# Carterra® LSA Emerging Applications

Noah T. Ditto

Carterra Technical Product Manager

# Outline

- Antibody Fc Receptor Screening
- Antibody Polyspecificity
- DNA Encoded Libraries
- Targeted Protein Degraders
- Future Applications

carterra®

# High Resolution Fc Receptor Characterization

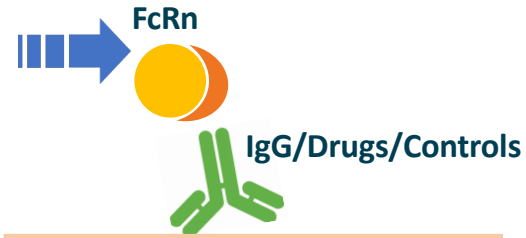
carterra

# Kinetics/steady state/potency single chip FcRn workflow

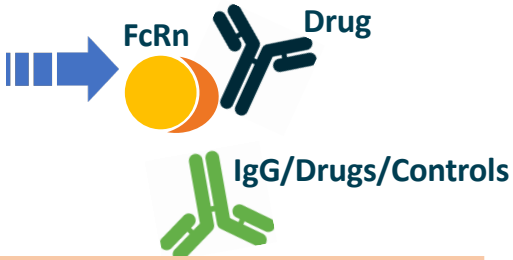
1. Create 96 spot array on sensor surface


- IgG1, IgG2, IgG3, IgG4
- Four drugs
- Fc Fragment, Fab fragment
- All in triplicate at three different surface capture densities: 5, 1, and 0.2ug/ml

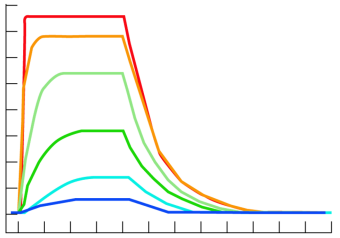
2. FcRn titration



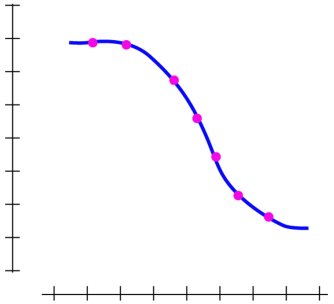

50nM FcRn in presence of increasing [drug]




3. Steady state & Kinetic affinity

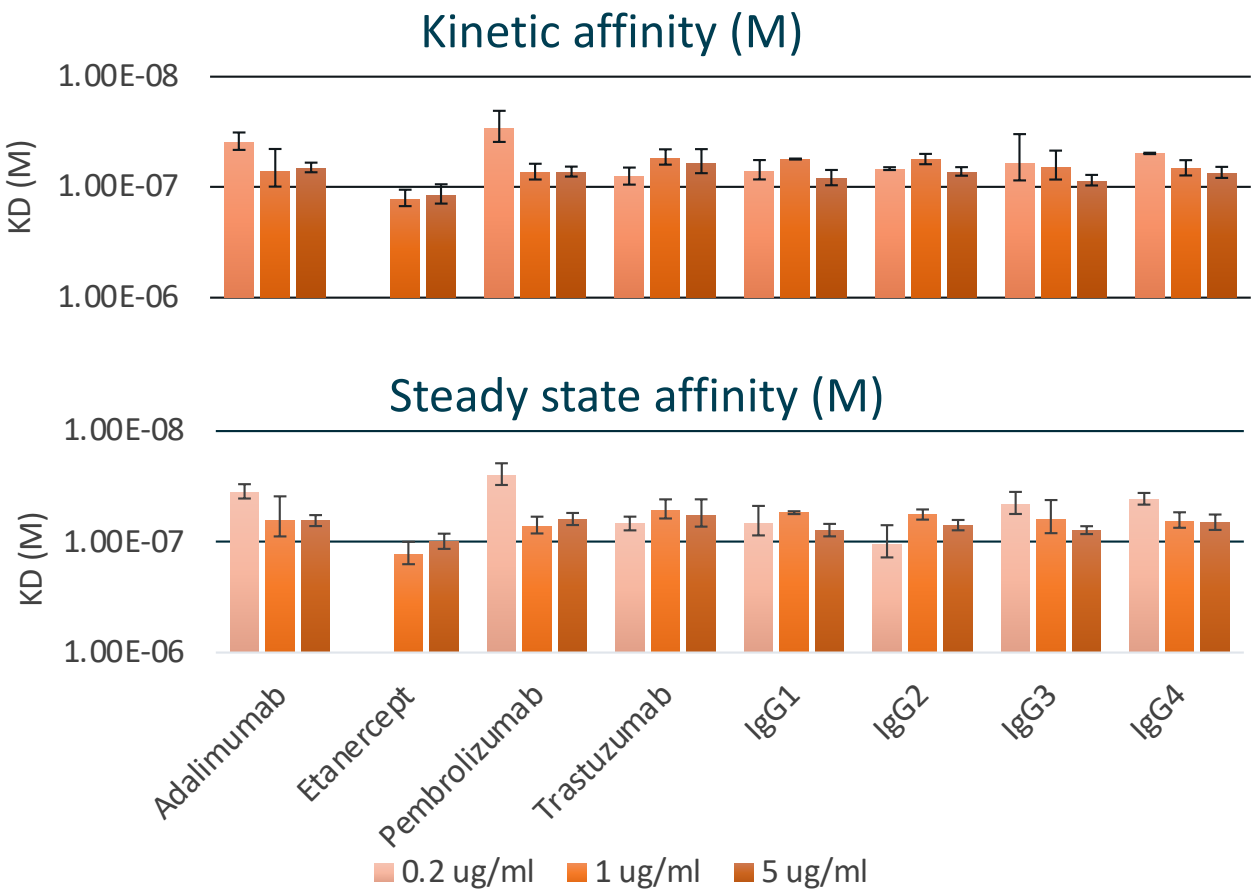


$IC_{50}$ Potency



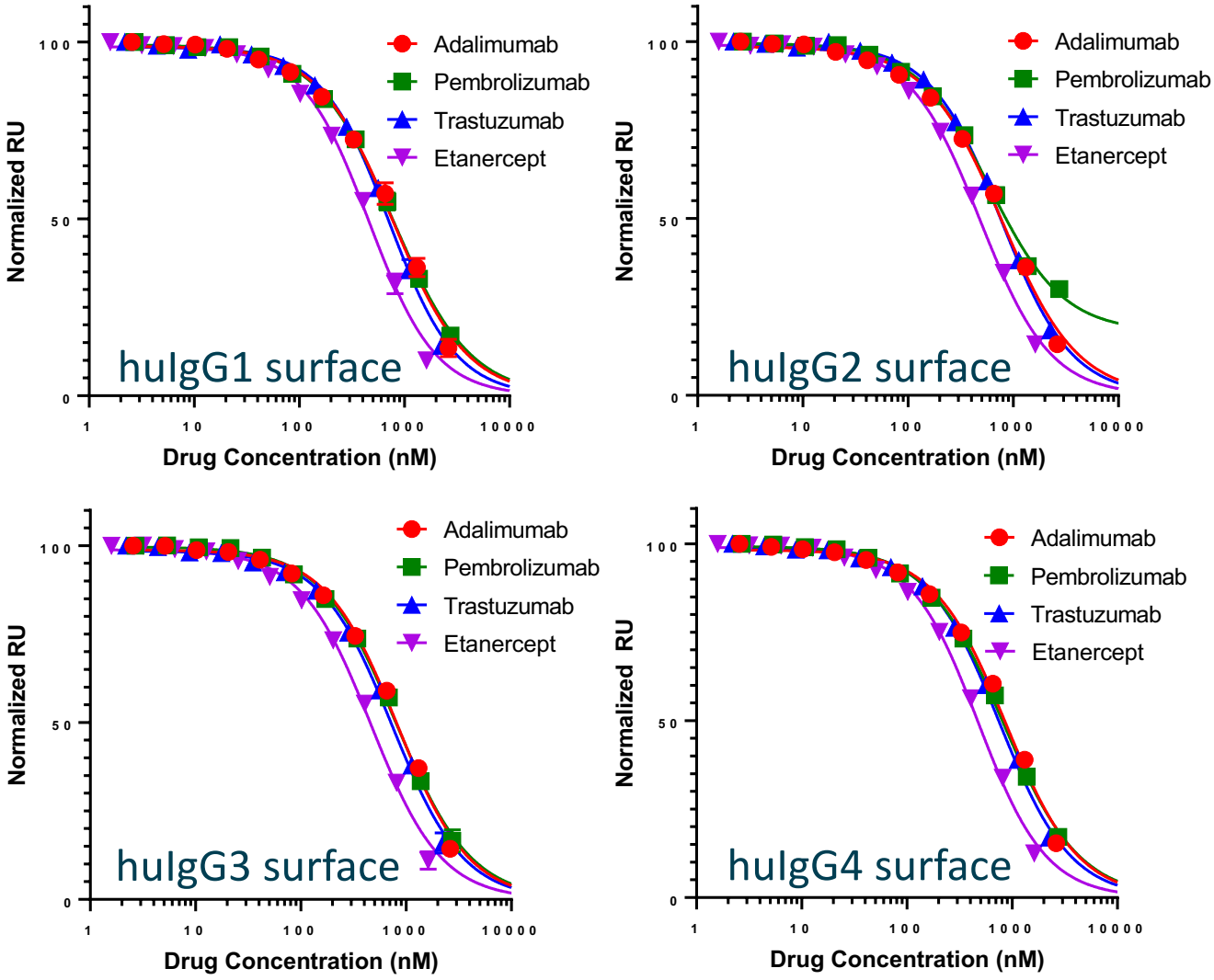
# FcRn kinetic and steady state affinities

- 1:1 Langmuir model
- Triplicate measures performed on three different surface densities



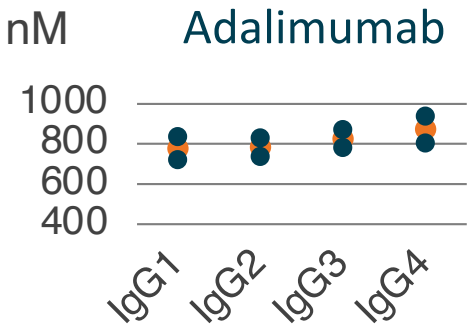
# Drug:FcRn $IC_{50}$ potency curves

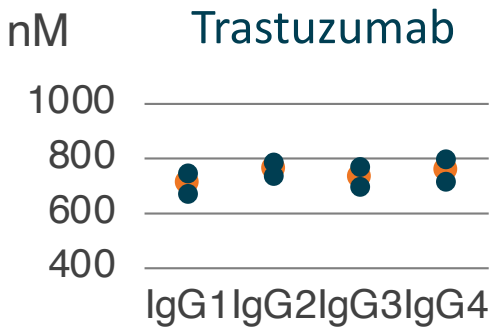
- All values measured in triplicate
- Shown are the higher density surfaces; similar results obtained on lower density surfaces
- Y-scale is response units (RU) normalized to the maximum signal for each replicate

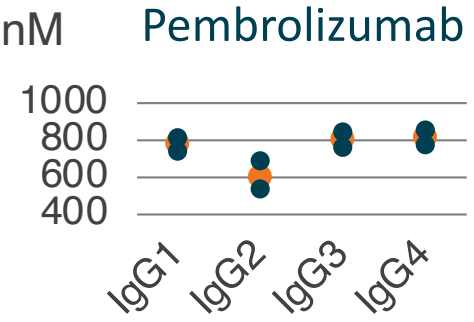


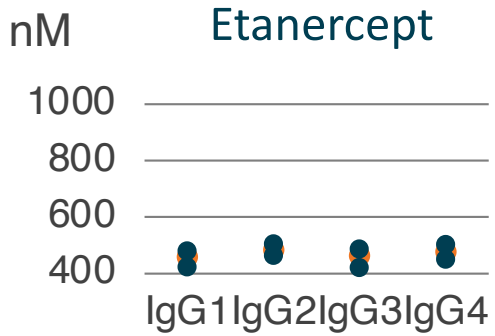
# FcRN potency values

- $IC_{50}$ values are generally comparable across drugs and subclasses
- Pembrolizumab had slightly more potency versus IgG2
- Etanercept showing slightly higher potency across all subclasses



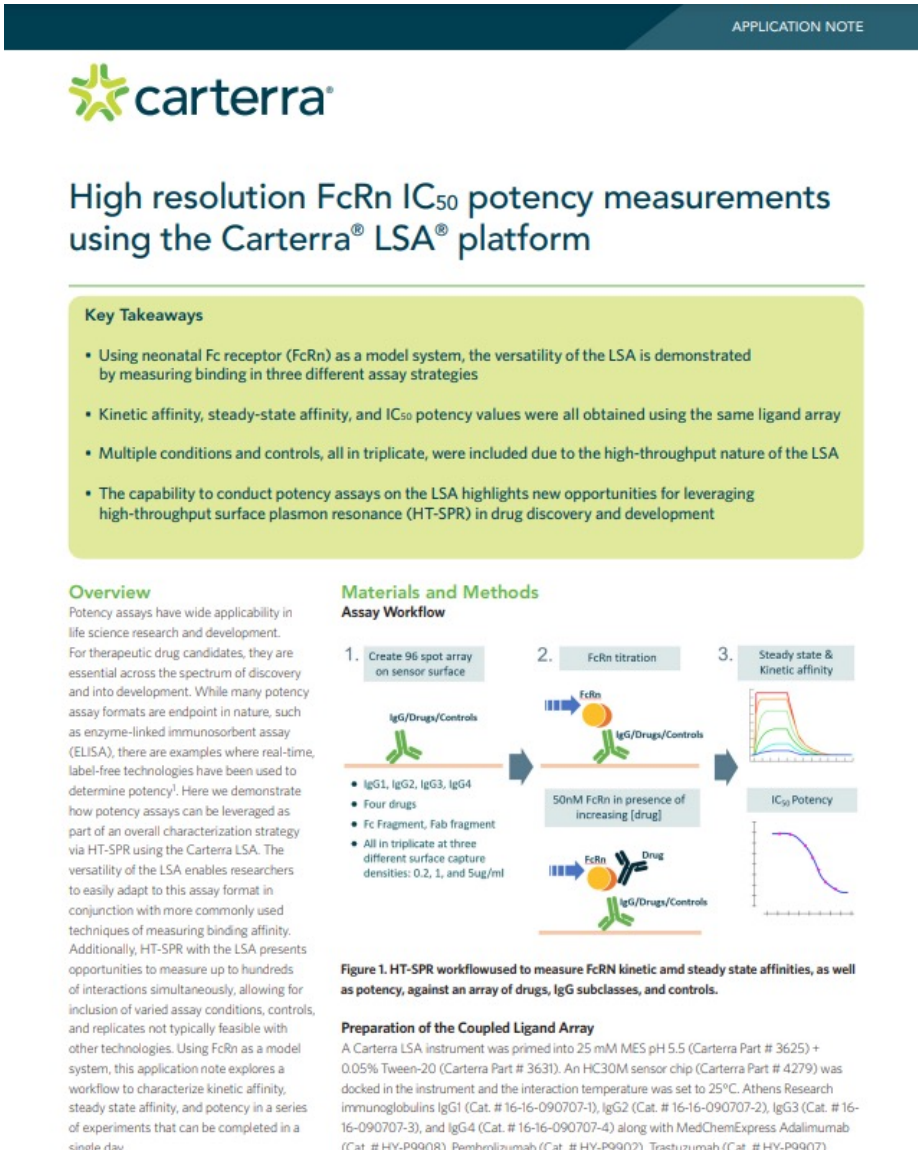




$IC_{50}$, nM  95% confidence interval

# Assessing Antibody Binding to Fc Receptors via HT-SPR

- Demonstrated here with FcRn, but easily expanded to Fc gamma receptors as well
- Inclusion of several densities and controls
- Multiple measures from the same chip: kinetics, steady state affinity, and $IC_{50}$ potency
- Advantageous as an orthogonal approach to complement other techniques



APPLICATION NOTE

carterra

## High resolution FcRn $IC_{50}$ potency measurements using the Carterra® LSA® platform

**Key Takeaways**

- Using neonatal Fc receptor (FcRn) as a model system, the versatility of the LSA is demonstrated by measuring binding in three different assay strategies
- Kinetic affinity, steady-state affinity, and $IC_{50}$ potency values were all obtained using the same ligand array
- Multiple conditions and controls, all in triplicate, were included due to the high-throughput nature of the LSA
- The capability to conduct potency assays on the LSA highlights new opportunities for leveraging high-throughput surface plasmon resonance (HT-SPR) in drug discovery and development

### Overview

Potency assays have wide applicability in life science research and development. For therapeutic drug candidates, they are essential across the spectrum of discovery and into development. While many potency assay formats are endpoint in nature, such as enzyme-linked immunosorbent assay (ELISA), there are examples where real-time, label-free technologies have been used to determine potency[1]. Here we demonstrate how potency assays can be leveraged as part of an overall characterization strategy via HT-SPR using the Carterra LSA. The versatility of the LSA enables researchers to easily adapt to this assay format in conjunction with more commonly used techniques of measuring binding affinity. Additionally, HT-SPR with the LSA presents opportunities to measure up to hundreds of interactions simultaneously, allowing for inclusion of varied assay conditions, controls, and replicates not typically feasible with other technologies. Using FcRn as a model system, this application note explores a workflow to characterize kinetic affinity, steady state affinity, and potency in a series of experiments that can be completed in a single day.

### Materials and Methods

**Assay Workflow**



**Figure 1. HT-SPR workflowused to measure FcRN kinetic amd steady state affinities, as well as potency, against an array of drugs, IgG subclasses, and controls.**

**Preparation of the Coupled Ligand Array**

A Carterra LSA instrument was primed into 25 mM MES pH 5.5 (Carterra Part # 3625) + 0.05% Tween-20 (Carterra Part # 3631). An HC30M sensor chip (Carterra Part # 4279) was docked in the instrument and the interaction temperature was set to 25°C. Athens Research immunoglobulins IgG1 (Cat. # 16-16-090707-1), IgG2 (Cat. # 16-16-090707-2), IgG3 (Cat. # 16-16-090707-3), and IgG4 (Cat. # 16-16-090707-4) along with MedChemExpress Adalimumab (Cat. # HY-P9908), Pembrolizumab (Cat. # HY-P9902), Trastuzumab (Cat. # HY-P9907)

# Antibody Polyspecificity Screening

carterra

# The case for comprehensive polyspecificity screening

- Much like epitope and affinity, specificity is essential to characterize early in antibody drug discovery
- Empirical polyspecificity screening is the only practical way to assess binding to relevant endogenous biomolecules
- The highly multiplexed nature of the LSA is well designed to screen drug candidates against hundreds of polyspecificity reagents

"For the development of improved workflows to be efficient, they will ideally work across both the **human and research animal** species proteomes, **be rapid, cost-effective** to perform, and applicable **early enough in the drug discovery process to maximize their value**. These technologies may be purely experimental, or a combination of **artificial intelligence- driven in silico and in vitro approaches**."

Finley et al. (2021) *Polyreactivity and polyspecificity in therapeutic antibody development: risk factors for failure in preclinical and clinical development campaigns*, mAbs, 13:1.

# App note: Screening antibodies against array of abundant blood proteins

- Each blood protein arrayed on anti-His coated HC200M in duplicate
- Antibodies injected at low (10nM) and high (100nM) concentrations
- Protein A and antigen used as positive controls
- Regeneration with 10mM glycine pH 1.5

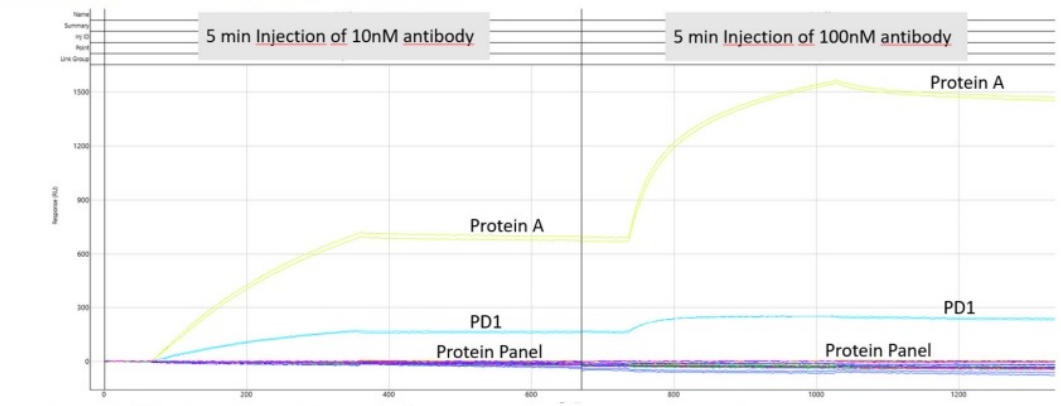
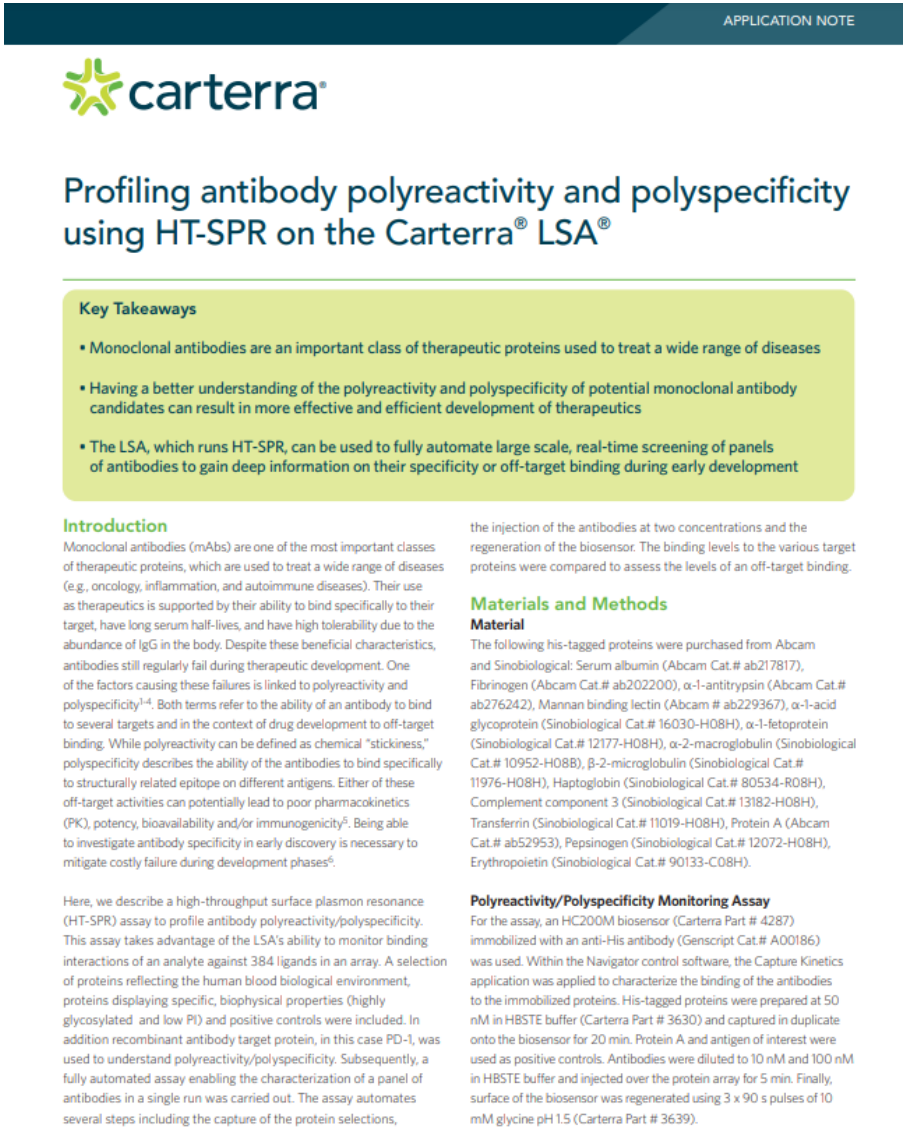



Figure 2. Sequential injections of an anti-PD1 antibody at 10 nM and 100 nM across the protein array.

| | Anti-RBD mAbs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ab1-10 | Ab1-100 | Ab2-10 | Ab2-100 | Ab3-10 | Ab3-100 | Ab4-10 | Ab4-100 | Ab5-10 | Ab5-100 |
| RBD | 0.22 | 0.41 | 0.16 | 0.35 | 0.35 | 0.41 | 0.24 | 0.35 | 0.22 | 0.36 |
| PD1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Protein A | 1.56 | 2.70 | 1.68 | 3.74 | 1.10 | 2.36 | 1.01 | 2.27 | 1.90 | 3.20 |
| α-2-macroglobulin | 0.02 | 0.03 | 0.03 | 0.04 | 0.02 | 0.05 | 0.03 | 0.00 | 0.07 | 0.05 |
| α-1-acid glycoprotein | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Pepsinogen | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 | 0.01 |
| Serum albumin | 0.01 | 0.02 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.02 | 0.00 | 0.00 |
| Haptoglobin | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| α-1-fetoprotein | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 |
| Erythropoietin | 0.01 | 0.01 | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 |
| ß-2 microglobulin | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Complement component 3 | 0.01 | 0.02 | 0.01 | 0.01 | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.01 |
| Fibrinogen 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 |
| Mannan binding lectin | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 |
| Transferrin | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 |
| α-1-antitrypsin | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Blank | 0.18 | 0.28 | 0.10 | 0.08 | 0.45 | 0.09 | 0.20 | 0.10 | 0.04 | 0.02 |
| | Anti-PD1 mAbs | | | | | | | | | |
| | Ab6-10 | Ab6-100 | Ab7-10 | Ab7-100 | Ab8-10 | Ab8-100 | Ab9-10 | Ab9-100 | Ab10-10 | Ab10-100 |
| RBD | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PD1 | 0.35 | 0.36 | 0.19 | 0.33 | 0.36 | 0.36 | 0.36 | 0.36 | 0.14 | 0.35 |
| Protein A | 1.13 | 2.58 | 0.83 | 2.03 | 0.87 | 2.04 | 0.57 | 1.69 | 0.67 | 1.85 |
| α-2-macroglobulin | 0.02 | 0.04 | 0.02 | 0.04 | 0.02 | 0.03 | 0.03 | 0.03 | 0.02 | 0.03 |
| α-1-acid glycoprotein | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Pepsinogen | 0.01 | 0.01 | 0.21 | 0.33 | 0.01 | 0.01 | 0.18 | 0.29 | 0.01 | 0.01 |
| Serum albumin | 0.00 | 0.00 | 0.07 | 0.07 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 |
| Haptoglobin | 0.00 | 0.00 | 0.15 | 0.23 | 0.00 | 0.00 | 0.12 | 0.23 | 0.00 | 0.00 |
| α-1-fetoprotein | 0.00 | 0.00 | 0.10 | 0.15 | 0.00 | 0.00 | 0.12 | 0.18 | 0.00 | 0.01 |
| Erythropoietin | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.01 |
| ß-2 microglobulin | 0.00 | 0.00 | 0.10 | 0.12 | 0.00 | 0.00 | 0.11 | 0.16 | 0.00 | 0.00 |
| Complement component 3 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 |
| Fibrinogen 1 | 0.00 | 0.00 | 0.22 | 0.34 | 0.00 | 0.00 | 0.22 | 0.32 | 0.00 | 0.00 |
| Mannan binding lectin | 0.00 | 0.00 | 0.11 | 0.14 | 0.00 | 0.00 | 0.08 | 0.14 | 0.00 | 0.00 |
| Transferrin | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 |
| α-1-antitrypsin | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Blank | 0.01 | 0.01 | 0.16 | 0.08 | 0.12 | 0.20 | 31.00 | 58.00 | 0.06 | 0.22 |

# Enhancing polyspecificity understanding using the LSA

- Screen against hundreds of relevant proteins simultaneously
- Common tag capture strategy makes assay constituents plug and play
- Proteins from both human and preclinical models can be integrated into the same screen



APPLICATION NOTE

carterra

## Profiling antibody polyreactivity and polyspecificity using HT-SPR on the Carterra® LSA®

**Key Takeaways**

- Monoclonal antibodies are an important class of therapeutic proteins used to treat a wide range of diseases
- Having a better understanding of the polyreactivity and polyspecificity of potential monoclonal antibody candidates can result in more effective and efficient development of therapeutics
- The LSA, which runs HT-SPR, can be used to fully automate large scale, real-time screening of panels of antibodies to gain deep information on their specificity or off-target binding during early development

### Introduction

Monoclonal antibodies (mAbs) are one of the most important classes of therapeutic proteins, which are used to treat a wide range of diseases (e.g., oncology, inflammation, and autoimmune diseases). Their use as therapeutics is supported by their ability to bind specifically to their target, have long serum half-lives, and have high tolerability due to the abundance of IgG in the body. Despite these beneficial characteristics, antibodies still regularly fail during therapeutic development. One of the factors causing these failures is linked to polyreactivity and polyspecificity[1-4]. Both terms refer to the ability of an antibody to bind to several targets and in the context of drug development to off-target binding. While polyreactivity can be defined as chemical "stickiness," polyspecificity describes the ability of the antibodies to bind specifically to structurally related epitope on different antigens. Either of these off-target activities can potentially lead to poor pharmacokinetics (PK), potency, bioavailability and/or immunogenicity[5]. Being able to investigate antibody specificity in early discovery is necessary to mitigate costly failure during development phases[6].

Here, we describe a high-throughput surface plasmon resonance (HT-SPR) assay to profile antibody polyreactivity/polyspecificity. This assay takes advantage of the LSA's ability to monitor binding interactions of an analyte against 384 ligands in an array. A selection of proteins reflecting the human blood biological environment, proteins displaying specific, biophysical properties (highly glycosylated and low PI) and positive controls were included. In addition recombinant antibody target protein, in this case PD-1, was used to understand polyreactivity/polyspecificity. Subsequently, a fully automated assay enabling the characterization of a panel of antibodies in a single run was carried out. The assay automates several steps including the capture of the protein selections, the injection of the antibodies at two concentrations and the regeneration of the biosensor. The binding levels to the various target proteins were compared to assess the levels of an off-target binding.

### Materials and Methods

**Material**

The following his-tagged proteins were purchased from Abcam and Sinobiological: Serum albumin (Abcam Cat.# ab217817), Fibrinogen (Abcam Cat.# ab202200), α-1-antitrypsin (Abcam Cat.# ab276242), Mannan binding lectin (Abcam # ab229367), α-1-acid glycoprotein (Sinobiological Cat.# 16030-H08H), α-1-fetoprotein (Sinobiological Cat.# 12177-H08H), α-2-macroglobulin (Sinobiological Cat.# 10952-H08B), β-2-microglobulin (Sinobiological Cat.# 11976-H08H), Haptoglobin (Sinobiological Cat.# 80534-R08H), Complement component 3 (Sinobiological Cat.# 13182-H08H), Transferrin (Sinobiological Cat.# 11019-H08H), Protein A (Abcam Cat.# ab52953), Pepsinogen (Sinobiological Cat.# 12072-H08H), Erythropoietin (Sinobiological Cat.# 90133-C08H).

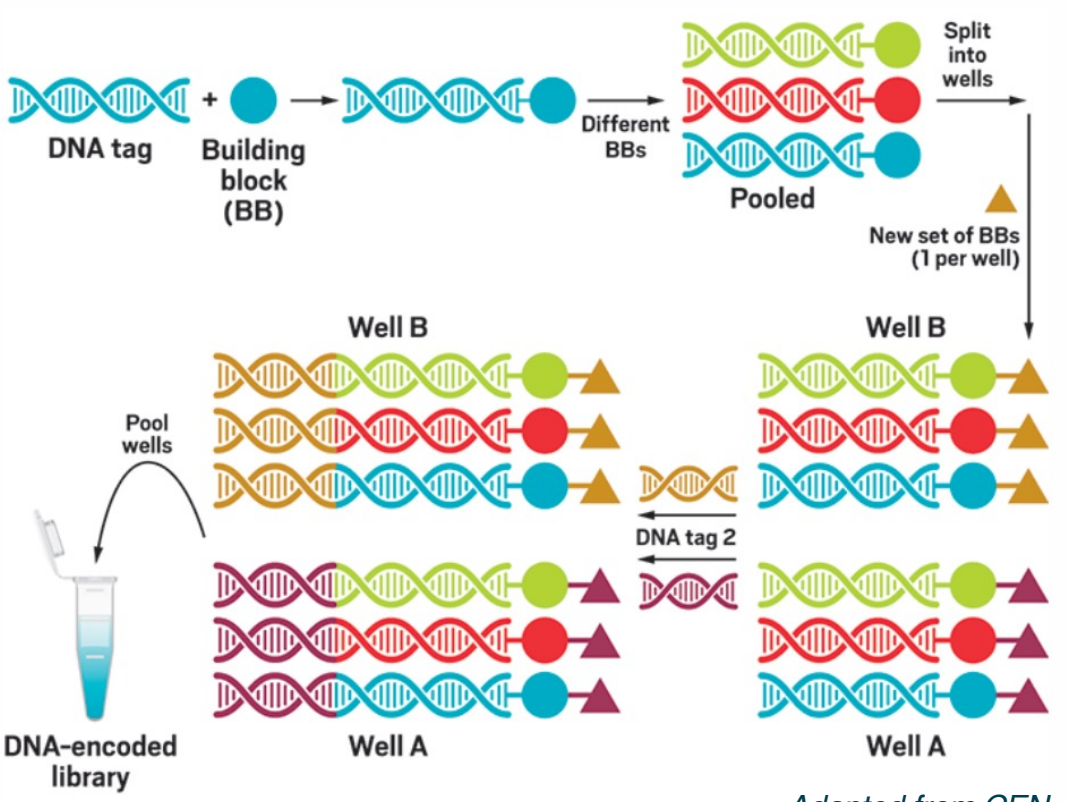
**Polyreactivity/Polyspecificity Monitoring Assay**

For the assay, an HC200M biosensor (Carterra Part # 4287) immobilized with an anti-His antibody (Genscript Cat.# A00186) was used. Within the Navigator control software, the Capture Kinetics application was applied to characterize the binding of the antibodies to the immobilized proteins. His-tagged proteins were prepared at 50 nM in HBSTE buffer (Carterra Part # 3630) and captured in duplicate onto the biosensor for 20 min. Protein A and antigen of interest were used as positive controls. Antibodies were diluted to 10 nM and 100 nM in HBSTE buffer and injected over the protein array for 5 min. Finally, surface of the biosensor was regenerated using 3 x 90 s pulses of 10 mM glycine pH 1.5 (Carterra Part # 3639).

# Screening of DNA Encoded Libraries

carterra

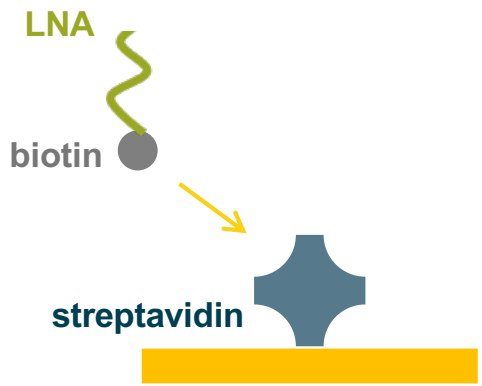
# DNA Encoded Library (DEL) Technology




*Adapted from CEN*

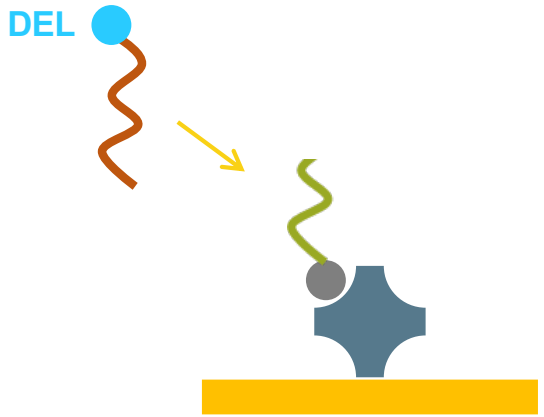
- Key Benefits
  - Rapid screening and identification of hits in a pooled format
  - No structural info required
- Challenges
  - Screening is low-resolution
  - Affinity is unknown
  - Weak binders can be lost

## Biotinylated LNA coated on streptavidin surface



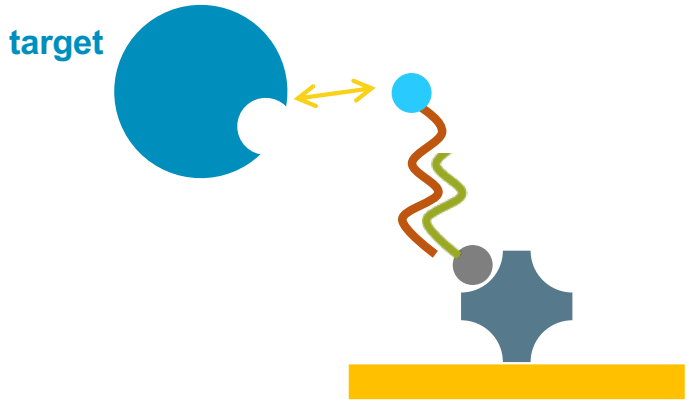

1

## 384 DEL compounds arrayed via LNA hybridization



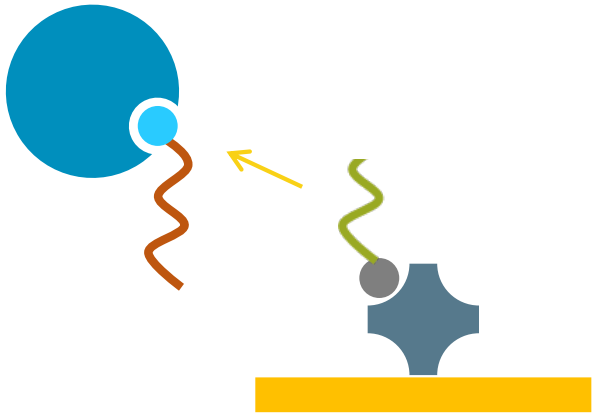

2

## Real-time binding of titrated target




3

## Regenerate surface and repeat



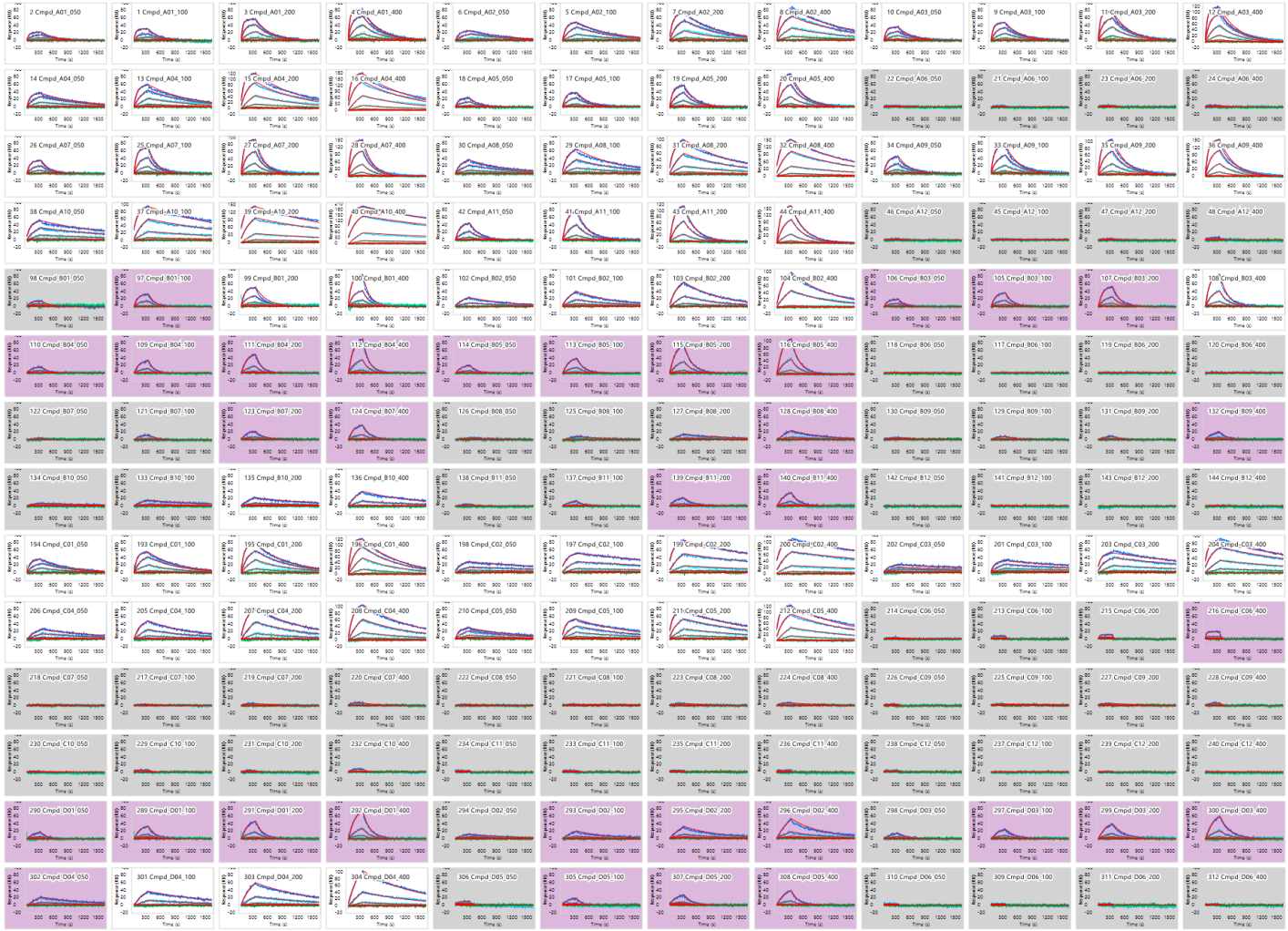
4

# HT-SPR DEL assay metrics

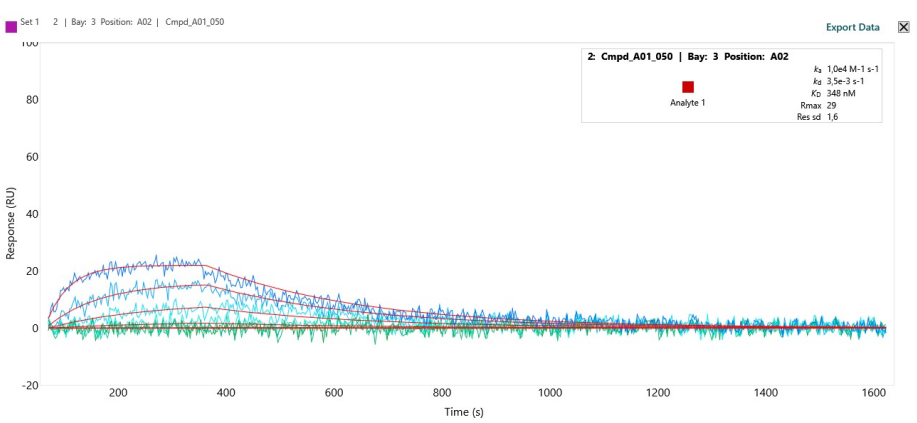
- Max throughput (detailed kinetic affinity)
  - 1,152 DELs/day
  - 5,760 DELs/week
- DEL quantity
  - 44 pmol each
- Target quantity (e.g., 20 kDa protein @ 6uM max conc)
  - 1,152 DELs: 165 ug
  - 5,760 DELs: 495 ug

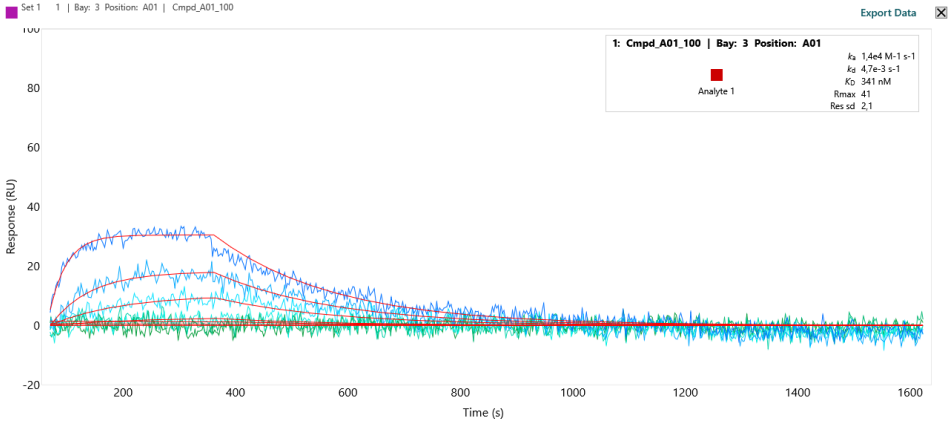
# Representative DEL kinetic screening data

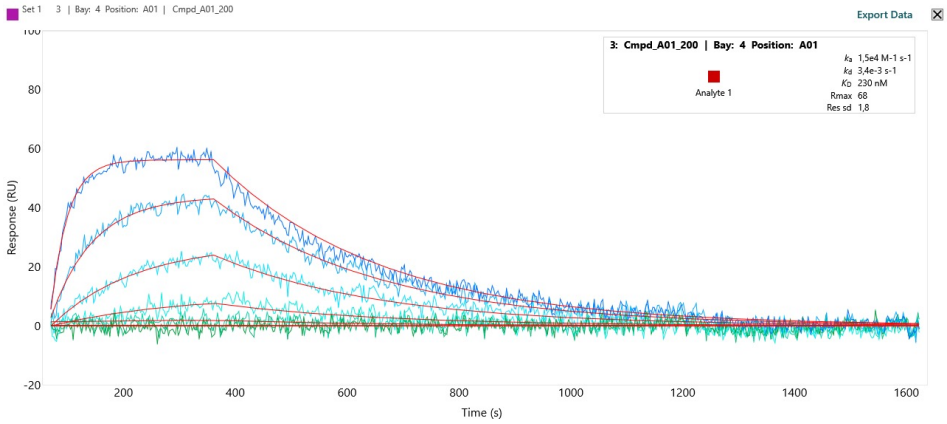
- 1:1 fit model lines in red
- Automatic data flagging in analysis software highlighting low activity (gray) and complex binders (purple)
- 1,152 affinities process in < 10 min

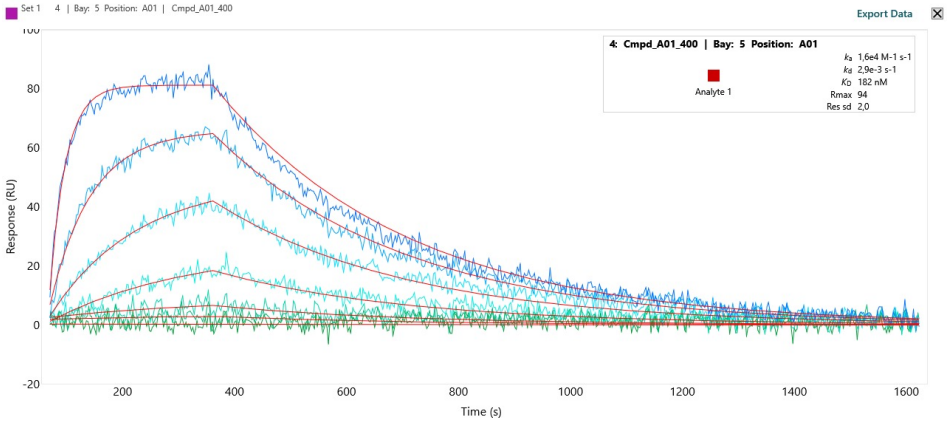


# Example of data quality at multiple densities



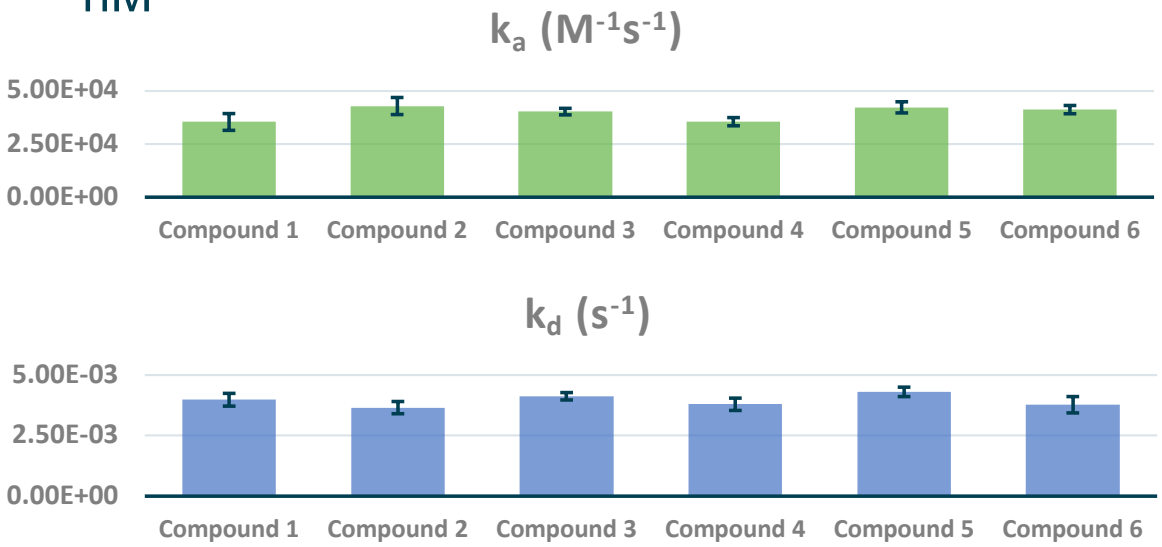



# Kinetic uniformity

- 6 DEL compounds tested as 8 replicates each
- 47 kDa protein target injected from 1000 to 0.24 nM



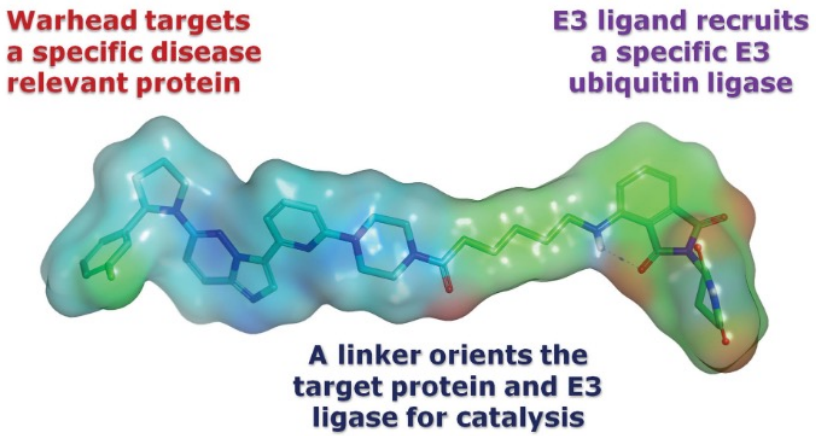

**≤ 10%** variation across all on and off-rates

# Targeted Protein Degrader (TPD) Characterization

carterra

# Therapeutic Value of TPDs

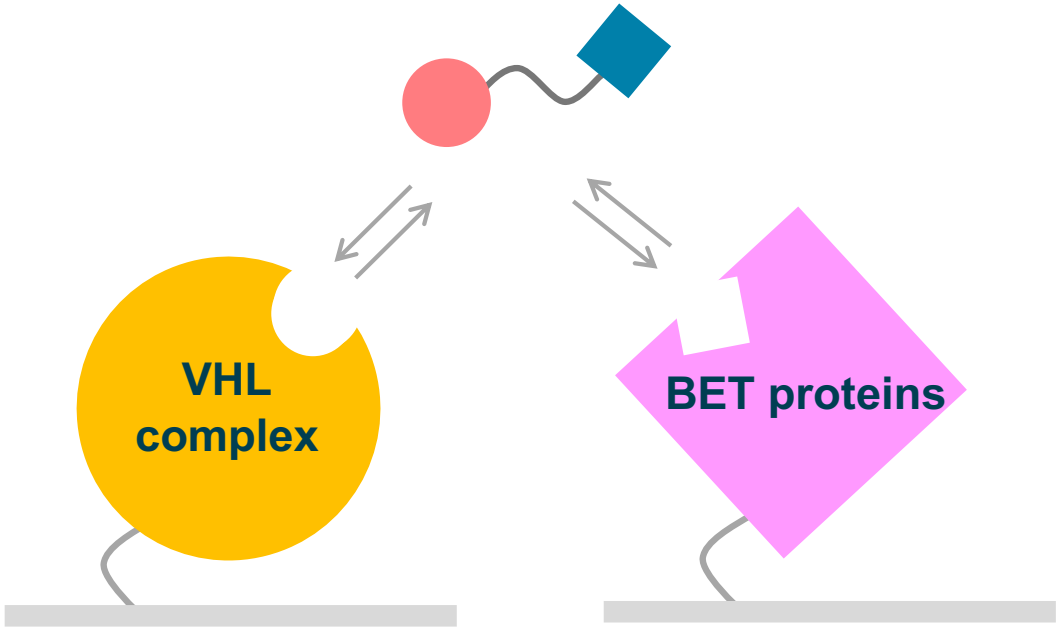
- AKA PROTACS (**PRO**teolysis **T**argeting **C**himeras)
- Harness native intracellular degradation pathways to remove disease-related proteins
- High therapeutic potential where traditional protein-protein inhibitory drug strategies are not appropriate




Typically 700-1000 Da

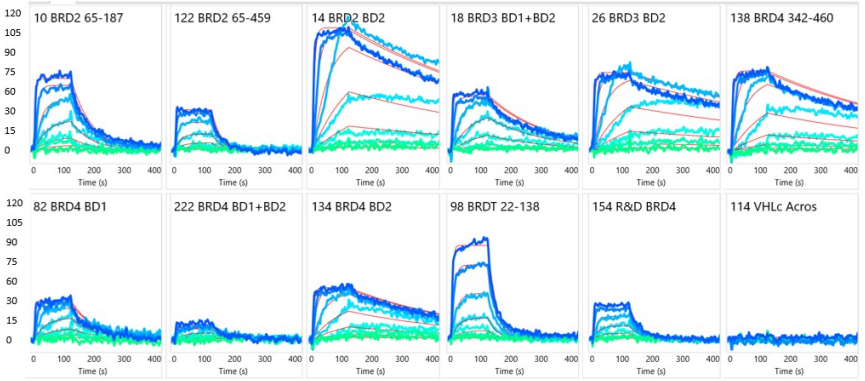
# Binary kinetics example: TPDs targeting VHL and BET proteins

- E3 ligases and target proteins arrayed in triplicate at several densities either covalently or non-covalently
- Multiple compounds injected as titrated series

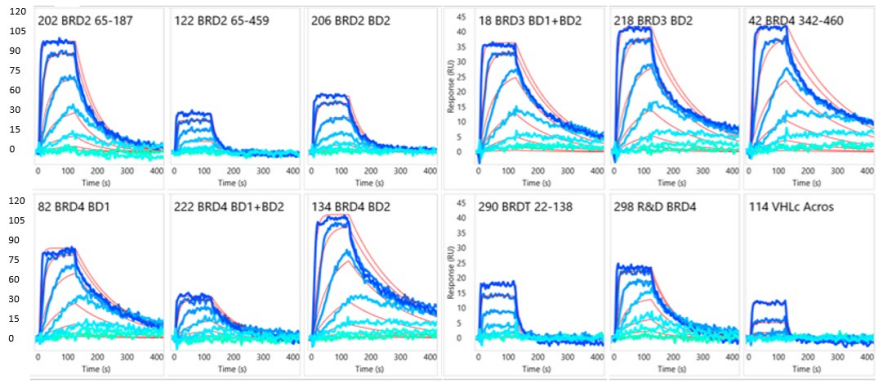


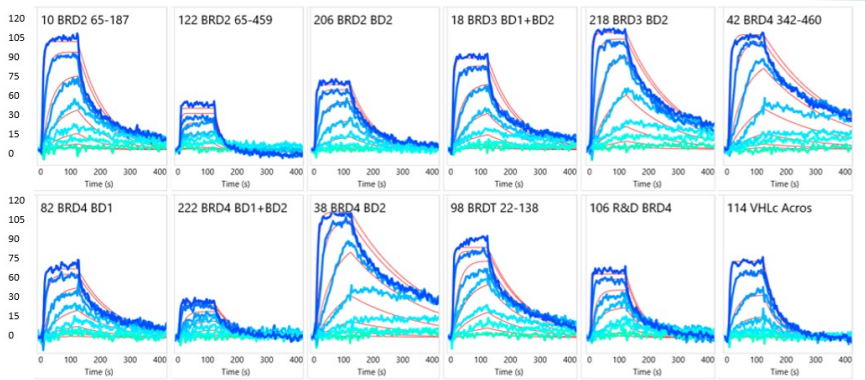
# Kinetics of four TPDs against panel of BET proteins and VHL

ZHX236

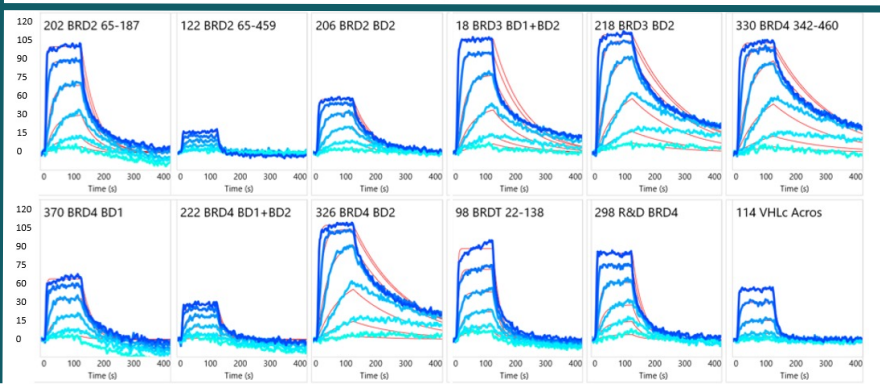



MZ1



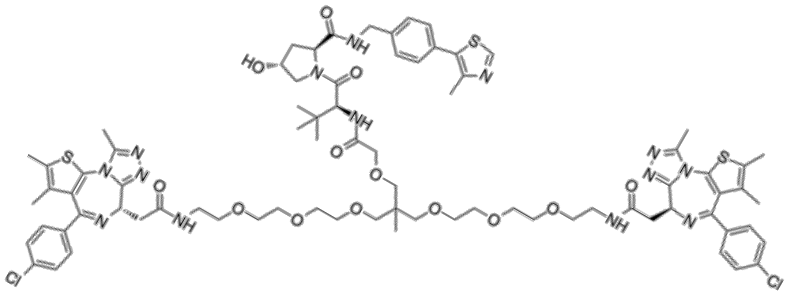




AT1



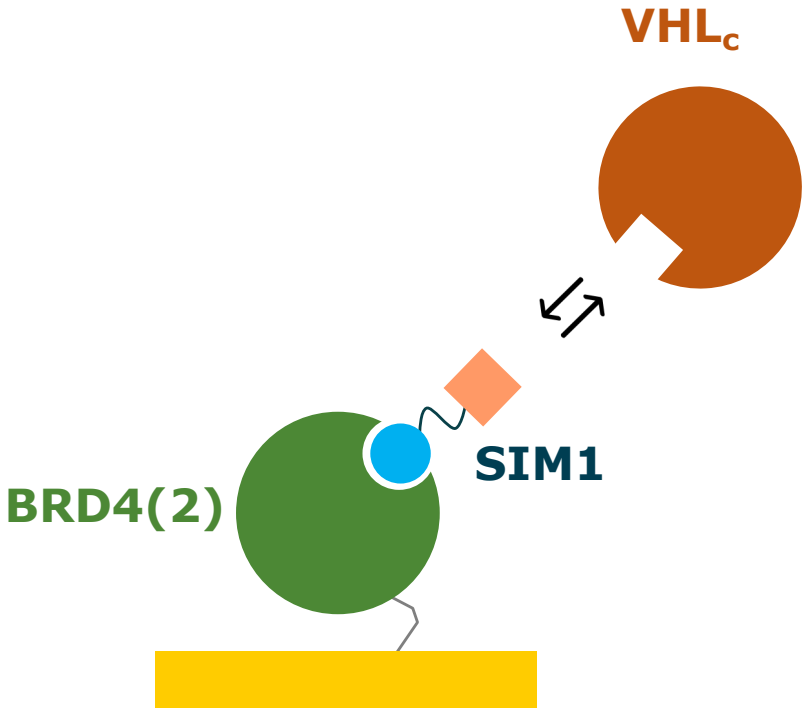

ARV771

# Ternary complex evaluation using SIM1, a high-affinity BRD binder

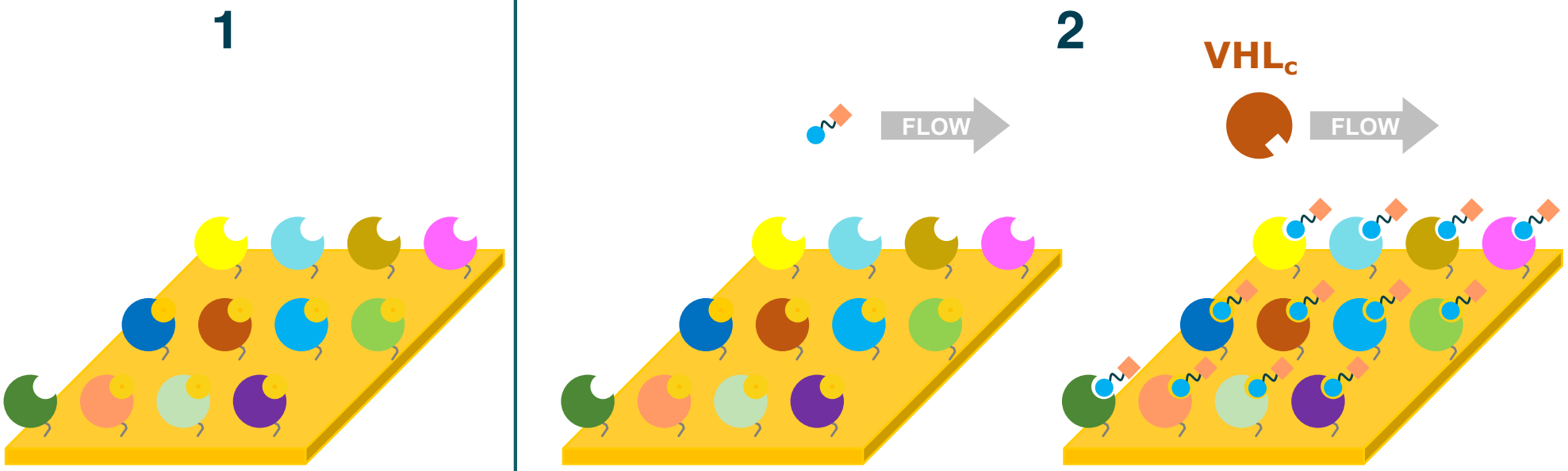


**SIM1**

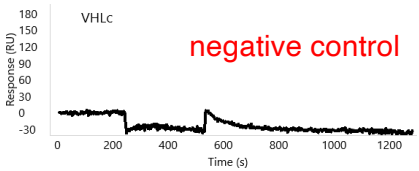
trivalent PROTAC
mw = 1619 Da
$K_D$(BRD4) < 1 nM

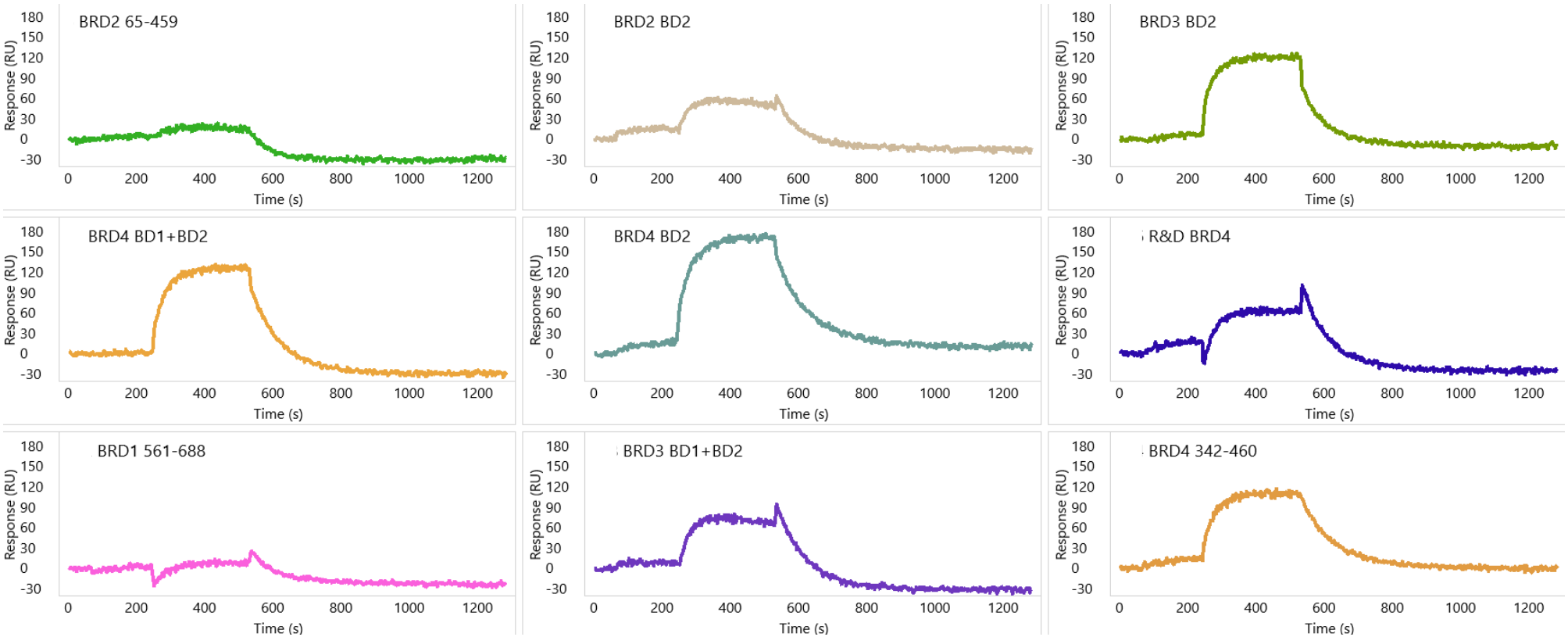


# Ternary specificity: $VHL_c$ + BRD/SIM1



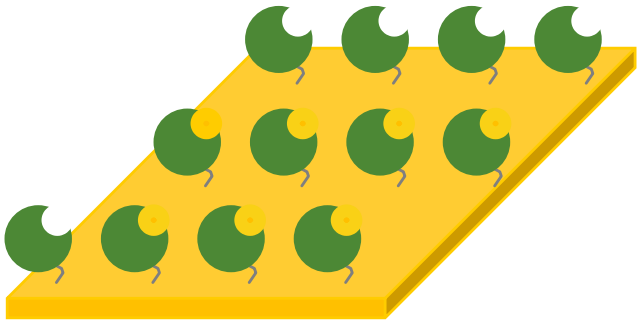
# Co-injection of SIM1 then $VHL_c$ across multiple BET proteins



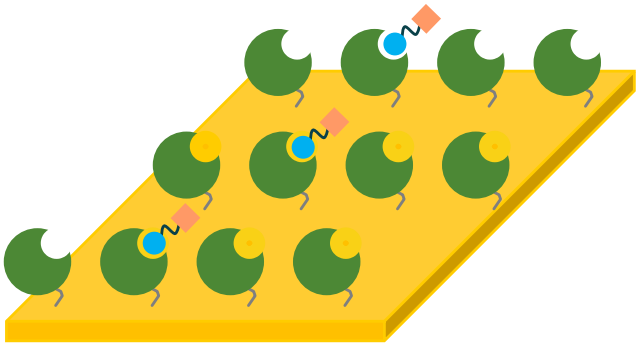
# Ternary kinetics: $VHL_c$ + BRD/SIM1

**1**



**Immob BRD4(2) lawn**

**2**

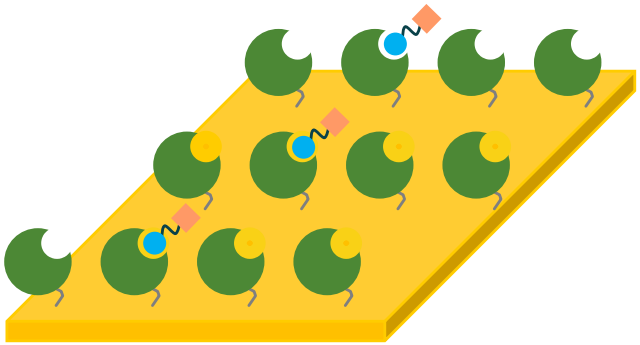


**Capture SIM1 array**

**3**

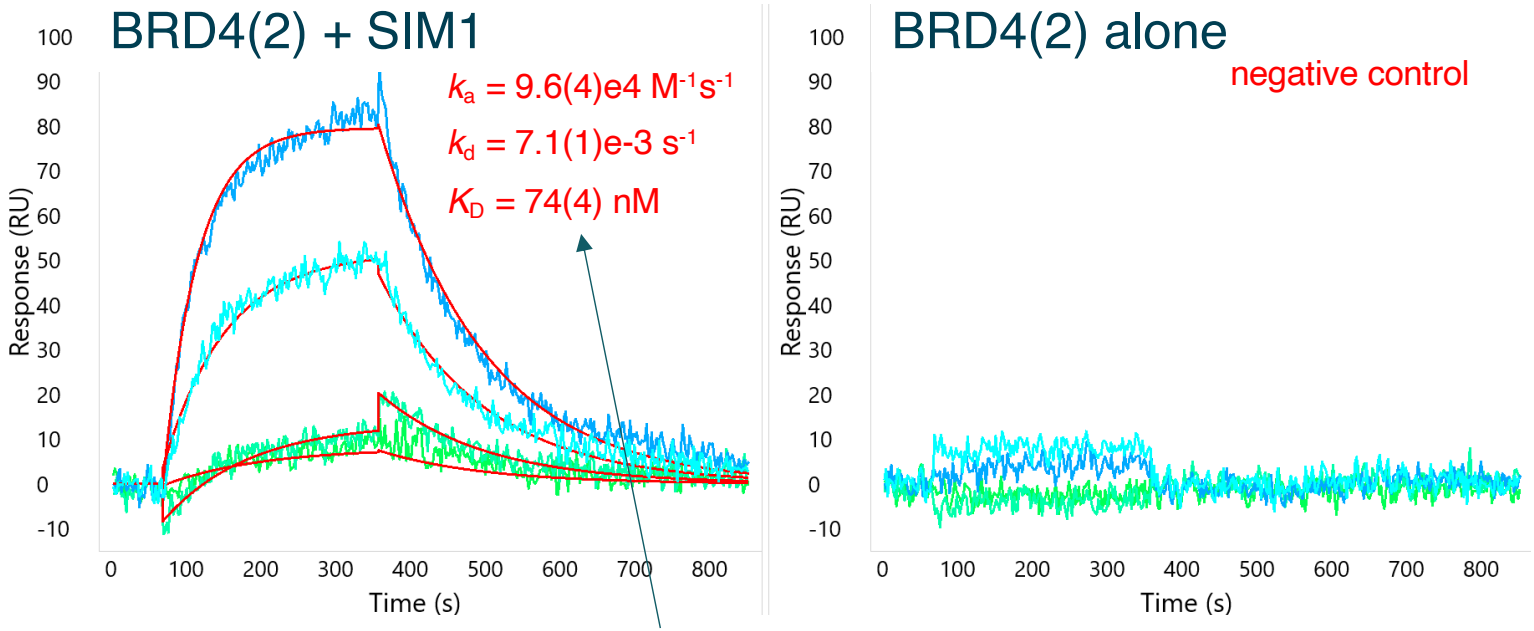
$VHL_c$

FLOW



**Inject VHLc**

# Ternary kinetics of $VHL_c$ binding to BRD4(2)/SIM1




Imaide et al. ternary $K_D$: 94 nM

Imaide S, et al. *Trivalent PROTACs enhance protein degradation via combined avidity and cooperativity.* Nat Chem Biol. 2021 Nov;17(11):1157-1167.

# What the Carterra LSA brings to TPD discovery

Throughput: Screen hundreds of warhead/linker/E3 ligand combinations for both binary and ternary binding/affinity

Specificity: Screen each TPD against up multiple E3 ligases, targets, isoforms etc. in parallel

Flexibility: Multiple assay configurations depending on project needs

# Applications on the horizon…..

**Aptamers**

**Membrane proteins including GPCRs**

# Thank You!

**The Carterra Scientific Team**

- Judicael Parisot
- Jonathan Popplewell
- Maria McGresham
- Ira Seferovich
- Alyssa Hughes
- Rebecca Rich
- Dan Bedinger
- Camille Troup

carterra®