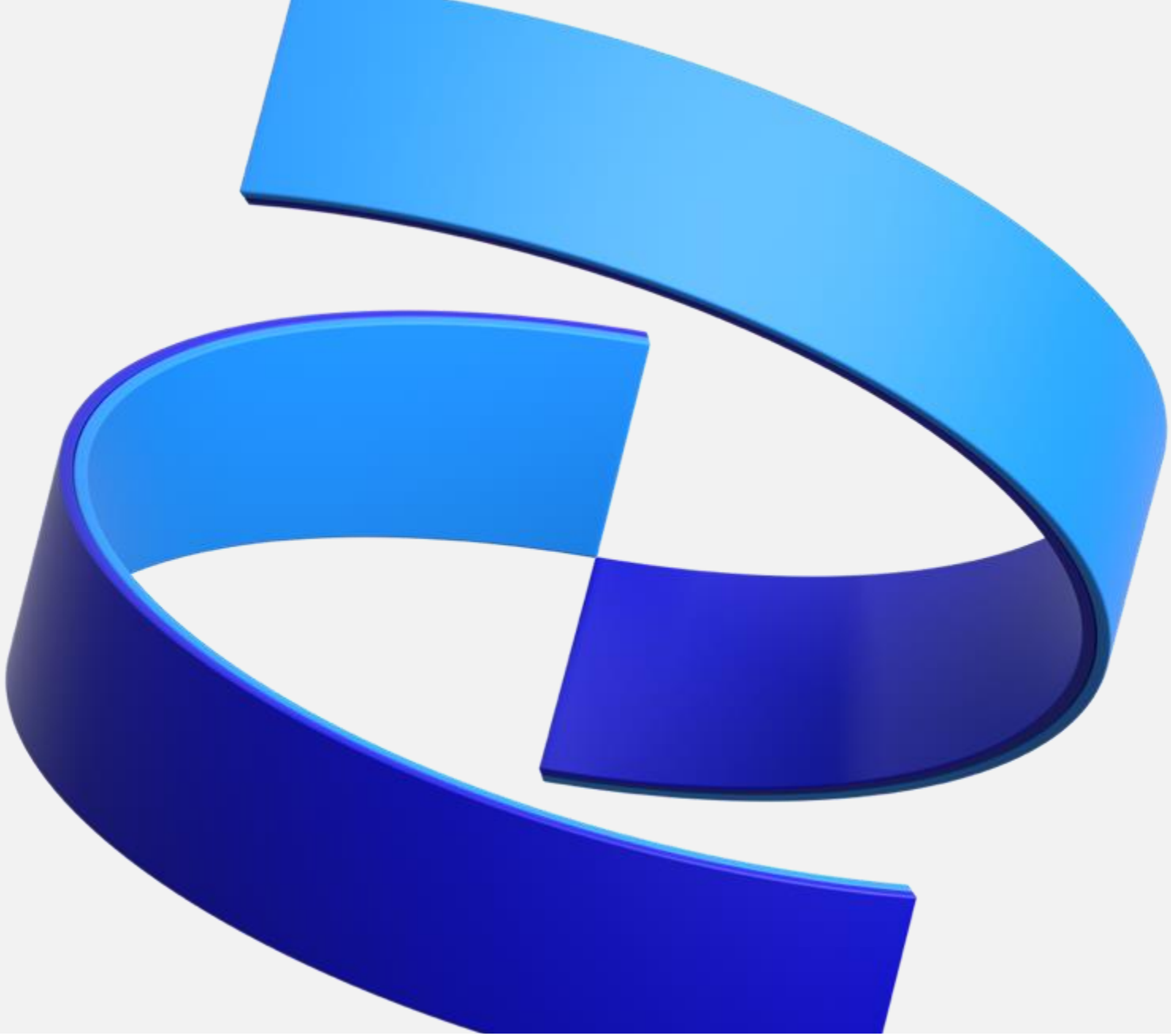
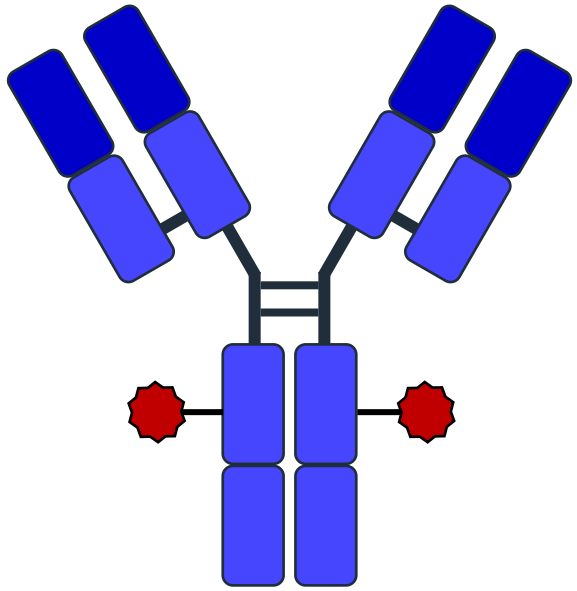
HT-SPR Evaluation of Critical Reagent Antibodies Yields Expected and Unexpected Benefits



James Fishburn

Carterra Symposium
June 24th, 2025

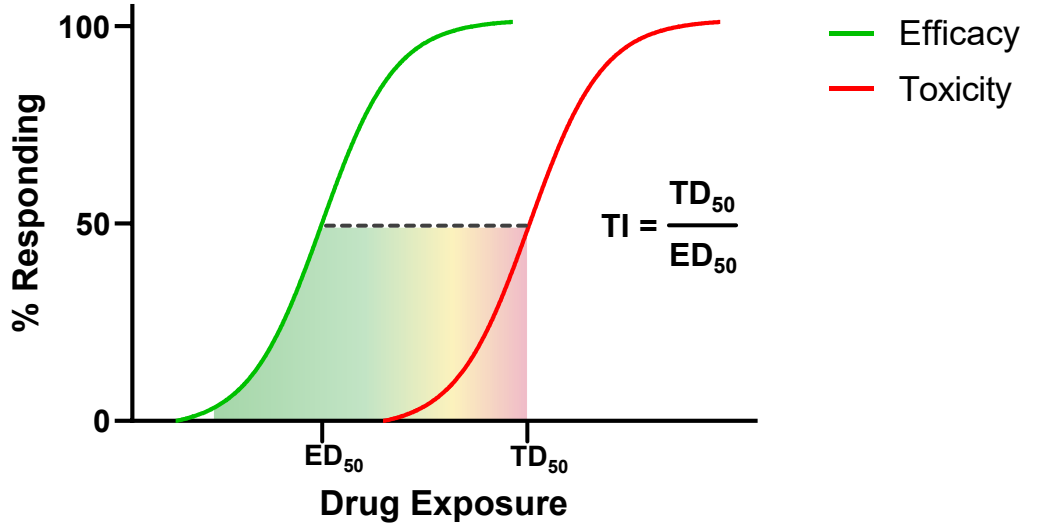
ADCs Target a Broad Therapeutic Index and Low Immunogenicity



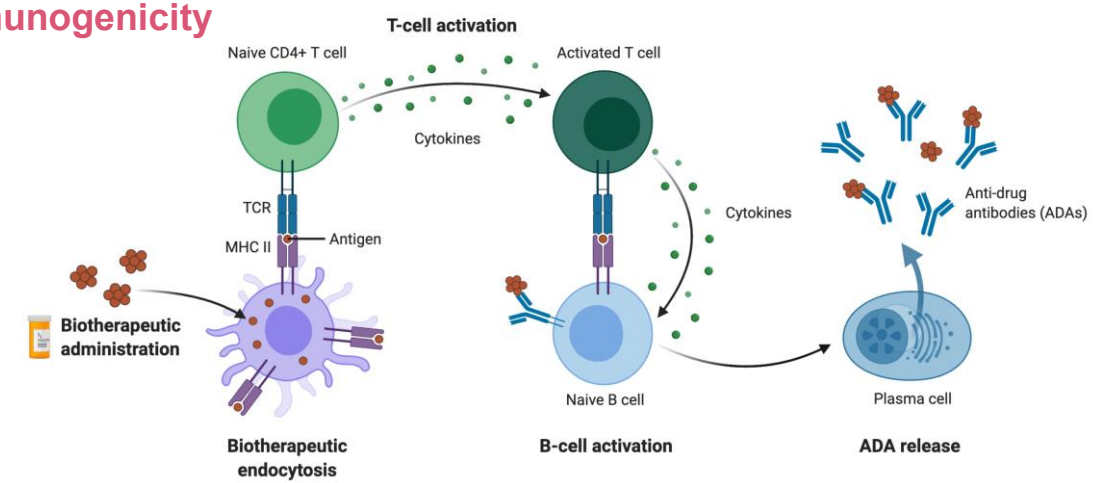
Antibody-Drug Conjugates

- Monoclonal Antibody
- Covalent Drug-Linker
- Bioactive Payload

Therapeutic Index



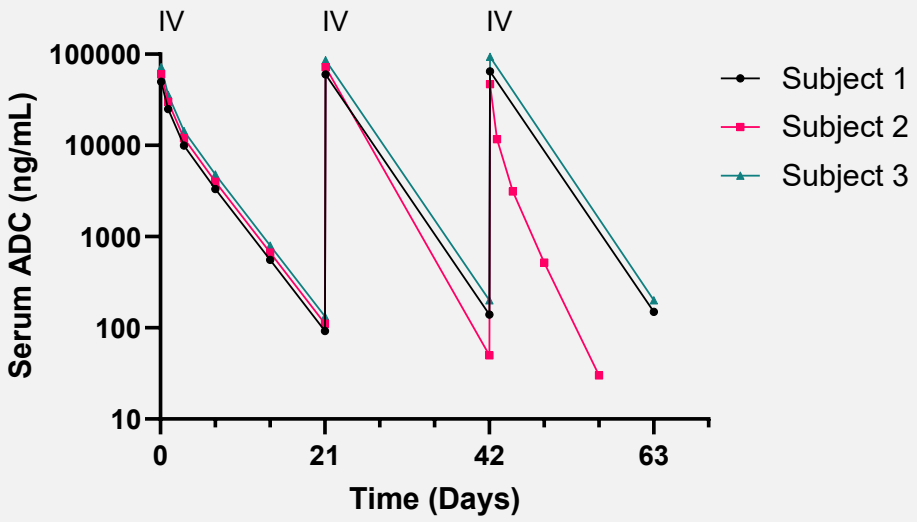
Immunogenicity



https://www.rapidnovor.com/anti-drug-antibody-assays-with-next-generation-protein-sequencing/

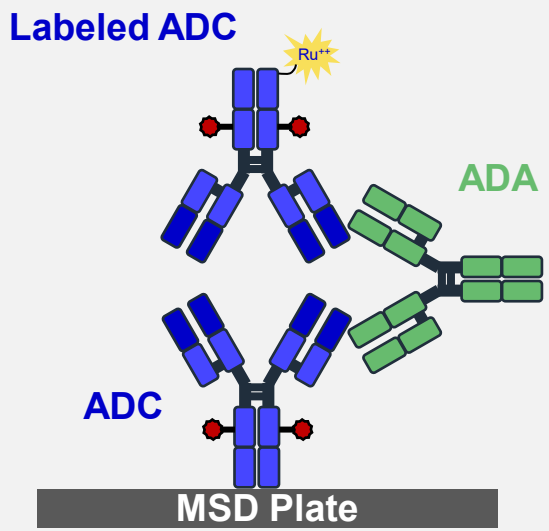
ADC Bioanalysis Monitors Drug Exposure and Immune Responses

Pharmacokinetics of a Treatment Cycle

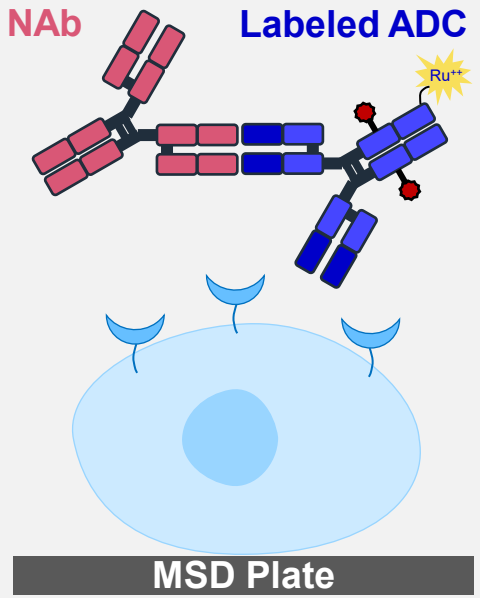


- BioA monitors drug exposure in clinical and nonclinical studies
- Rapid ADC clearance may indicate anti-drug antibodies (ADA)

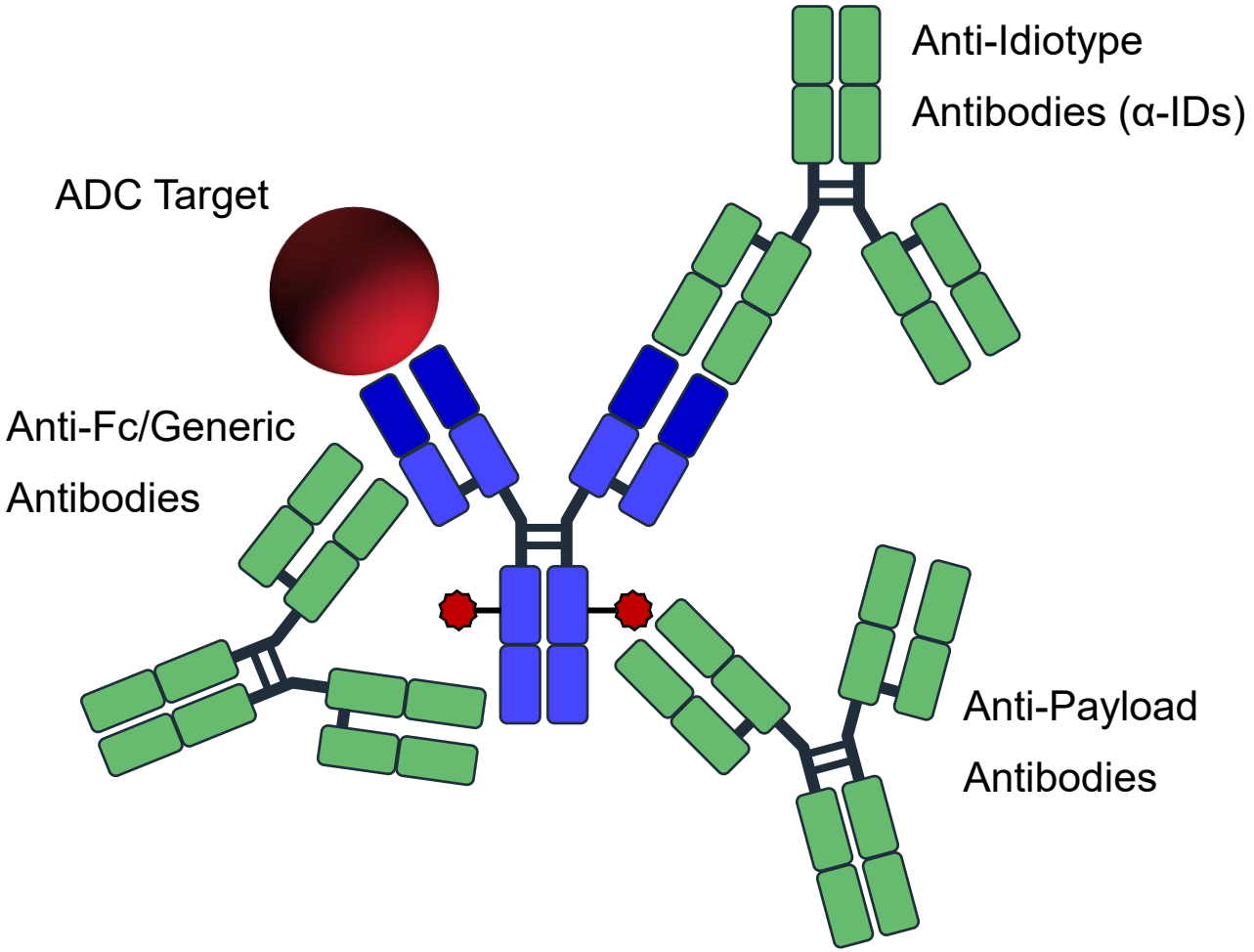
Anti-Drug Antibodies
Bridging Assay



Neutralizing Antibodies
Competition Assay



Critical Reagents are Foundational to Bioanalytical Assays



BioA Assays	Required Reagents
gTAb	Generic
sTAb	α-IDs or ADC Target
ADC	α-Payload
ADA	Generic or α-ID
NAb	α-ID

Desired Assay Characteristics

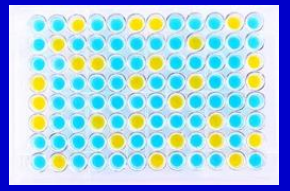
- Sensitive
- Specific
- Selective
- Accurate
- Precise
- Robust

Assessable on the LSA

Traditional Approaches to Screening are Costly and Inefficient

Checkerboard ELISAs: test every combination of antibodies as capture and detect reagents

- Test a minimum of three analyte conditions: high, low, and blank
- Vendor screened hybridoma campaigns produce up to 96 antibodies for further evaluation
- 96 captures x 96 detects x 3 analyte concentrations = 27,648 reactions = 288 ELISA plates



Traditional Screening

13.7 feet

288

ELISA

Plates

14 feet

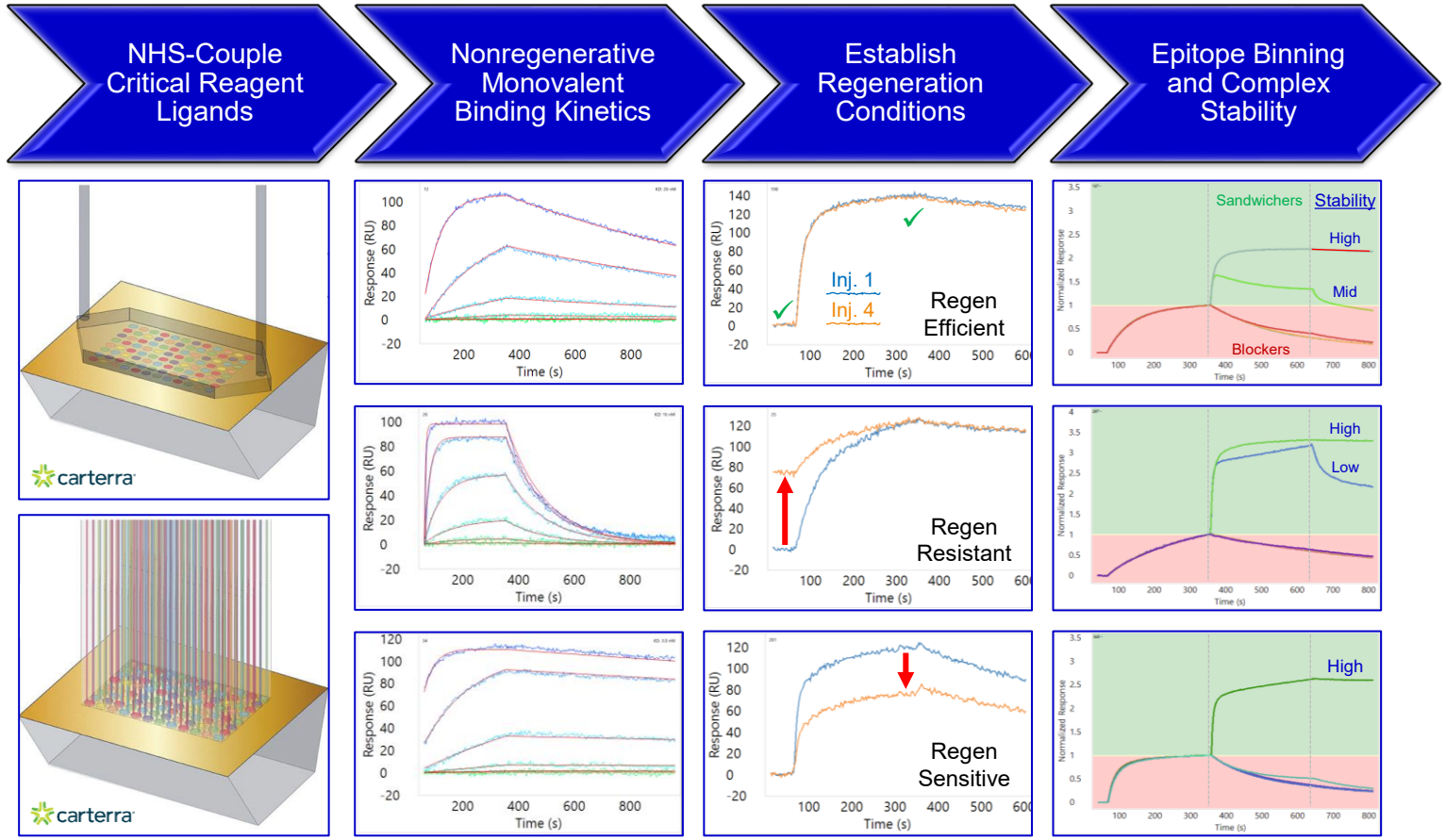
Adult

Female

Giraffe

Contemporary Screening

Critical Reagents are Evaluated Using High-Throughput SPR



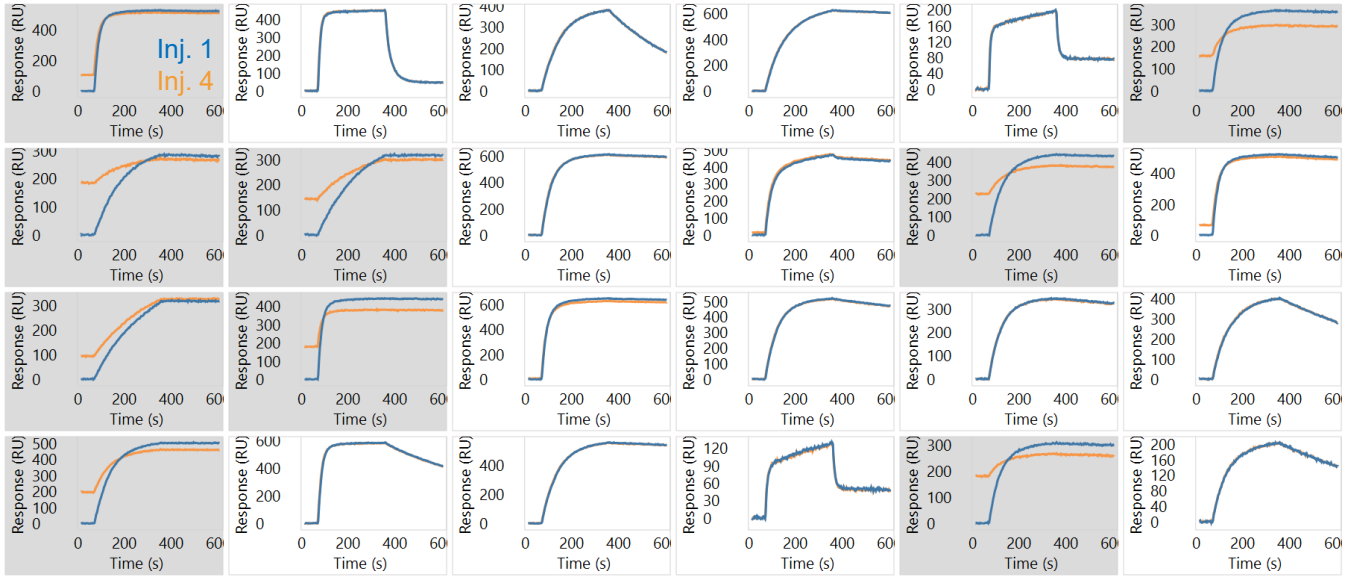
Integrate binding kinetics, regeneration efficiency, epitope binning, and complex stability data to identify critical reagent leads and predict immunoassay reagent orientations

Regen Scouting Informs the Selection of ADA Positive Controls

- Excessive ADC in samples necessitates dissociation of ADA:ADC complexes and capture of the ADAs
- Acidification of samples to facilitate ADA:ADC dissociation generally lowers the sample pH to 2.7 – 3.0
- α-idiotype mAbs function as positive controls in many immunogenicity assays, especially NAb assays
- The α-ID PCs should dissociate efficiently from the ADC to facilitate assay development and reproducibility

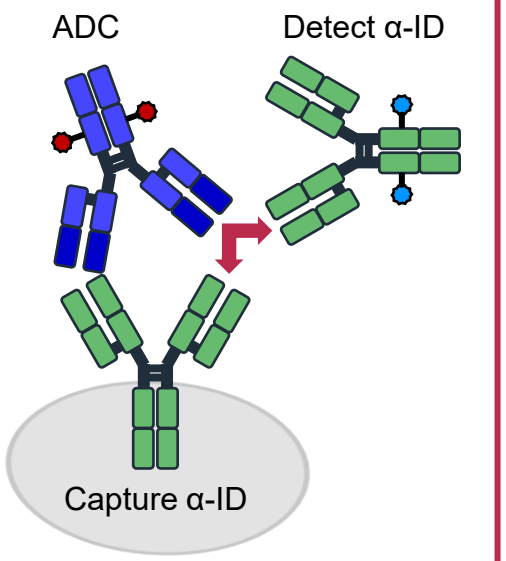
Regen scouting α-IDs using glycine pH 2.25

- 100 nM ADC Fab was injected 4 times
- 38% of ligands assayed were resistant to regeneration in these conditions (gray ROIs)
- ADA PCs could be selected from the 62% of ligands that regenerated efficiently

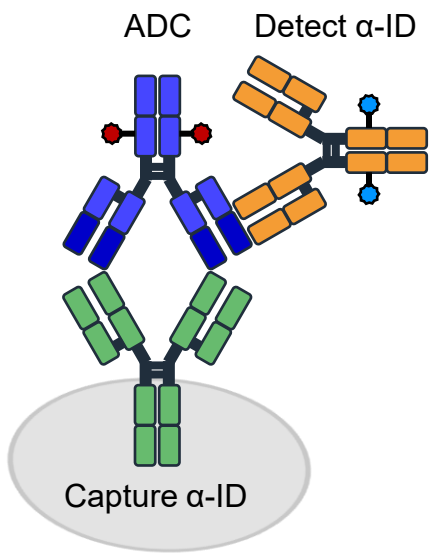


Sandwiching Reagent Pairs can Expedite Assay Development

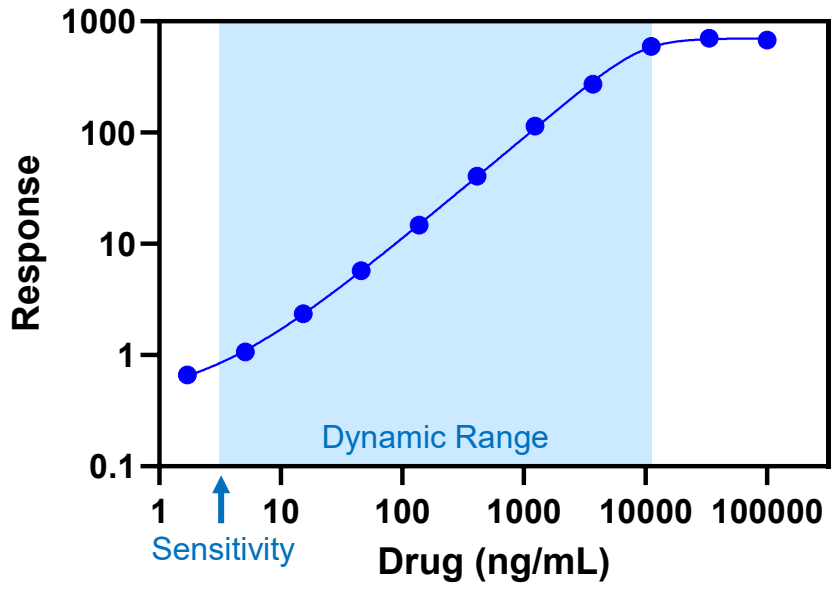
Blocking α-IDs Require Greater Optimization of Assay Conditions



Sandwiching α-IDs Pair Efficiently and Facilitate Immunoassay Development



Immunoassays Ideally are Highly Sensitive with a Broad Dynamic Range

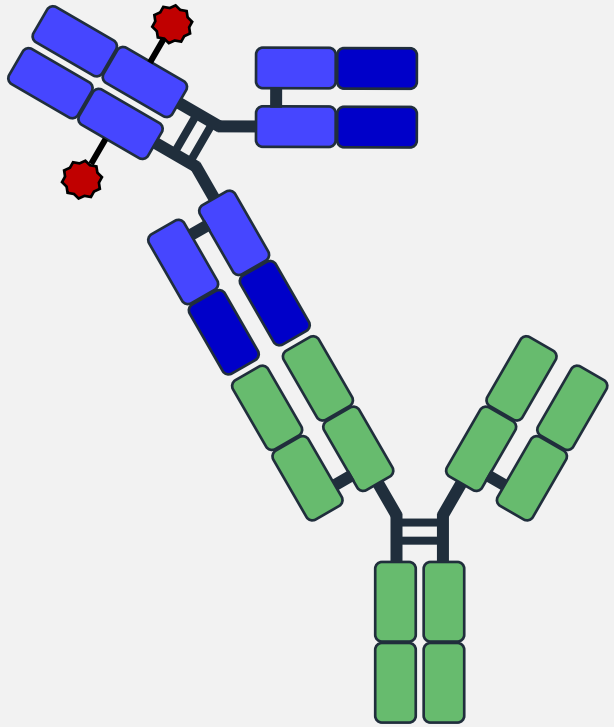


- Assay sensitivity is largely determined by capture kinetics
- Dynamic range is influenced by sandwiching efficiency
- Increased range minimizes sample dilutions and improves testing accuracy

The Presented Campaign Produced Types 1 and 2 Anti-Idiotype Antibodies

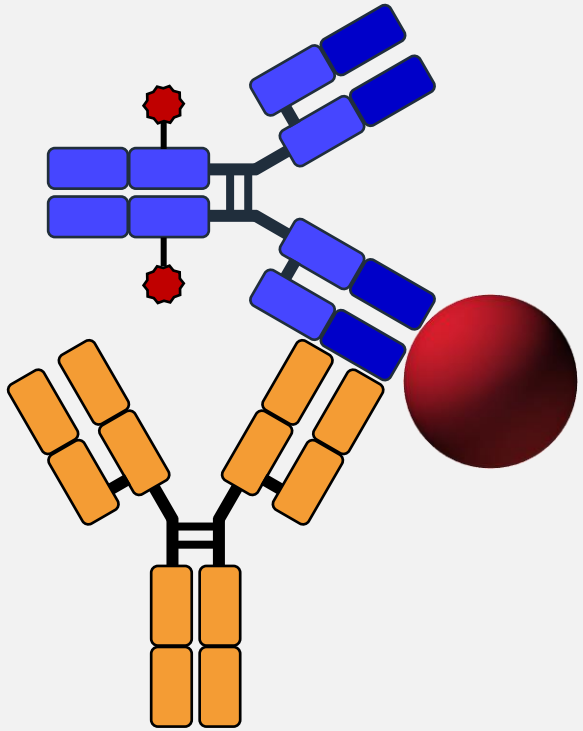
Type 1 – Blocking

Detects Free ADC



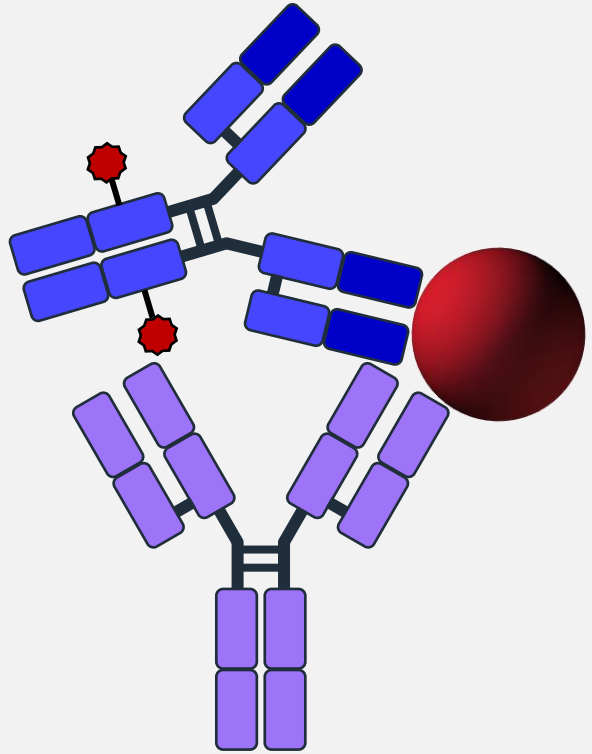
Type 2 – Nonblocking

Detects Total ADC



Type 3 – Complex Specific

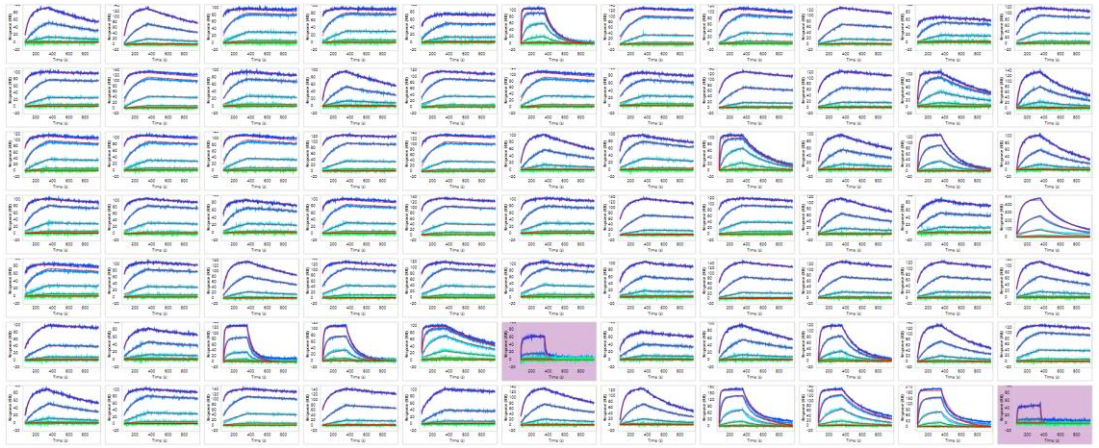
Detects Target-Bound ADC

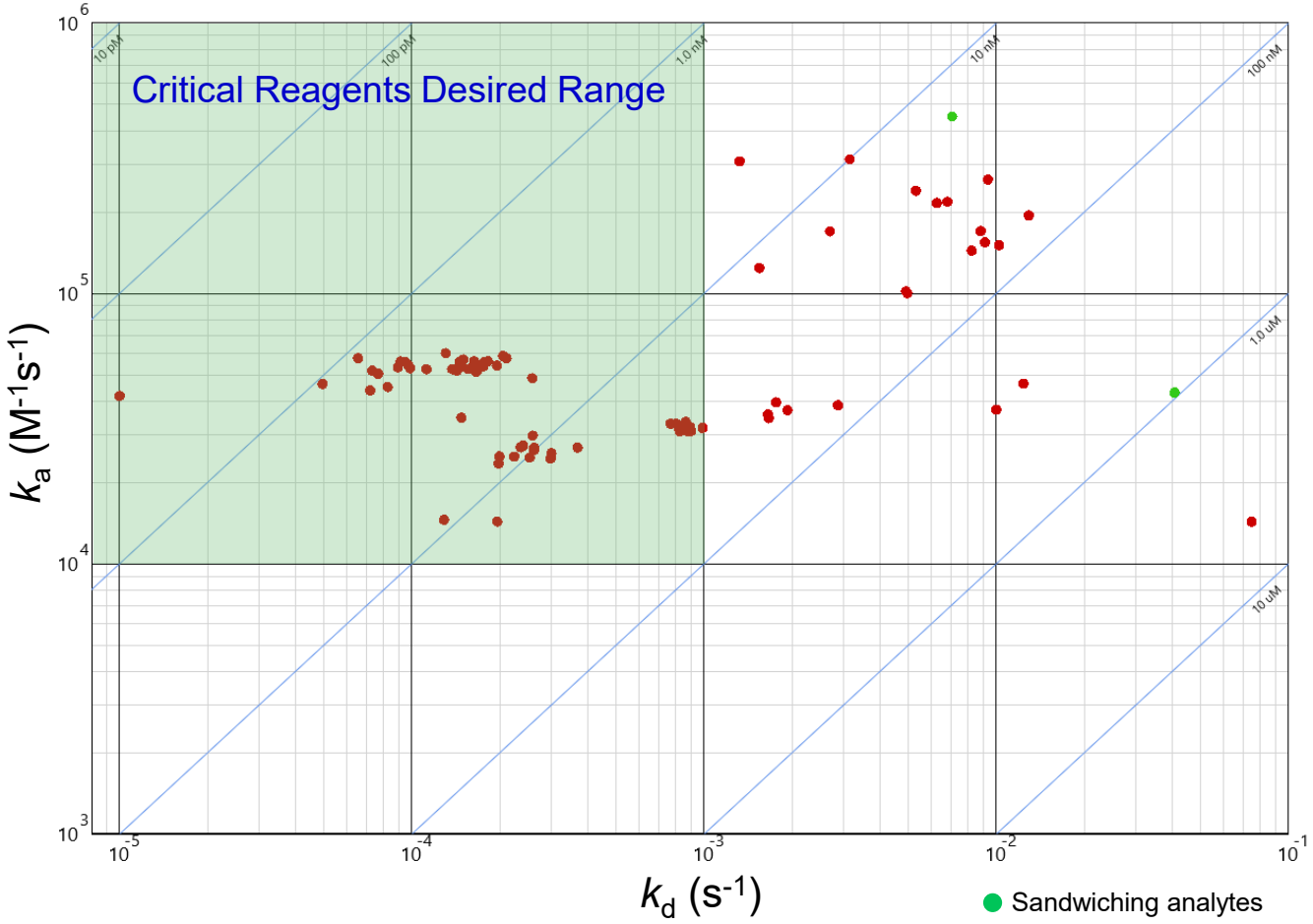


α-ID Campaign Produced Antibodies with Diverse Kinetics and Affinities

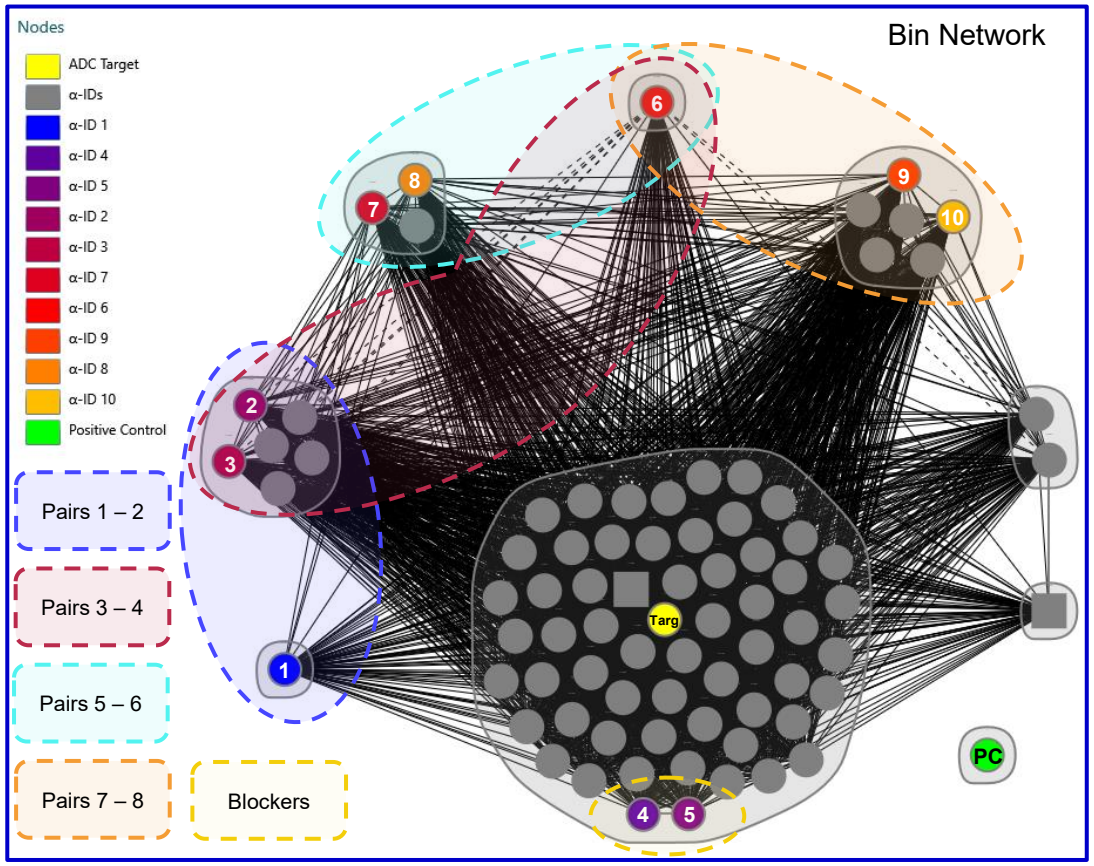
Clones Expressed	HT-SPR Evaluated	Antigen Specific	On-Rates k_a ($M^{-1}s^{-1}$)	Off-Rates k_d (s^{-1})	Affinities K_D
94	85	79	1.4×10^4 – 4.5×10^5	7.5×10^{-2} – 1.0×10^{-5}	0.2 nM – 5.0 uM

Nonregenerative Monovalent Binding Kinetics





Epitope Binning Reveals 20 Sandwiching Pairs Involving 3 Analytes



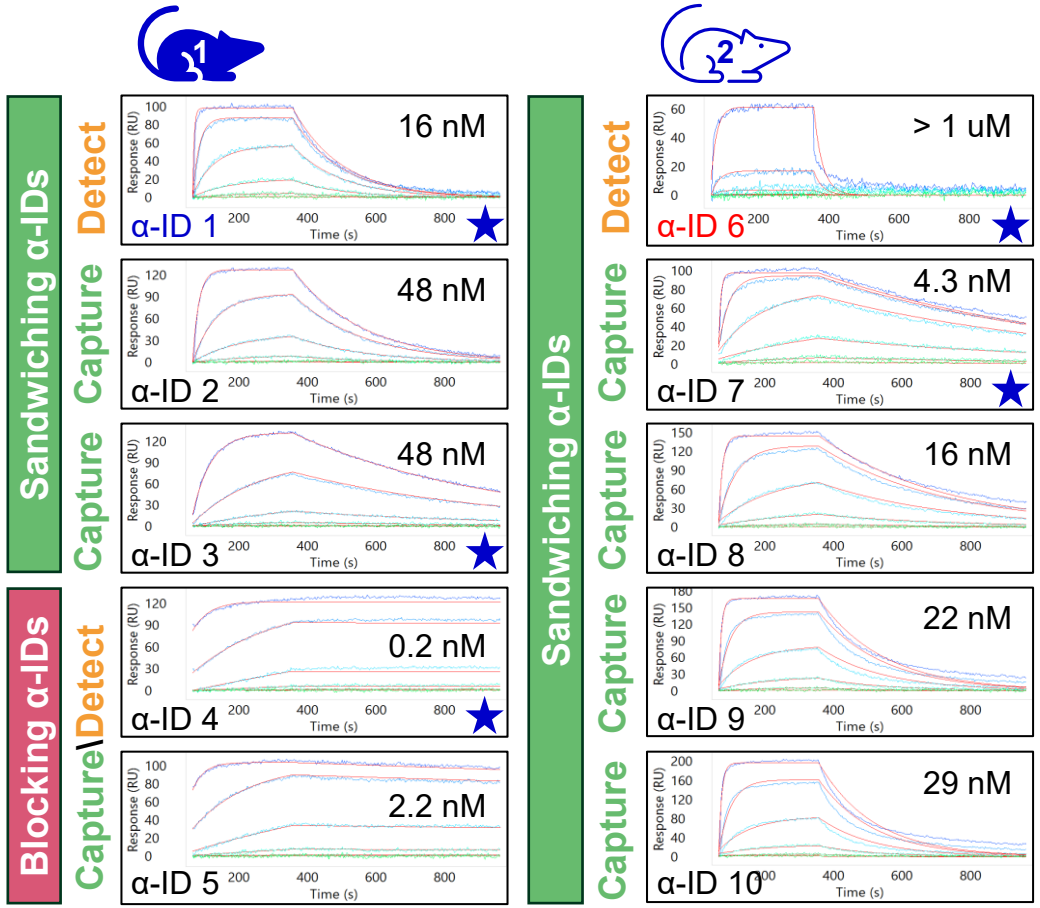
- 6083 unique interactions were interrogated through epitope binning
- 20 sandwiching interactions involving 20 ligands and 3 analytes from 2 mice
- 10 α-IDs were selected for further evaluation as immunoassay reagents

Dissociation Rates for 8 Sandwich Complexes

Pair	Ligand k_d (s^{-1})	Analyte k_d (s^{-1})	Sandwich k_d (s^{-1})	Ligand k_d / Sandwich k_d	Analyte k_d / Sandwich k_d
1	α-ID 2 4.9E-03	α-ID 1 7.1E-03	7.1E-04	7	10
2	α-ID 3 1.7E-03	α-ID 1 7.1E-03	8.1E-05	21	**87**
3	α-ID 2 4.9E-03	α-ID 6 4.1E-02	3.6E-03	1	11
4	α-ID 3 1.7E-03	α-ID 6 4.1E-02	3.0E-03	1	14
5	α-ID 7 1.3E-03	α-ID 6 4.1E-02	5.7E-05	23	**711**
6	α-ID 8 2.7E-03	α-ID 6 4.1E-02	2.5E-04	11	165
7	α-ID 9 5.3E-03	α-ID 6 4.1E-02	4.8E-04	11	85
8	α-ID 10 4.9E-03	α-ID 6 4.1E-02	2.1E-04	29	190

- α-IDs 1 – 5 were produced by mouse 1, α-IDs 6 – 10 originated in mouse 2
- k_d was much slower for some sandwich complexes than the engaged analyte
- Large changes in k_d were observed for α-IDs originating in the same mouse
- Magnitude of changes in k_d suggested a potential for cooperative interactions

Lead Reagents Produce Excellent Assay Sensitivity



- Nonregenerative kinetics and affinities of the 10 α-ID leads
- Orientations predicted from kinetics, binning, and stability data

Reagent Performance Validation ELISAs

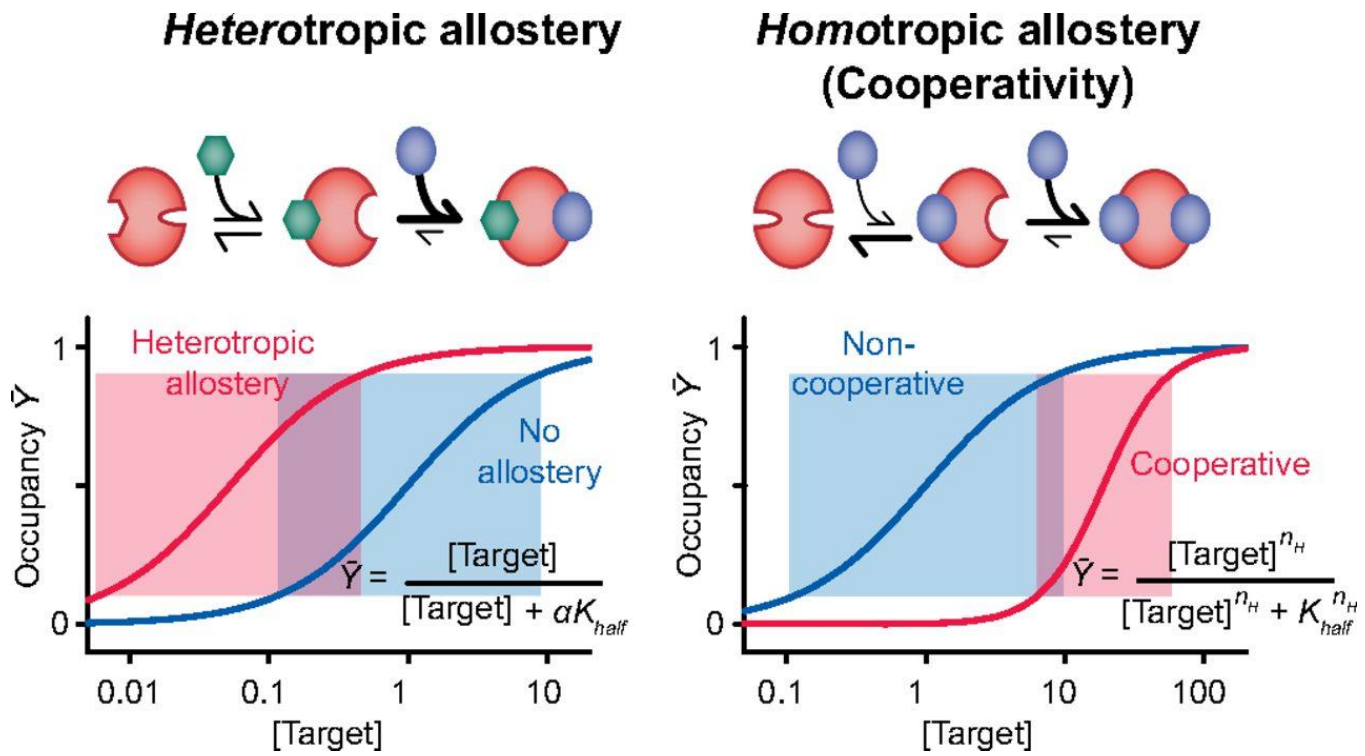
Matched α-ID Pairs (Signal/Noise Shown Below)															
Capture α-IDs	α-ID 4 Detect			α-ID 1 Detect			α-ID 3 Detect			α-ID 8 Detect			α-ID 6 Detect		
α-ID 4	83	4	1	24	1	1	21	1	1	41	1	1	7	1	1
α-ID 5	68	3	1	26	1	1	16	1	1	58	1	1	8	1	1
α-ID 1	8	1	1	5	1	1	54	24	1	15	1	1	9	1	1
α-ID 2	5	1	1	96	13	1	13	1	1	8	1	1	24	1	1
α-ID 3	15	1	1	107	15	1	28	2	1	13	1	1	11	1	1
α-ID 6	4	1	1	2	1	1	4	1	1	54	4	1	2	1	1
α-ID 7	23	1	1	21	1	1	12	1	1	46	1	1	74	19	1
α-ID 8	12	1	1	11	1	1	4	1	1	22	1	1	70	14	1
α-ID 9	5	1	1	5	1	1	2	1	1	9	1	1	69	14	1
α-ID 10	6	1	1	4	1	1	2	1	1	10	1	1	70	15	1
ADC (ng/mL):	100	1	0	100	1	0	100	1	0	100	1	0	100	1	0

Arlan Martin

Blockers | Pairs 1 – 2 | Pairs 3 – 4 | Pairs 5 – 6 | Pairs 7 – 8

★ 5 clones scaled up for assay development

Allosteric Interactions Between Antibody Clones are Heterotropic

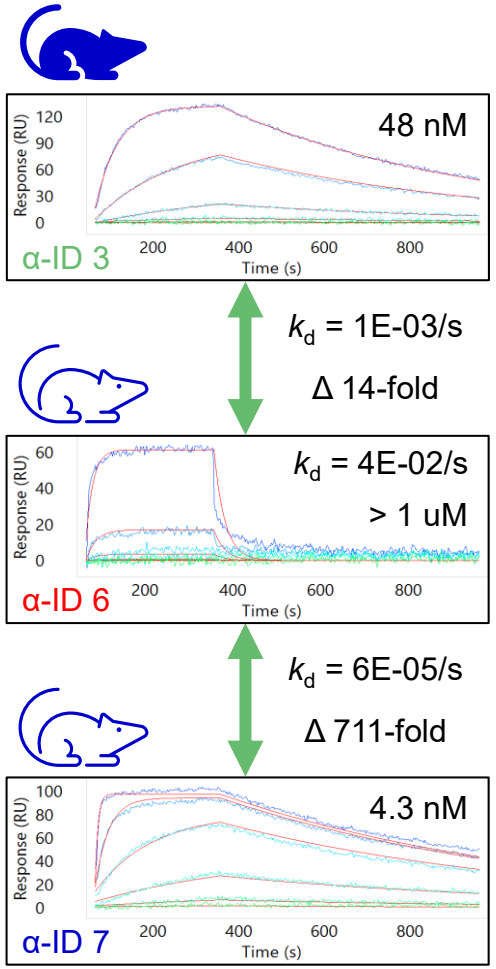


Proc. Natl. Acad. Sci. U.S.A. 111 (42) 15048-15053 (2014)

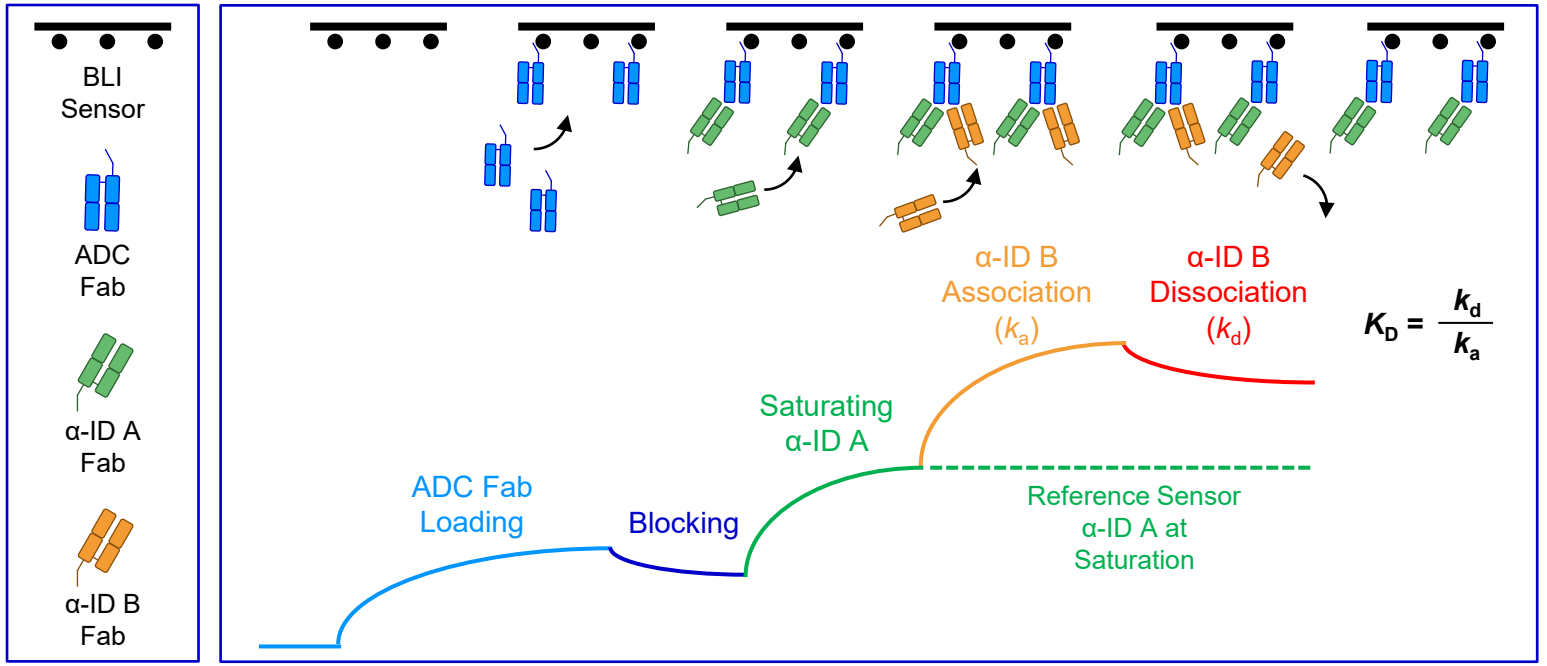
Heterotropic Allostery
Ligands are distinct and can be introduced separately
Binding of ligand A alters the affinity for ligand B
Effect can be positive (greater affinity) or negative (lower affinity)
Binding curve shape (ligand sensitivity) remains the same

Homotropic Allostery
Receptor possesses multiple binding sites for the same ligand
Ligand binding at site 1 alters the affinity for binding at site 2
Cooperativity can be positive or negative
Sensitivity to ligand concentration (Hill coefficient) is changed

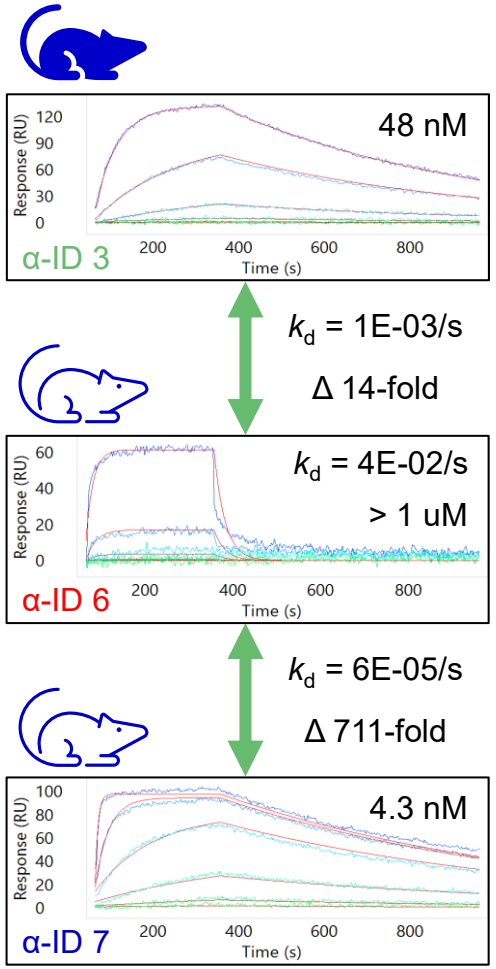
Allosteric Effects Were Interrogated for Two α-ID Pairs



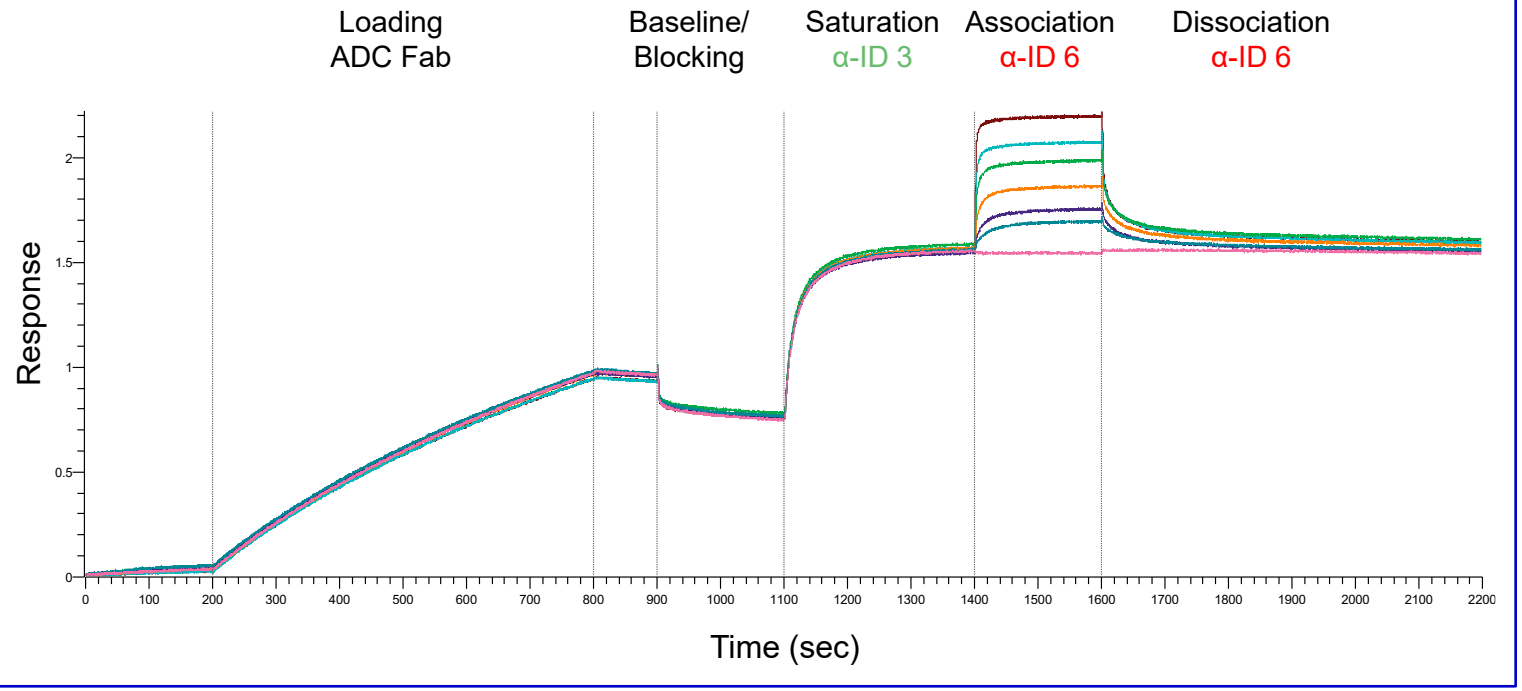
- Binding kinetics were evaluated on an Octet HTX or Red 384 using FAB2G biosensors
- ADC and α-ID Fabs are utilized to eliminate avidity effects and simplify interpretation
- Heterotropic cooperativity exists if the ADC affinity of α-ID B is altered by α-ID A



Full Experimental Data for the α-ID 3:ADC Interaction with α-ID 6



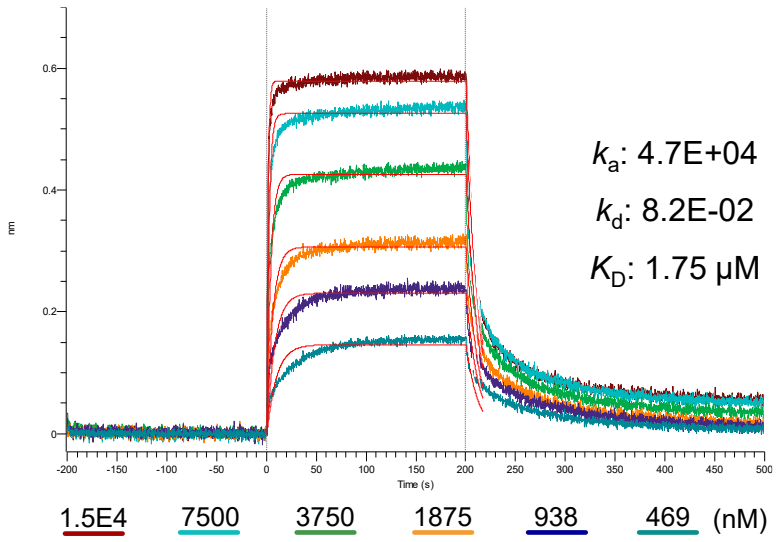
Interaction of α-ID 6 and ADC Prebound with α-ID 3



α-ID 7 Exerts a Positive Allosteric Effect on the α-ID 6:ADC Interaction

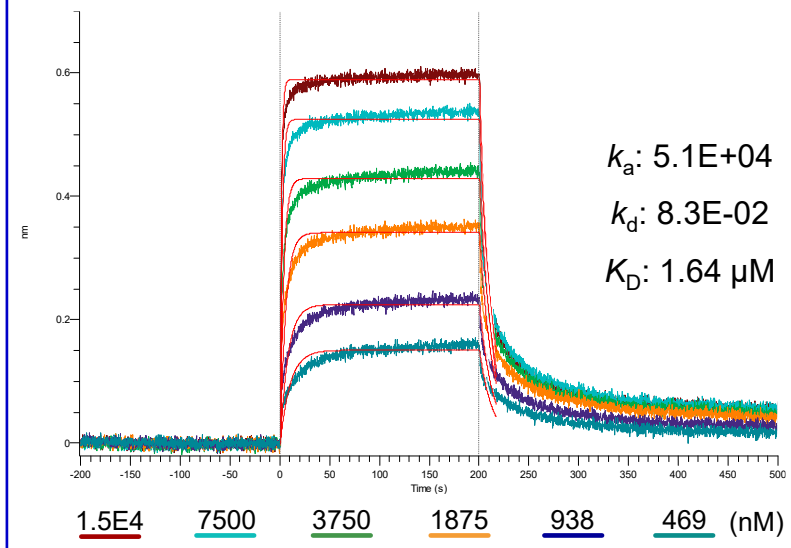
ADC + α-ID 6 Binding Kinetics

ADC + α-ID 6



α-ID 3:ADC + α-ID 6 Binding Kinetics

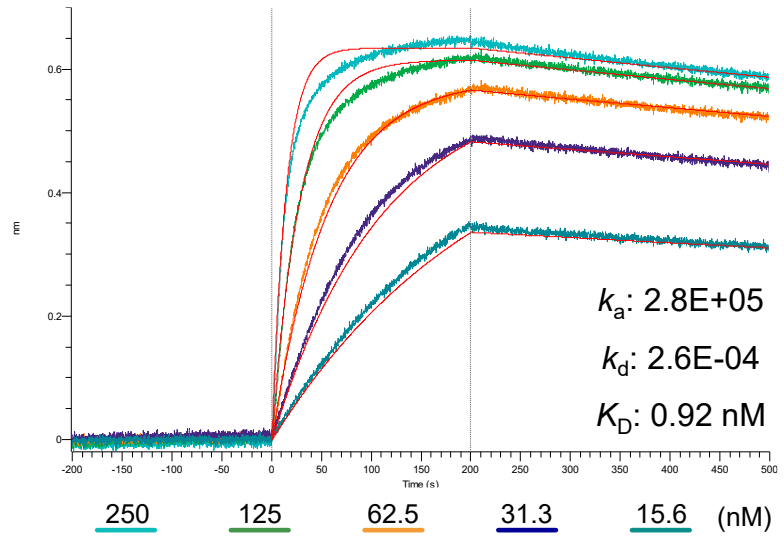
α-ID 3:ADC + α-ID 6



α-ID 6:ADC interaction is unaffected by α-ID 3

α-ID 7:ADC + α-ID 6 Binding Kinetics

α-ID 7:ADC + α-ID 6



α-ID 6:ADC affinity increased nearly 2000-fold by α-ID 7

Summary

Benefits of evaluating reagent candidates on the LSA

- Reduced risk of premature elimination of quality reagents (α-ID 6)
- Comprehensive characterization of all reagent candidates
- Valuable regeneration data for selecting ADA/NAb assay PCs
- Saved time and money compared to traditional approach

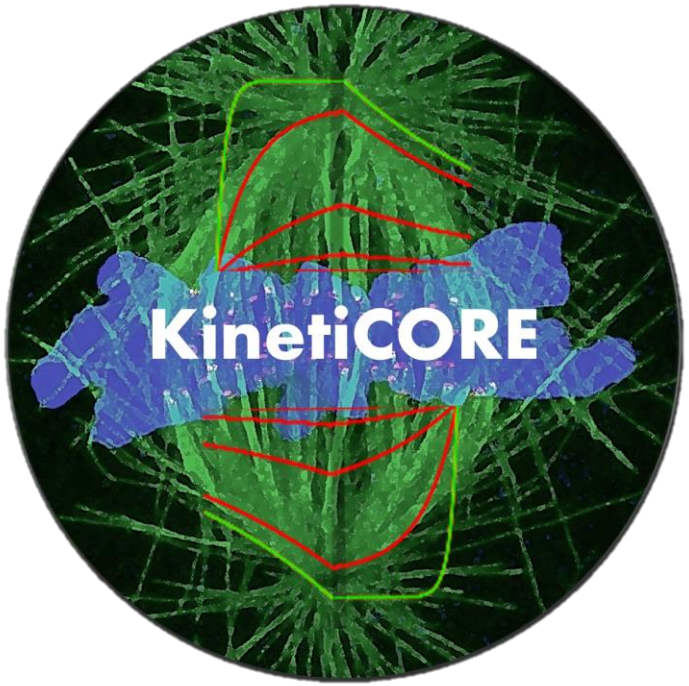
Food for thought regarding cooperativity…

If α-ID 6 was affinity mature at the time of isolation, then evaluating low affinity clones through epitope binning may facilitate the identification of allosterically interacting antibody pairs

Screening Method*	Clones to Evaluate	Total Cost	Cost Per Clone	Dataset Content
LSA	94	$3,800	$40	Rich
Traditional	94	$13,500	$144	Limited
Outsourced	40	$54,050	$1,351	Limited

*LSA evaluation performed in 2023, outsourcing quote provided in 2020

Acknowledgments



Key Contributors

Arlan Martin

Markus Carlson

Serena Wo

Leadership

John Lindsay

Christine O'Day

Rob Webster

Thank You

All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and Use Committee or through an ethical review process.