



The Use of HTRF in Biologics Discovery

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Protein Engineering

HTRF Symposium

26th April 2013

Overview

- 1 Benefits of HTRF in Biologics discovery
- 2 Assay formats employed at MedImmune
- 3 Case study – Anti IL-6 antibody
- 4 Summary

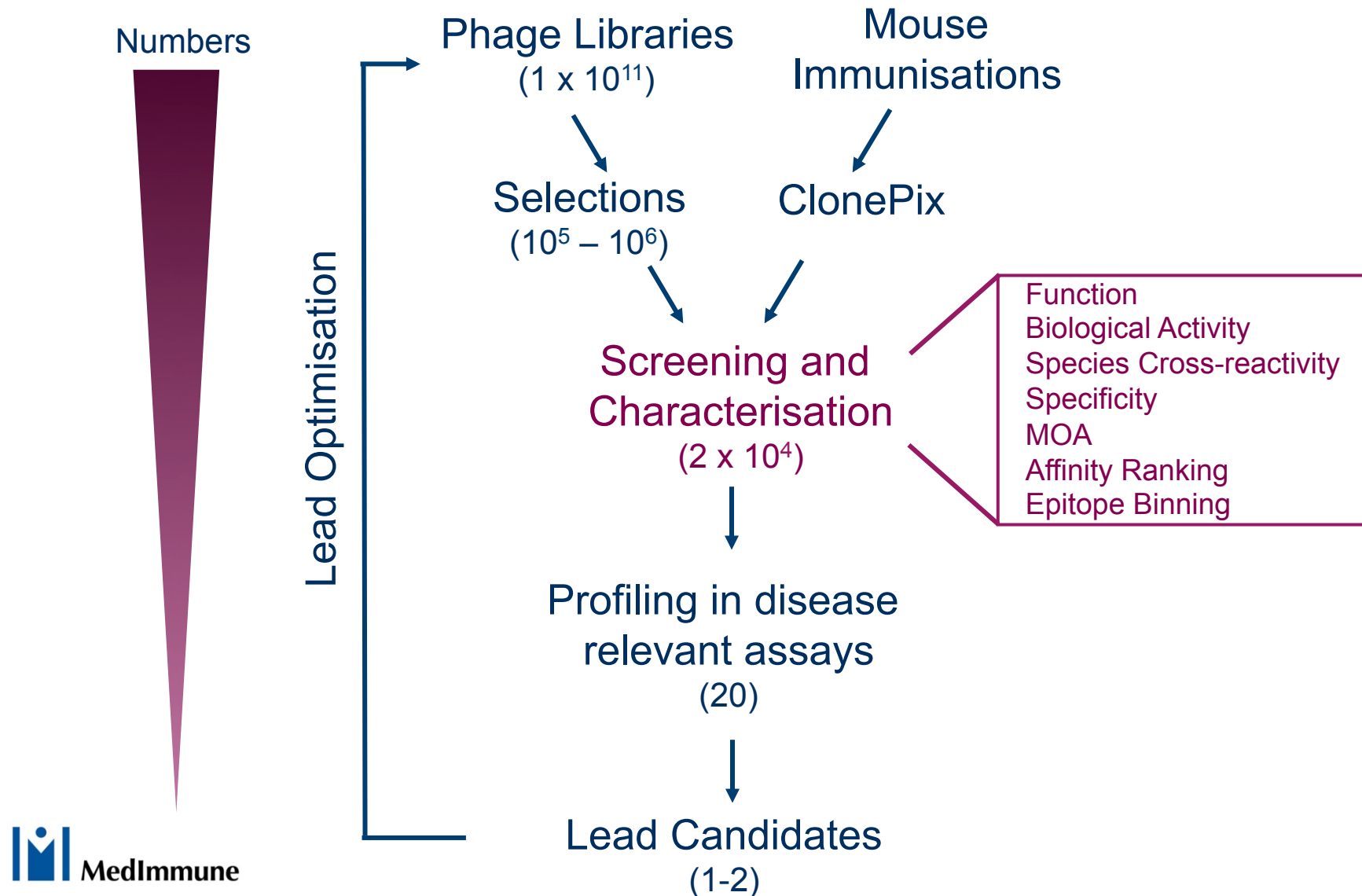
Profile of a Therapeutic Antibody

Therapeutic Antibody
Function
Mechanism of Action
Affinity
Specificity
Species Cross-reactivity
Lack of toxicity - on target - off target
Chemical, thermal and structural stability
Novelty

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Lead Generation Workflow



Benefits of HTRF in Biologics Discovery

◆ Homogeneous

- Reduces assay variability
- Reduces assay development time
- Reduces hands on lab time

◆ High throughput

- Routinely use 384 well
- Low volume assays (10 μ L)

◆ Available 'toolbox' reagents

◆ Sensitive assay format

◆ Tolerance to unpurified antibody samples

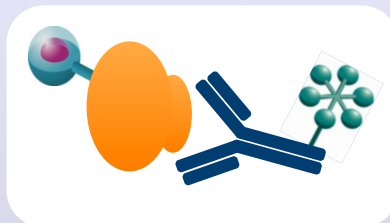
- Bacterial and mammalian cell expressed

◆ Adaptable format for many targets

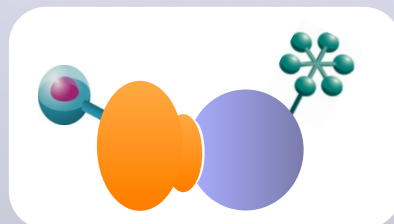
Adaptable Formats

Direct Antibody
Binding

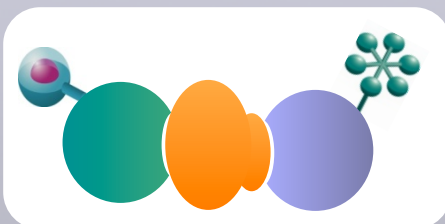
Direct Labelling



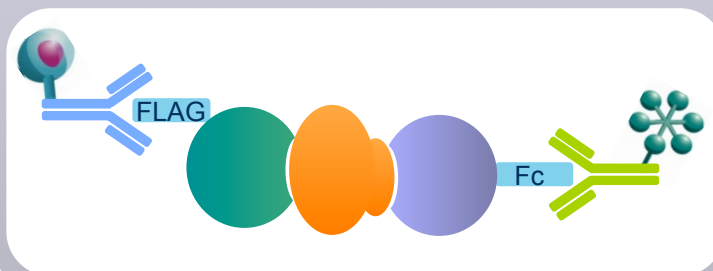
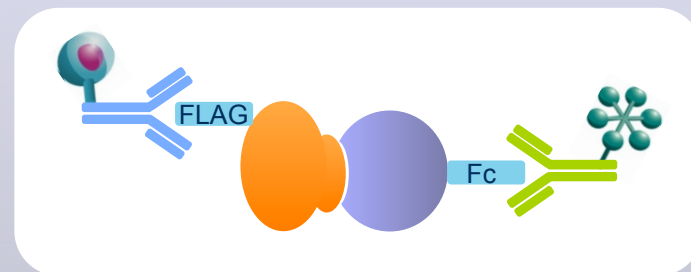
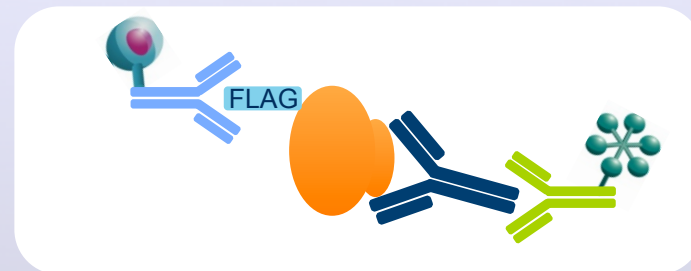
Receptor:Ligand
Binding



Co-receptor:
Ligand Binding



Indirect Labelling



Points to Consider

Direct Labelling

Pros

- ◆ Minimal reagent addition steps
- ◆ Avoids unwanted tag effects
- ◆ Can facilitate use of very low concentrations of reagents

Cons

- ◆ Effects of labelling on biological activity
- ◆ Batch variation
- ◆ Non-homogeneous labelling

Indirect Labelling

- ◆ Toolbox reagents available
- ◆ Maximise assay flexibility

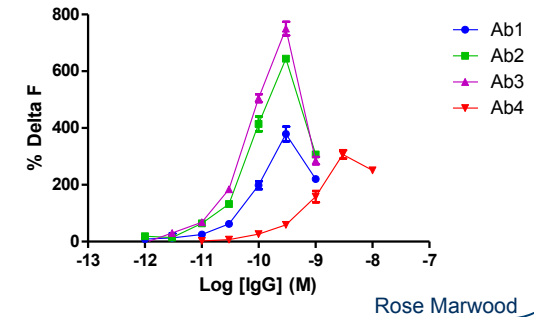
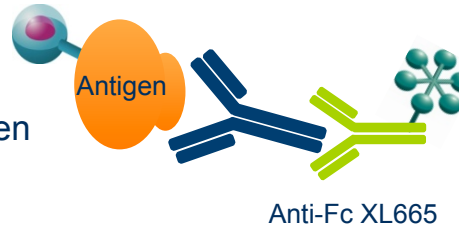
- ◆ Can require pre-incubation of reagent and detection pairs
- ◆ Possible 'tag' effects
- ◆ Possibility of steric hindrance

- ◆ Some detection reagent or tag pairs can't be used, e.g. anti-Human Fc if inhibitor is a human IgG, anti-Flag in combination with anti-mouse Fc
- ◆ The orientation of donor and acceptor can be optimised for specific assay requirements

Assay Formats for HTS

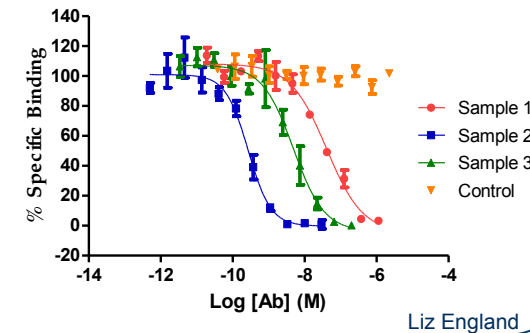
Direct Binding

- ◆ Simplest form of HTRF Assay
- ◆ Measures binding of antibody to antigen
- ◆ Will observe hook effect as detection reagent becomes saturated



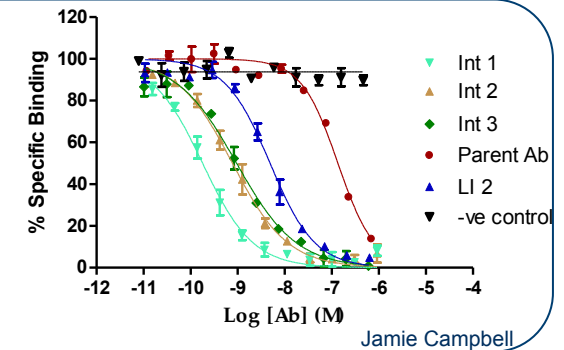
Receptor-Ligand Inhibition

- ◆ Competition Assay
- ◆ Measures binding of ligand to receptor
- ◆ Measures inhibition of interaction by competing antibodies



Epitope Competition

- ◆ Competition Assay
- ◆ Measures binding of antibody to antigen
- ◆ Measures inhibition of interaction by competing antibodies



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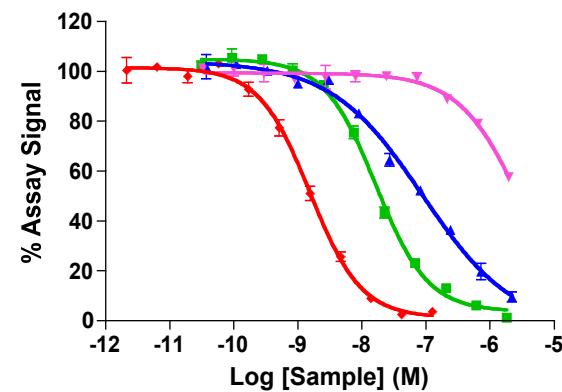
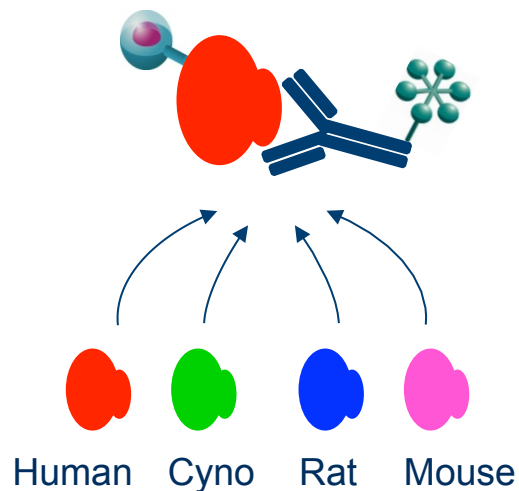
Cross-Reactivity

◆ Ligand receptor assays

- E.g. human ligand:human receptor versus cyno ligand:cyno receptor
- Use in parallel single point screening assays and for potency determination

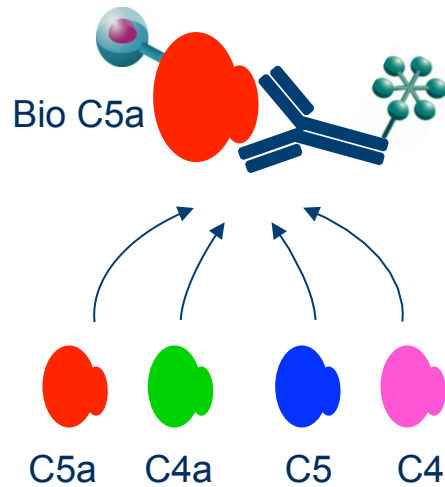
◆ Epitope competition assay format

- Compares ability to bind to antigen with a single assay format
- Fold difference between species for toxicology purposes

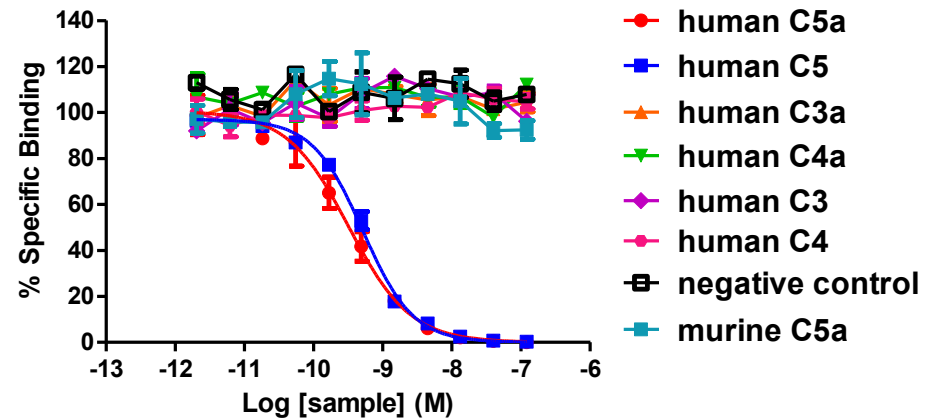


Data produced by
Carl Matthews

Specificity



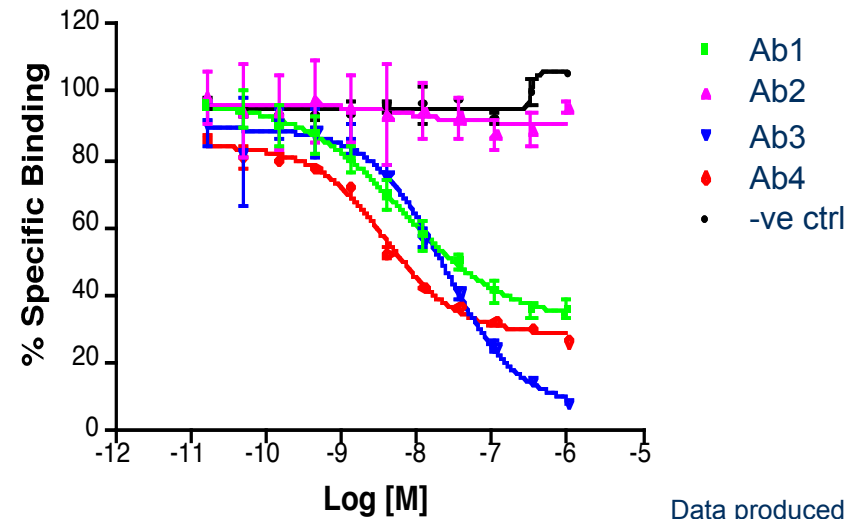
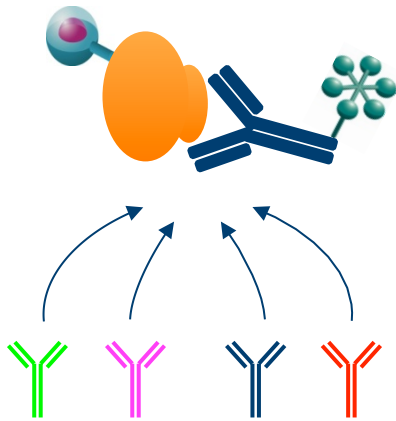
Competition assay
binding of bio human C5a to
optimised anti-C5/C5a mAb



Data produced by
Caz Colley

- ◆ Epitope competition assay format
- ◆ Confirm antibody specificity
- ◆ Early screen for potential off target affects

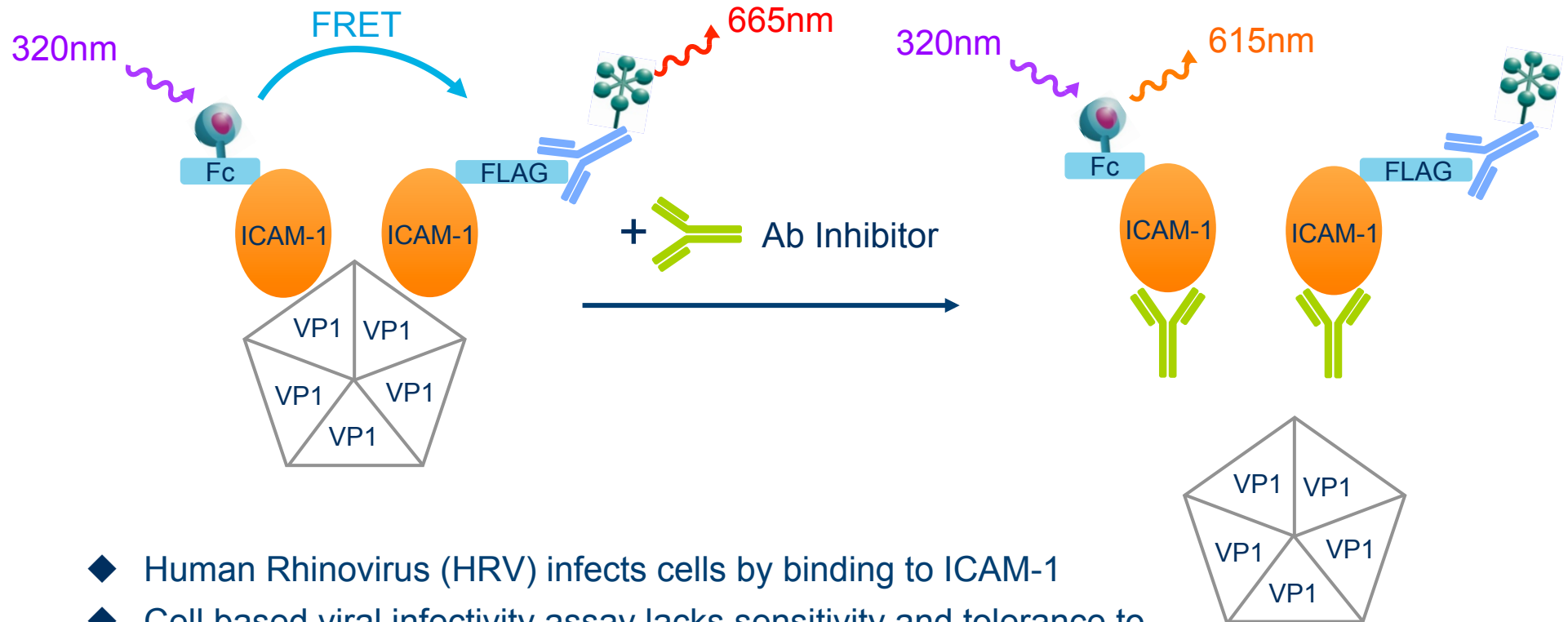
Epitope Binning



Data produced by
Carl Matthews

- ◆ Assay set up to measure binding of Ab3 to antigen
- ◆ Compete in all antibodies
- ◆ Ab1 and Ab4 have overlapping epitope relative to Ab3
- ◆ Ab2 has a completely different epitope to Ab3

Viral Inhibition Assay

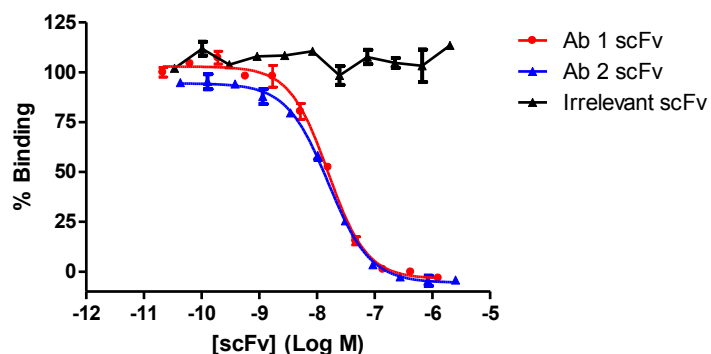


- ◆ Human Rhinovirus (HRV) infects cells by binding to ICAM-1
- ◆ Cell based viral infectivity assay lacks sensitivity and tolerance to bacterial samples
- ◆ HTRF assay designed to detect inhibitors of the HRV-ICAM:1 interaction
 - Good correlation to viral infectivity assay

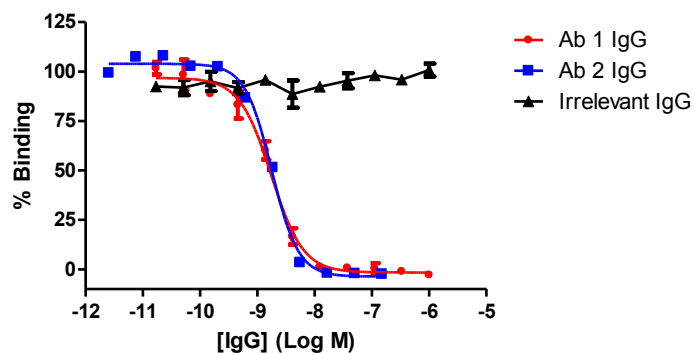
Identification of Inhibitors of HRV Infection

HTRF Assay

scFv

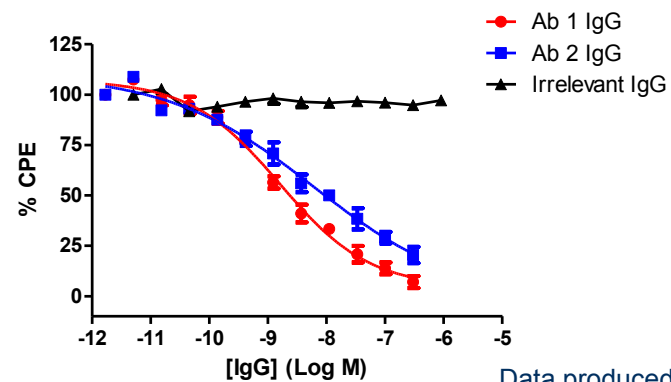
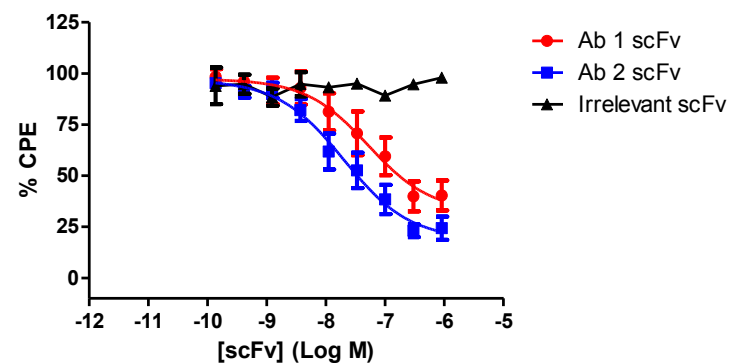


IgG



Data produced by Phil Newton

Viral Infectivity Assay



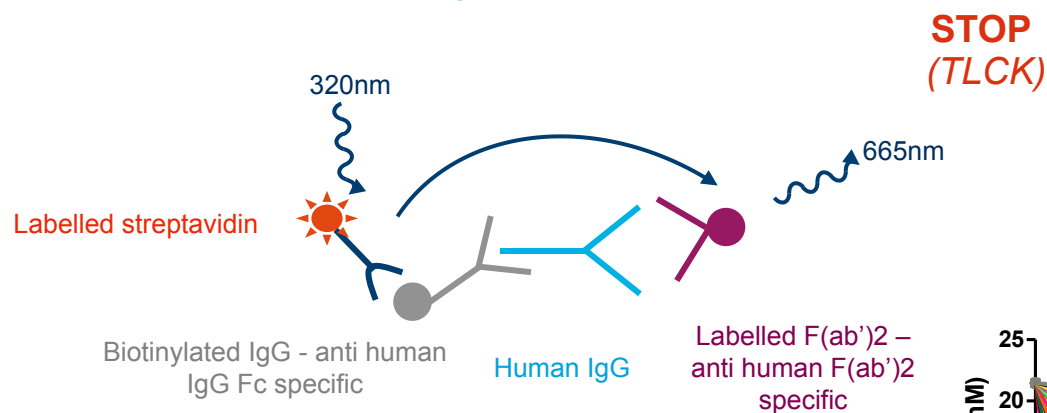
Data produced by Rebecca Dunmore

Enzyme Activity Assays

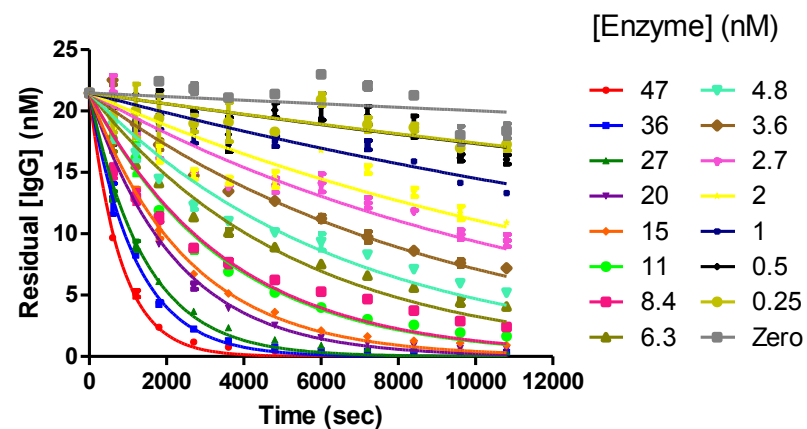
Step 1 = IgG cleavage reaction



Step 2 = HTRF residual IgG quantification

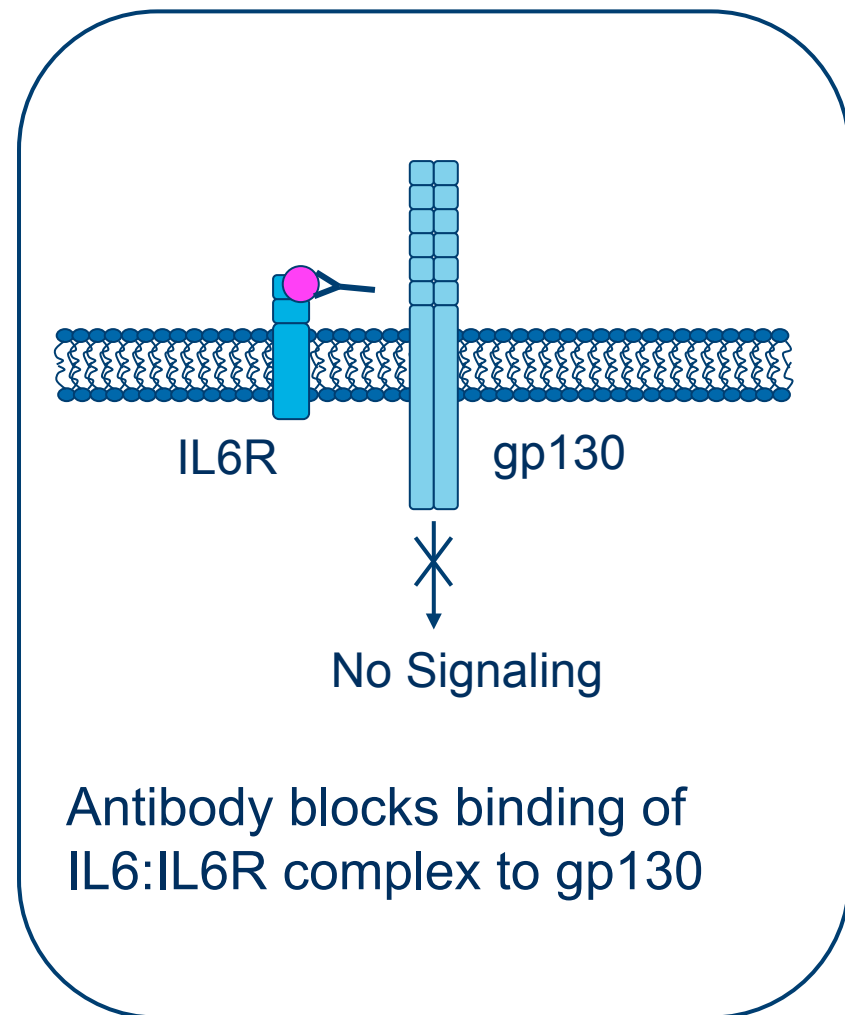
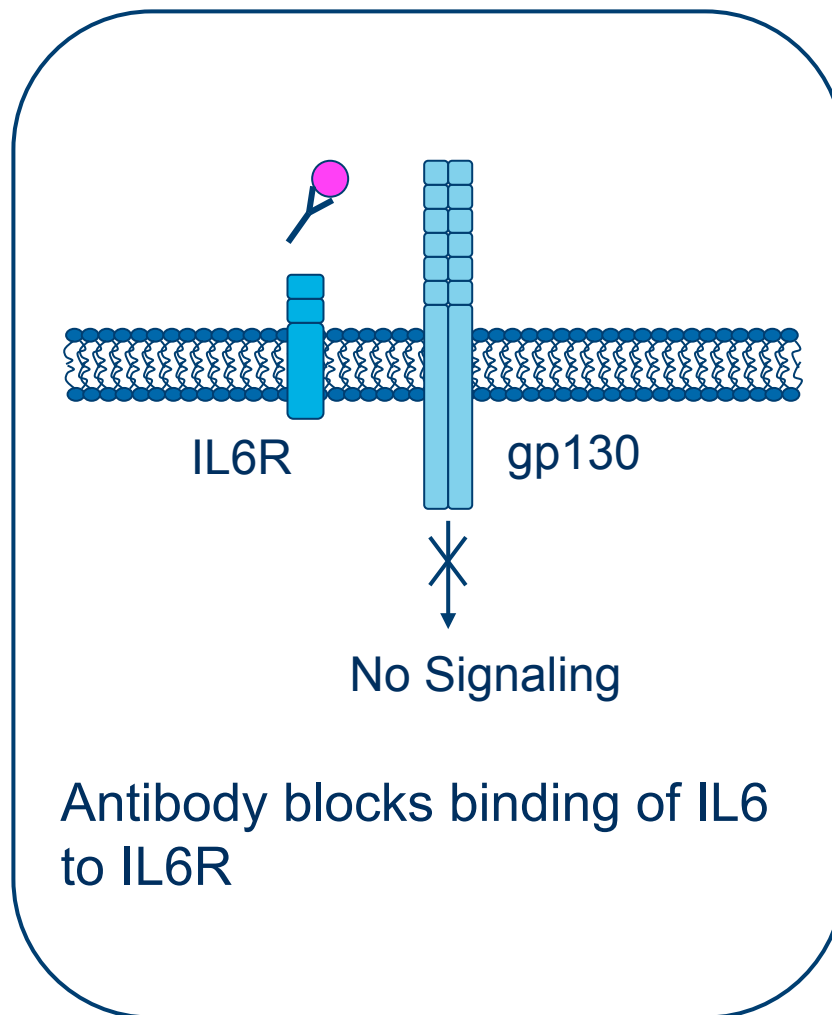


Measures 'k_{cat} / K_m'



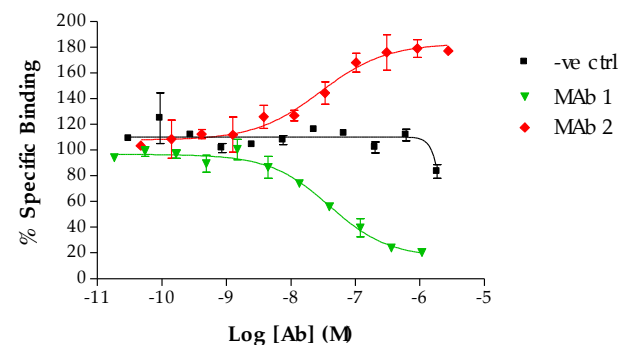
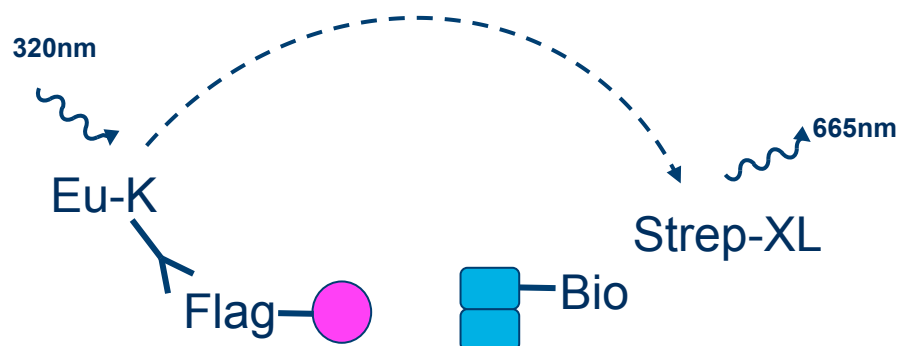
Case Study: Anti-IL6

Antibody Mechanism of Action



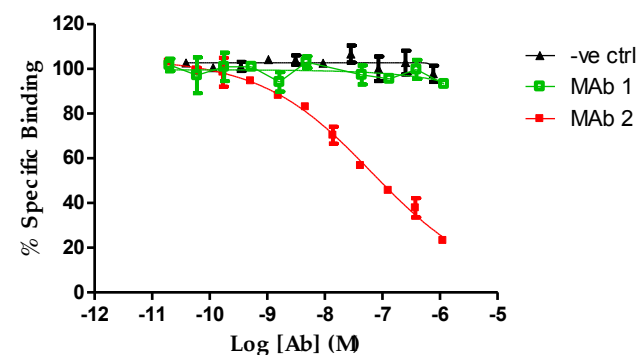
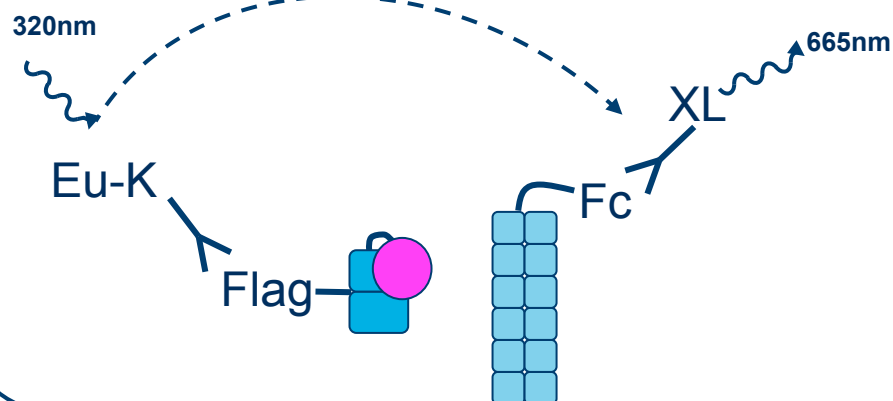
Identifying Alternative Mechanisms of Action Screening Assays

Assay Configuration 1 – IL6: IL6R



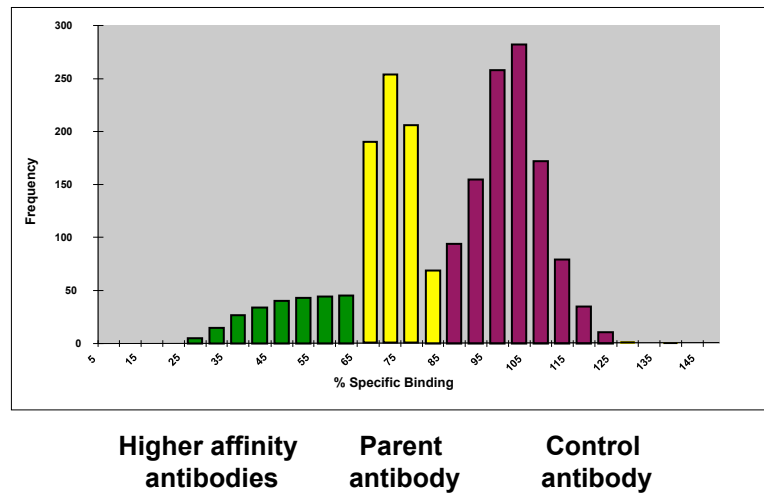
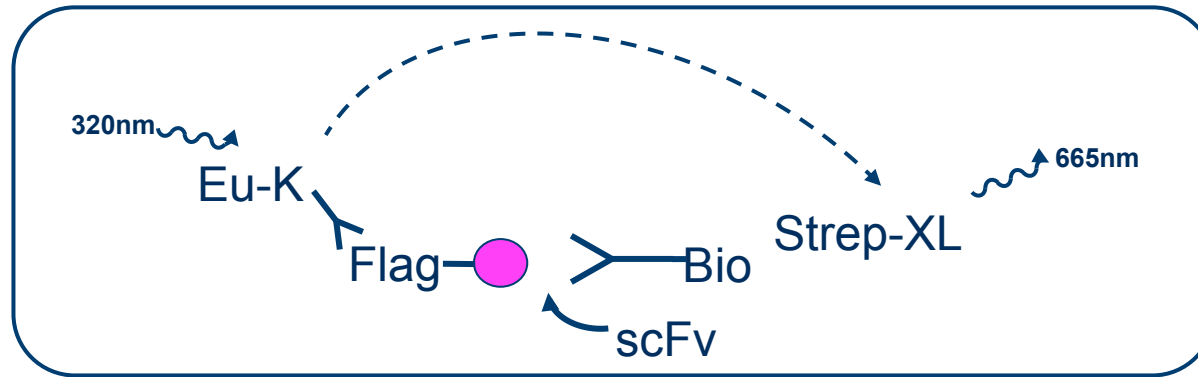
- ◆ MAb 1 – binds to IL6 and inhibits IL6 binding to IL6R

Assay Configuration 2 – Tethered IL6_IL6R: gp130

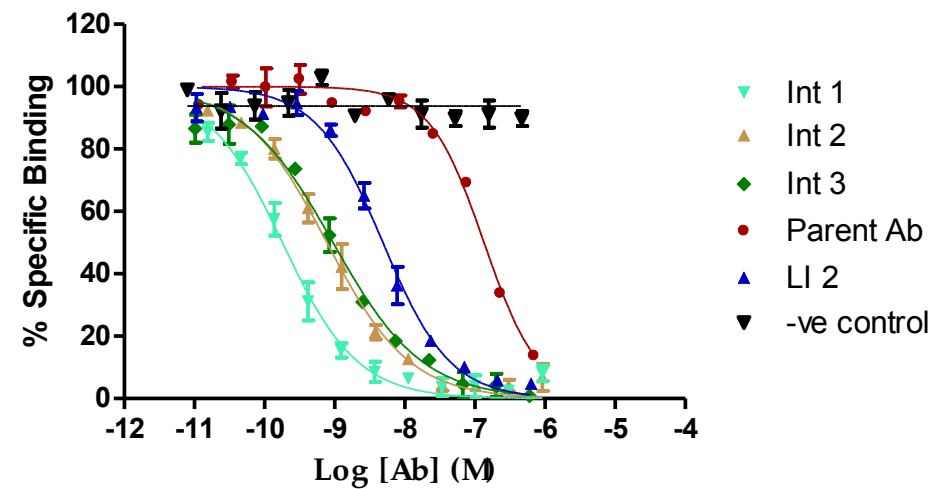


- ◆ MAb 2 – binds to the complex of IL6 and IL6R and inhibits recruitment of gp130

Lead Optimisation – Epitope Competition Assay



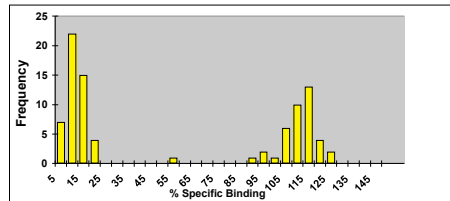
Epitope Competition Assay



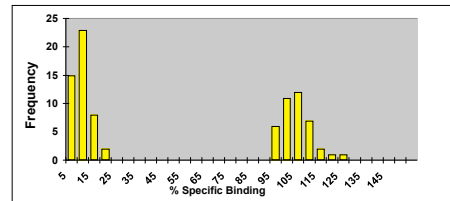
Improving Assay Sensitivity to Identify High Affinity Antibodies

Parent Epitope Competition HTS

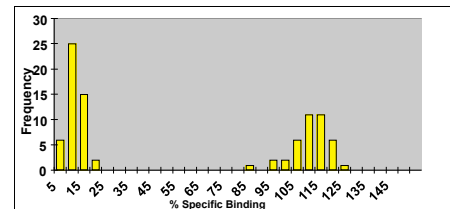
5.0% sample
(approx 2-20nM
scFv)



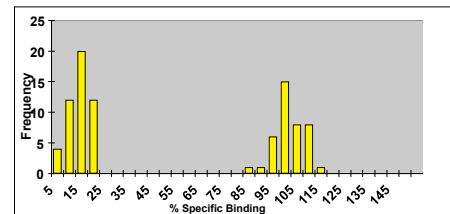
1.25% sample
(approx 0.5-5nM
scFv)



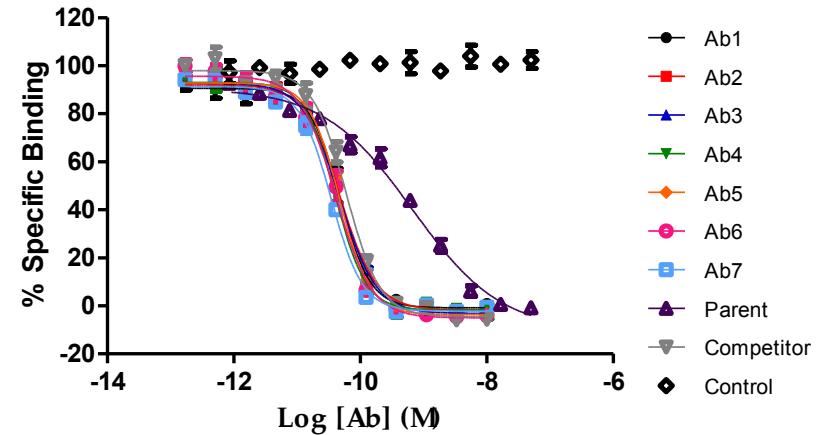
0.3% sample
(approx 0.12-1.2nM
scFv)



0.08% sample
(approx 0.03-0.32nM
scFv)



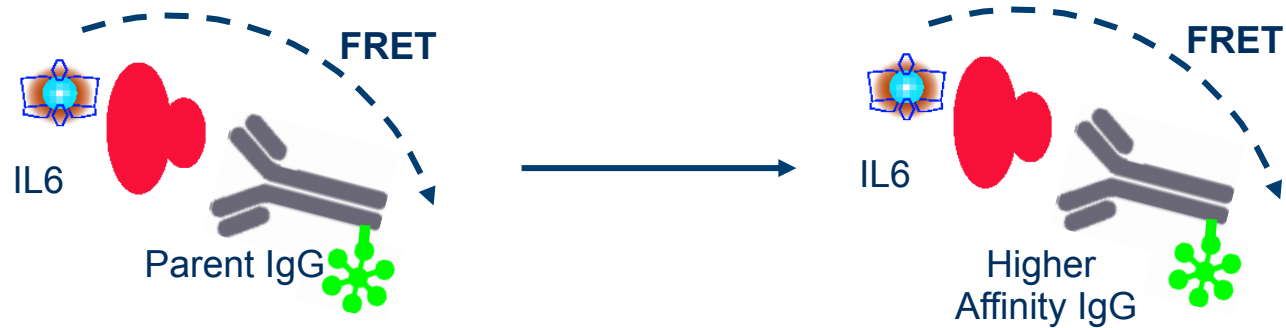
↑ Higher affinity antibodies ↑ Control antibody



Data produced by
Jamie Campbell

- ◆ Lack of discrimination of very high affinity antibodies
- ◆ Caused by antibody depletion or too much IL6 in assay
 - Decrease [IL6]
 - Increase [bio-IgG]
- ◆ Limited by amount concentrations can be varied

Second Generation Assays

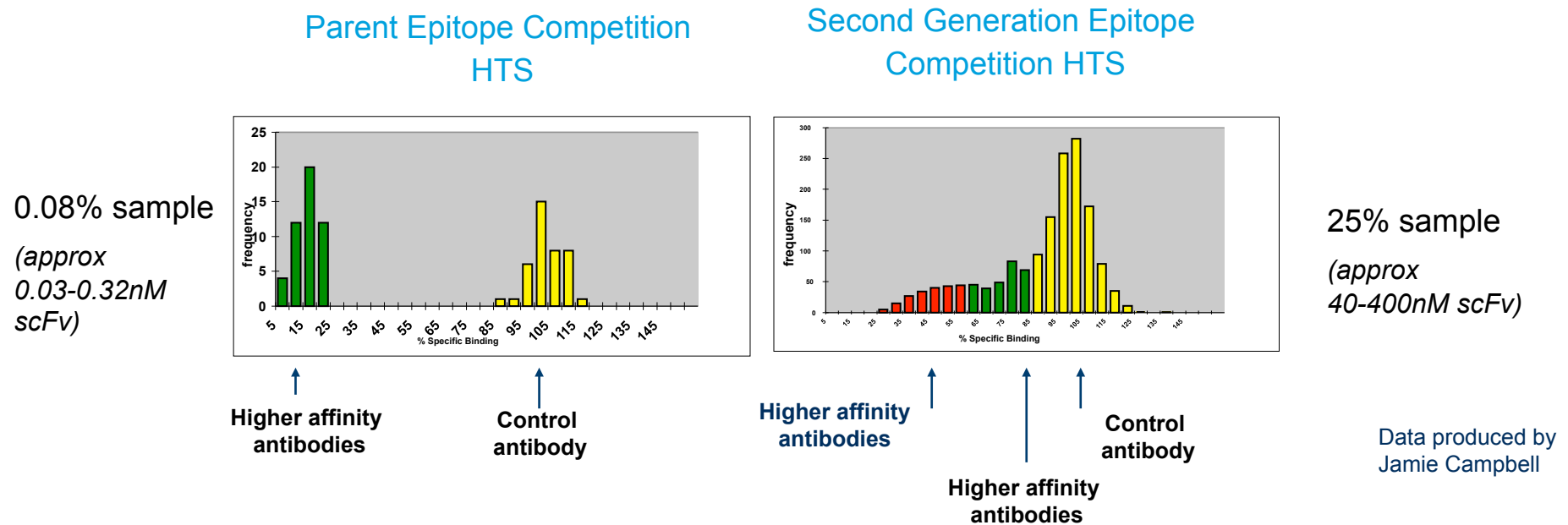


Assay	Affinity (nM)	[IL6] (nM)	[Bio-IgG] (nM)	[bio-IgG] relative to K_D	K_i scFv (nM)	IC_{50} scFv (nM)
Parent	20	0.05	2	x 0.1	0.1	0.11
Higher Affinity	0.1	0.05	2	x 20	0.1	2.1

Cheng Prusoff – $K_i = \frac{IC_{50}}{1 + \frac{[L]}{K_D}}$

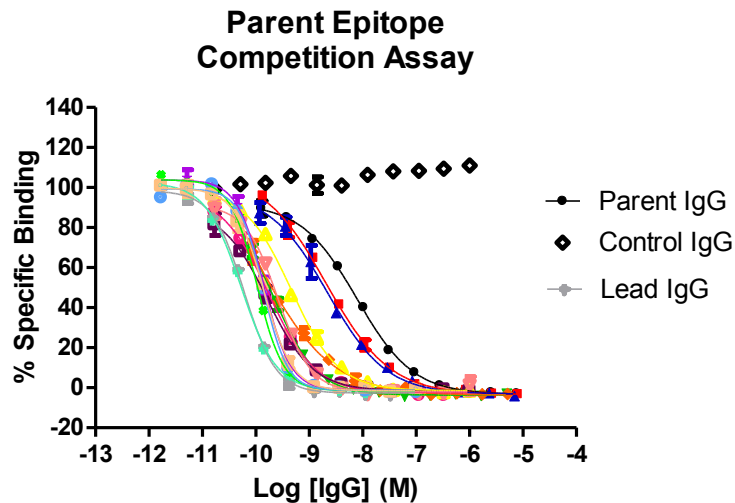
Second Generation Assays

Enables Differentiation of Higher Affinity ScFv



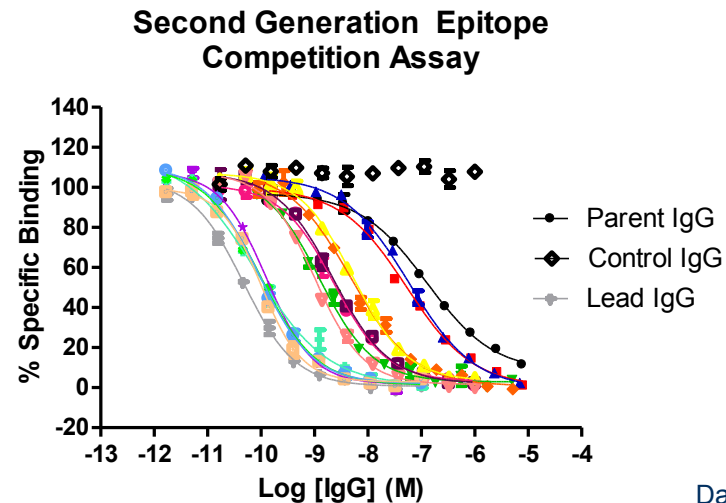
- ◆ Antibodies identified with affinity increased by >10,000x
 - < pM affinity

Improving Assay Sensitivity to Identify High Affinity Antibodies – Example 2



Higher affinity
antibodies

Parent



Higher affinity
antibodies

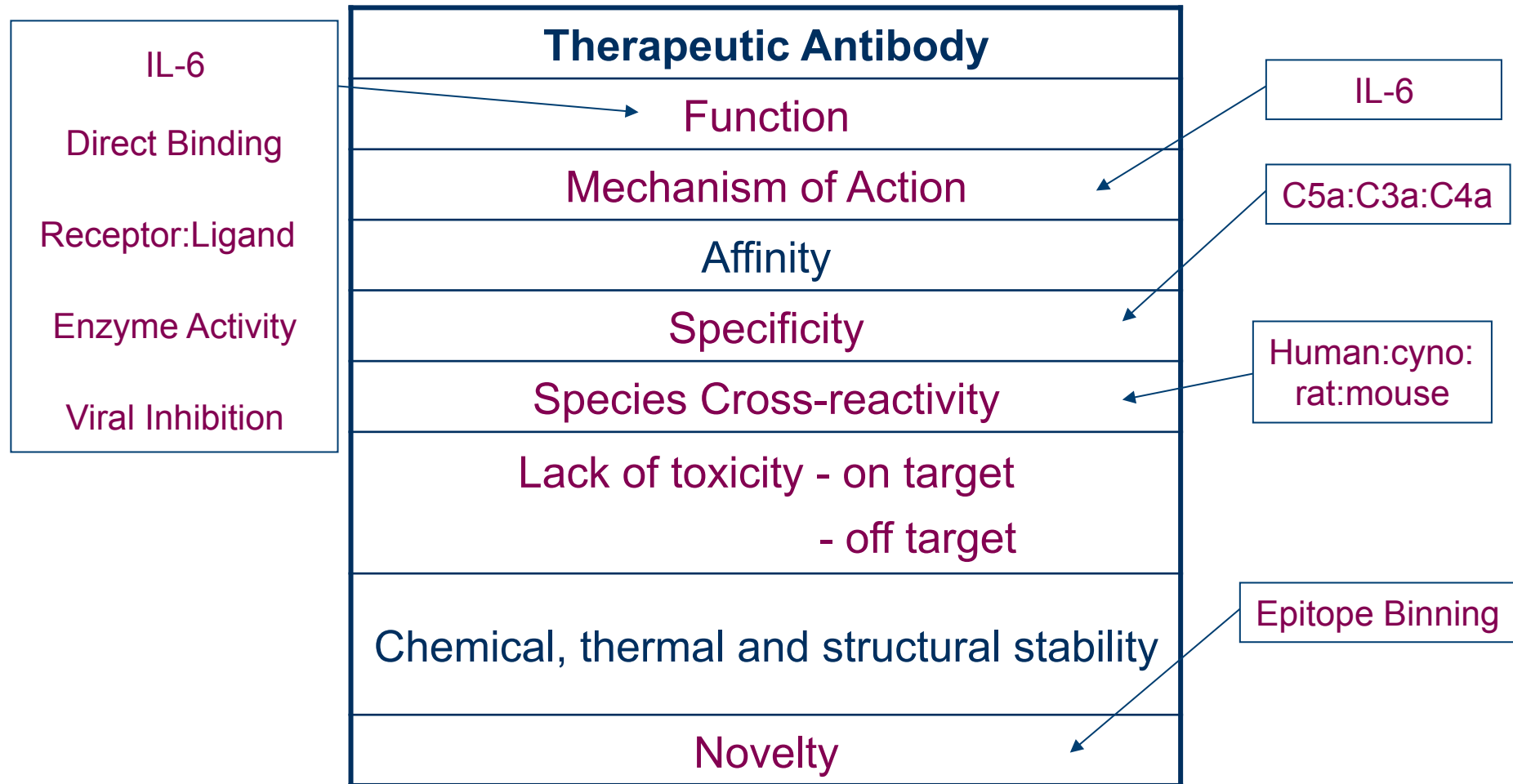
Higher affinity
antibodies

Parent

Data produced by
Izzy Boyfield

- ◆ Second generation epitope competition assays
- ◆ Enable differentiation of most potent antibodies

HTRF: Providing a Robust, Versatile Platform for Biologics Discovery



Use of HTRF assays