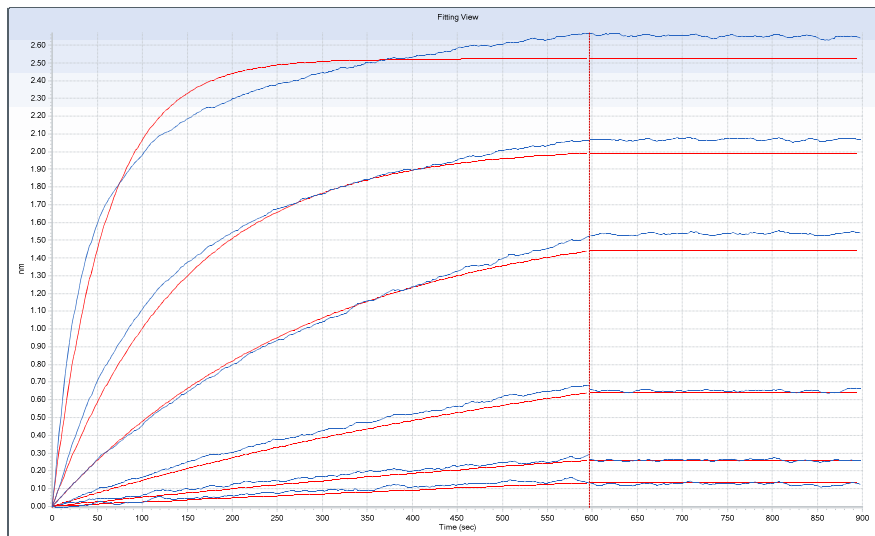
CDR1 point mutations identified with improved on-rate characteristics

Binding Affinity Measurements of Ligand Lead in Octet



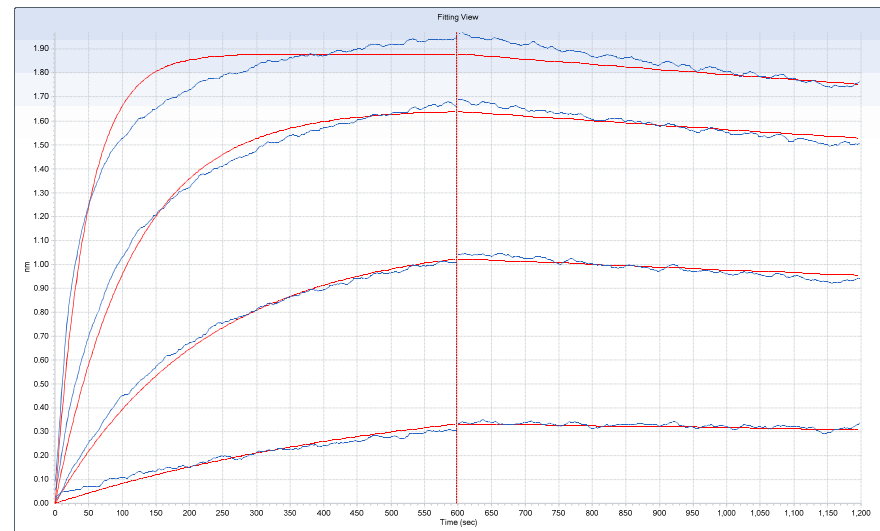
- Lead Affinity Ligand: anti human LC-kappa
 - affinity measurements for IgG and Fab

Biotin anti-LC-kappa (Hu): rHlgG-1 kappa



KD (M)	kon(1/Ms)	kon Error	kdis(1/s)	kdis Error
7.59E-11	9.61E+04	1.83E+03	7.29E-06	2.29E-05

Biotin anti-LC-kappa (Hu): rHlgG-1 kappa Fab#



KD (M)	kon(1/Ms)	kon Error	kdis(1/s)	kdis Error
2.73E-09	4.29E+04	5.30E+02	1.17E-04	7.29E-06

- Good correlation with Biacore
 - clear effect on off-rate due to bivalent binding

- ♫ BLI enables set-up of a diverse set of tools for ligand screening relating to the final application: affinity chromatography
 - specificity
 - affinity profiles
 - elution behavior

- ♫ Flexible system
 - quick and easy
 - idea -> answer within an hour

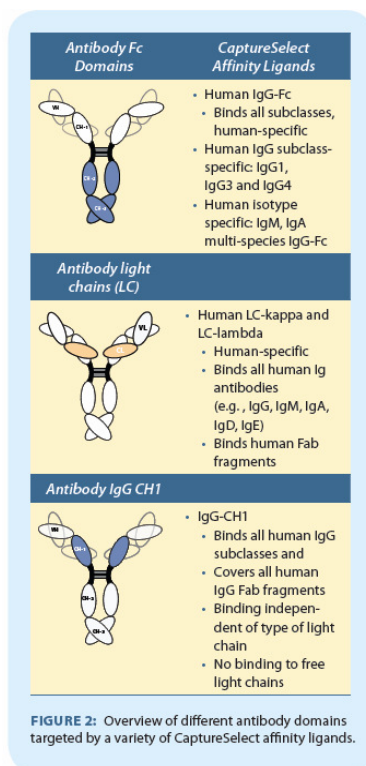
- ♫ Has speed up the process of identifying leads for final affinity products

Analytical Applications in Octet



Introducing CaptureSelect Affinity Ligands for Antibody Detection and Characterization on the Octet Platform

– article in up coming Fall 2011 “interactions” newsletter



Antibody Target	Isotype/ Subclass	CaptureSelect Biotin Conjugates				
		Biotin IgG-Fc (Hu)	Biotin IgG-CH1	Biotin LC-kappa (Hu)	Biotin LC-lambda (Hu)	Protein L Biosensor
IgG Subclasses	Hu IgG-1 kappa	√	√	√	–	–
	Hu IgG-2 lambda	√	√	–	√	–
	Hu IgG-3 kappa	√	√	√	–	√
	Hu IgG-4 lambda	√	√	–	√	–
Ab Isotypes	Hu IgM kappa	–	–	√	–	√
	Hu IgD kappa	–	–	√	–	√
	Hu IgE kappa	–	–	√	–	√
	Hu IgA (poly)	–	–	√	√	√
Ab Fragments	Hu IgG Fab (poly)	–	√	√	√	√
	Hu IgG Fc (poly)	√	–	–	–	–
	Free Hu LC kappa	–	–	√	–	√
	Free Hu LC lambda	–	–	–	√	–
Species	Bovine IgG	–	–	–	–	–
	Mouse IgG	–	–	–	–	√

TABLE 1: Binding selectivity of CaptureSelect Biotin Conjugates.

CaptureSelect Biotin Conjugates



- New reagents for setting up a variety of analytical tools for detection, quantitation and characterization/screening of antibodies

CaptureSelect Biotin Conjugates	Product Code (100 µg)	Antibody Domain Targeted
Biotin anti-LC-kappa (Hu)	710.0833.100	human kappa light chain (constant domain)
Biotin anti-LC-lambda (Hu)	710.3080.100	human lambda light chain (constant domain)
Biotin anti-IgG-Fc (Hu)	710.0822.100	Fc of human IgG (CH3 domain)
<i>Biotin anti-IgG-CH1</i>	<i>in development</i>	<i>CH1 of human IgG (including e.g. dog, cat)</i>
<i>Biotin anti-IgA</i>	<i>in development</i>	<i>Fc of human IgA (dimeric- and secretory IgA)</i>
<i>Biotin anti-IgM</i>	<i>in development</i>	<i>µ-chain of human IgM (including mouse and rat)</i>
<i>Biotin anti-IgG-Fc (ms)</i>	<i>in development</i>	<i>Fc of IgG (multi-species)</i>

For more information on CaptureSelect affinity products, please visit our website - www.captureselect.com - or send an E-mail to ligands@captureselect.com



The **Affinity** Experts

Thanks for your attention !