Driving discovery of novel medicines using Carterra's HT-SPR technology

carterra®

About Carterra

- Founded 2005
- Headquartered in Salt Lake City, UT
- Leader in gold-standard, high-throughput SPR instrumentation and analysis technology
- Customer Experience Centers in SFO, SLC, Boston, MAN, Munich, Tokyo, Seoul, & Shanghai
- Distributed by Revvity in APAC

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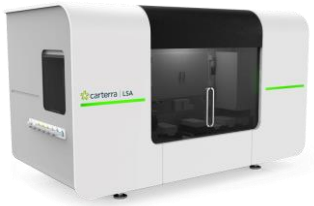
HT-SPR is embedded in discovery & development across pharma & biotech

Plug and play ready for any workflow



LSA

LSA^{XT}

Instrumentation

Control and analysis software

Biosensor chips and consumables

LSA and LSA^{XT}: Broad application space

Antibodies

- Antigen kinetics/affinity
- Epitope characterization
- FcγR/FcRn
- Thermodynamics
- Polyreactivity
- Quality benchmarking
- Immune profiling

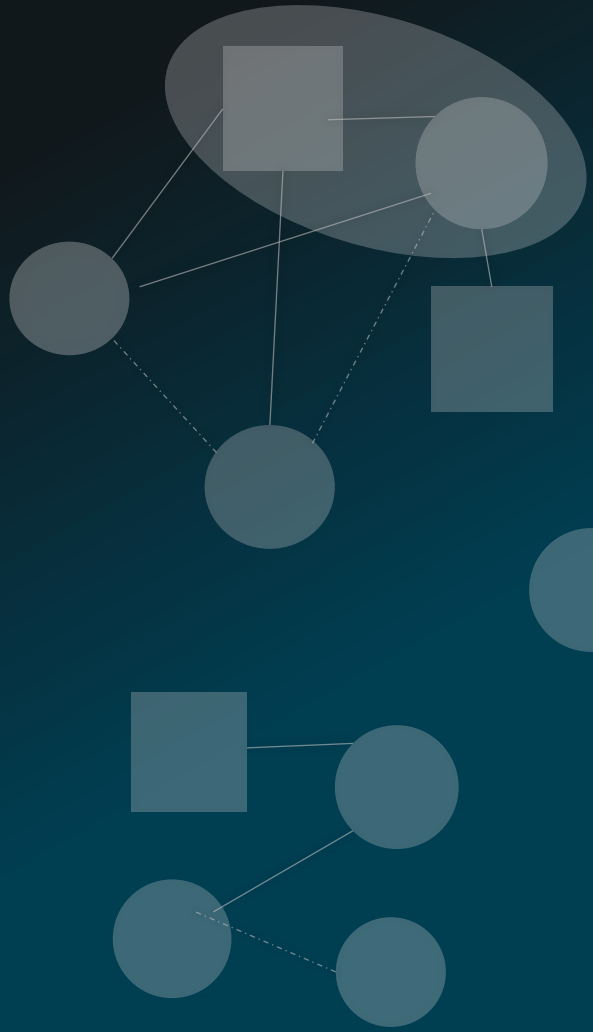
Other

- Peptides
- Macrocycles
- Aptamers
- Glycovariants
- Membrane proteins

Small molecules

- Omics-level screening
- Selectivity
- Target class profiling
- DNA Encoded Library (DEL) Compounds
- Glue/degraders

Kinase inhibitor profiling using Carterra LSA^{XT}



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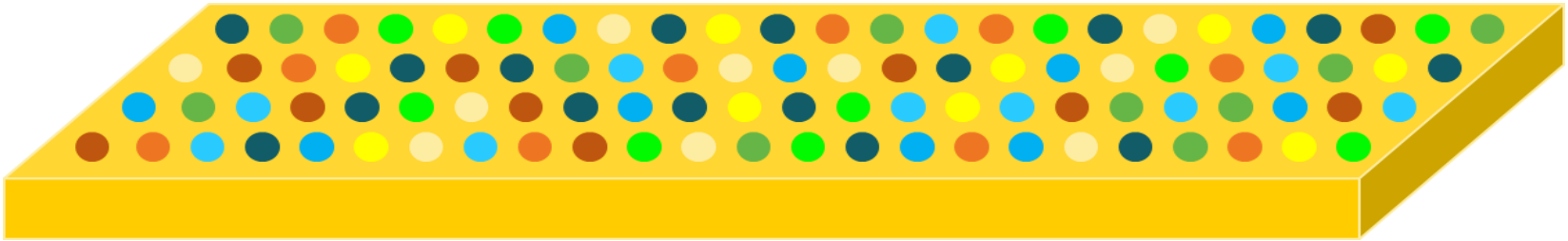
Kinome-level screening of inhibitors

- Collaboration between Carna Bio, Tocris (BioTechne), and Carterra.
- Unveiled as Spotlight presentation at SLAS 2024 in Boston.
- Binding kinetics of **210 compounds c**ompared against **104 biotinylated protein kinases.**

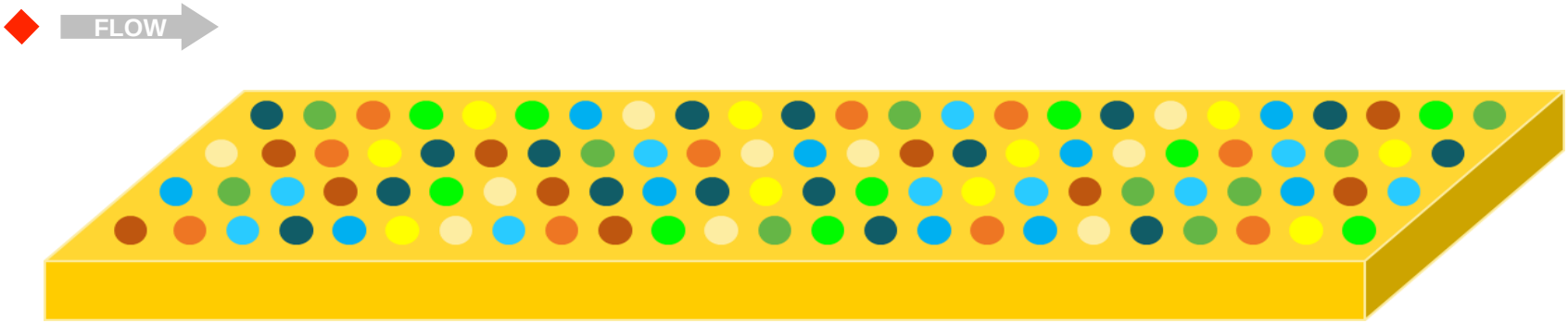
Adam Shutes, PhD Carna Bio

Step 1: Create the kinase array



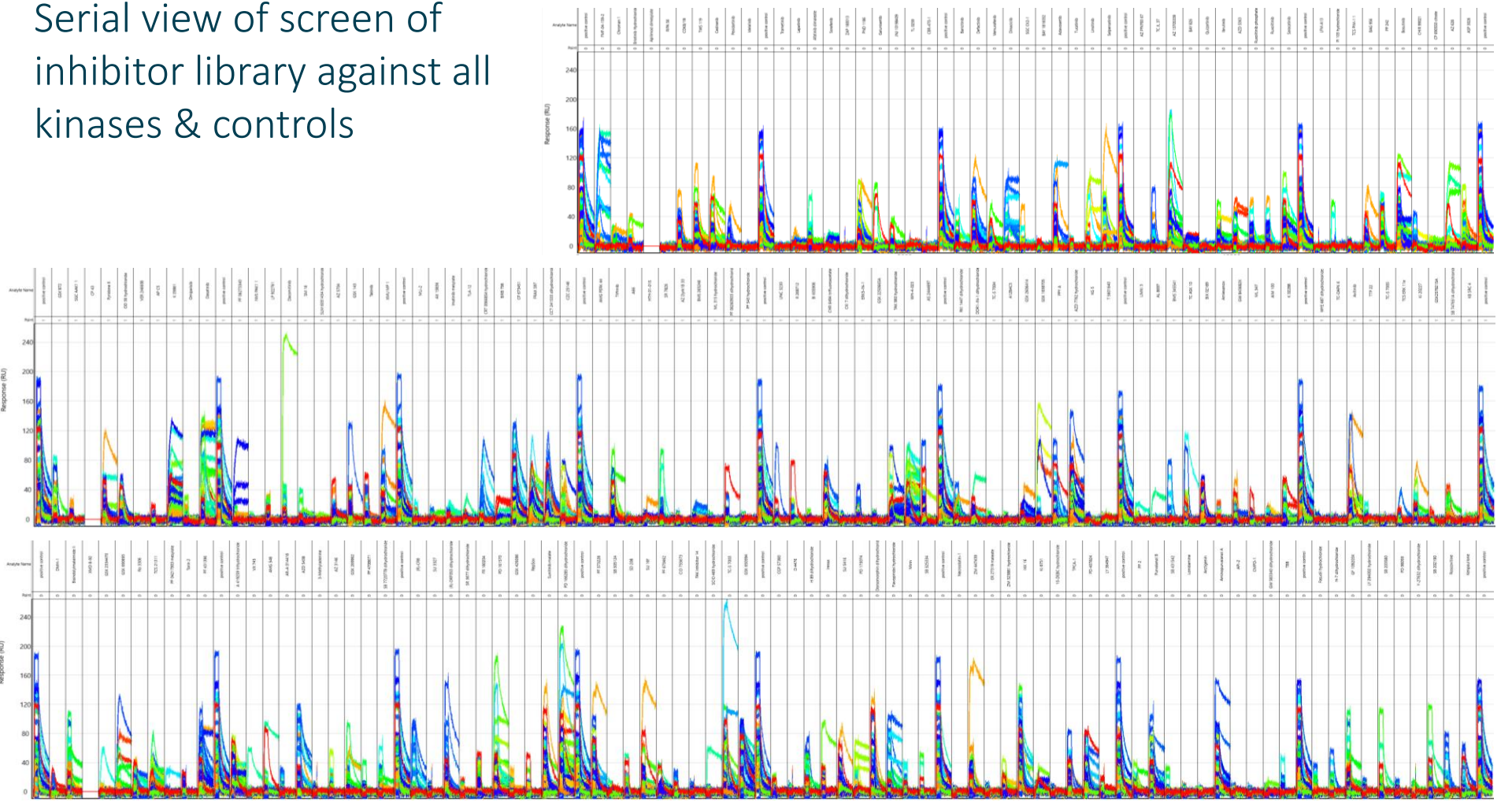
- 104 Carna Bio biotinylated kinases captured on SAD200M sensor chip
- Buffer: HBS, 5% glycerol, 0.005% Tween-20, 0.5 mg/mL BSA
- Temp: 15ºC
- 50 min capture
- Between 1,000 and 11,000 RU captured

Step 2: Inject Tocris Kinase Inhibitor library



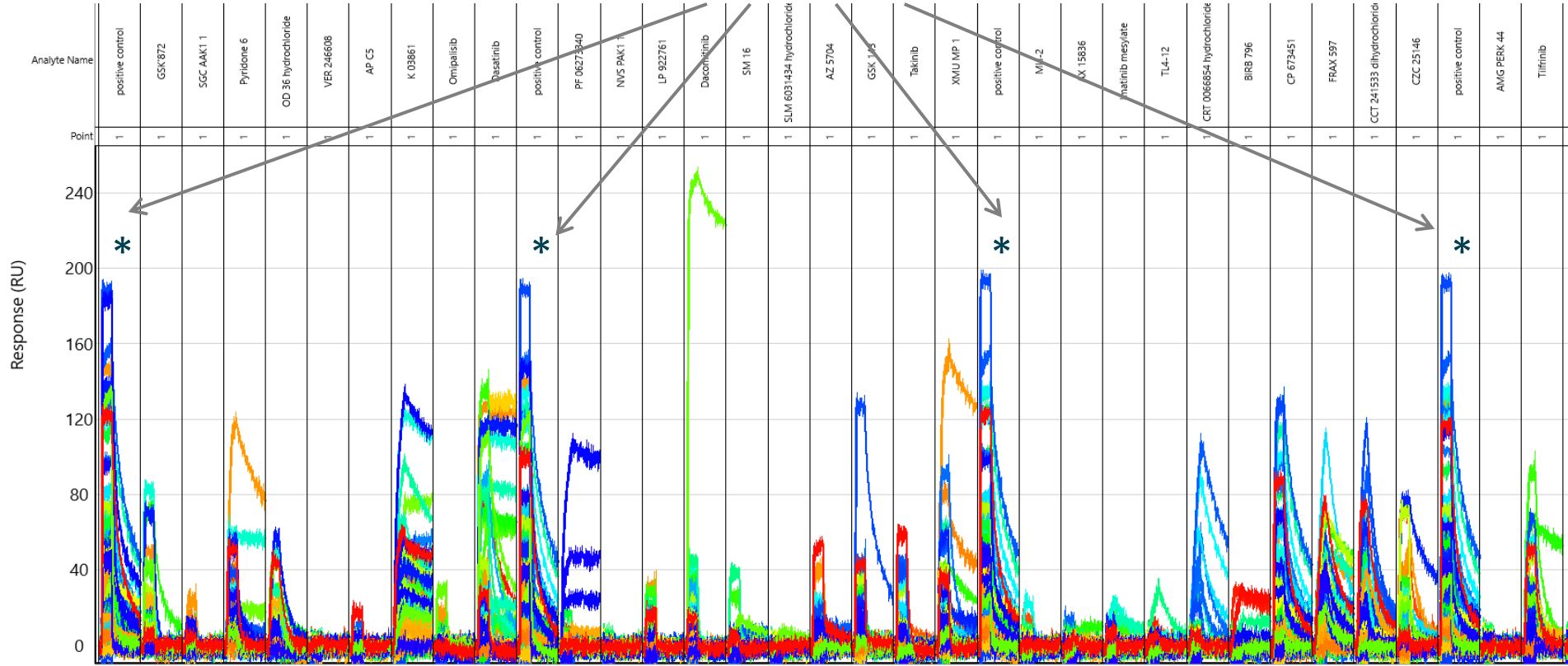
- Single point screen at 1 uM, total of 210 compounds.
- Select hits re-tested as two-fold dilution series ending at 2 uM.
- Buffer: HBS, 5% glycerol, 5 mM $MgCl_2$, 1 mM DTT, 0.005% Tween 20, 3% DMSO.
- Temp:15˚C.

Serial view of screen of inhibitor library against all kinases & controls



Zoomed view of 35 injections of screen

CP 673451 (440Da) injected periodically to track activity throughout screen



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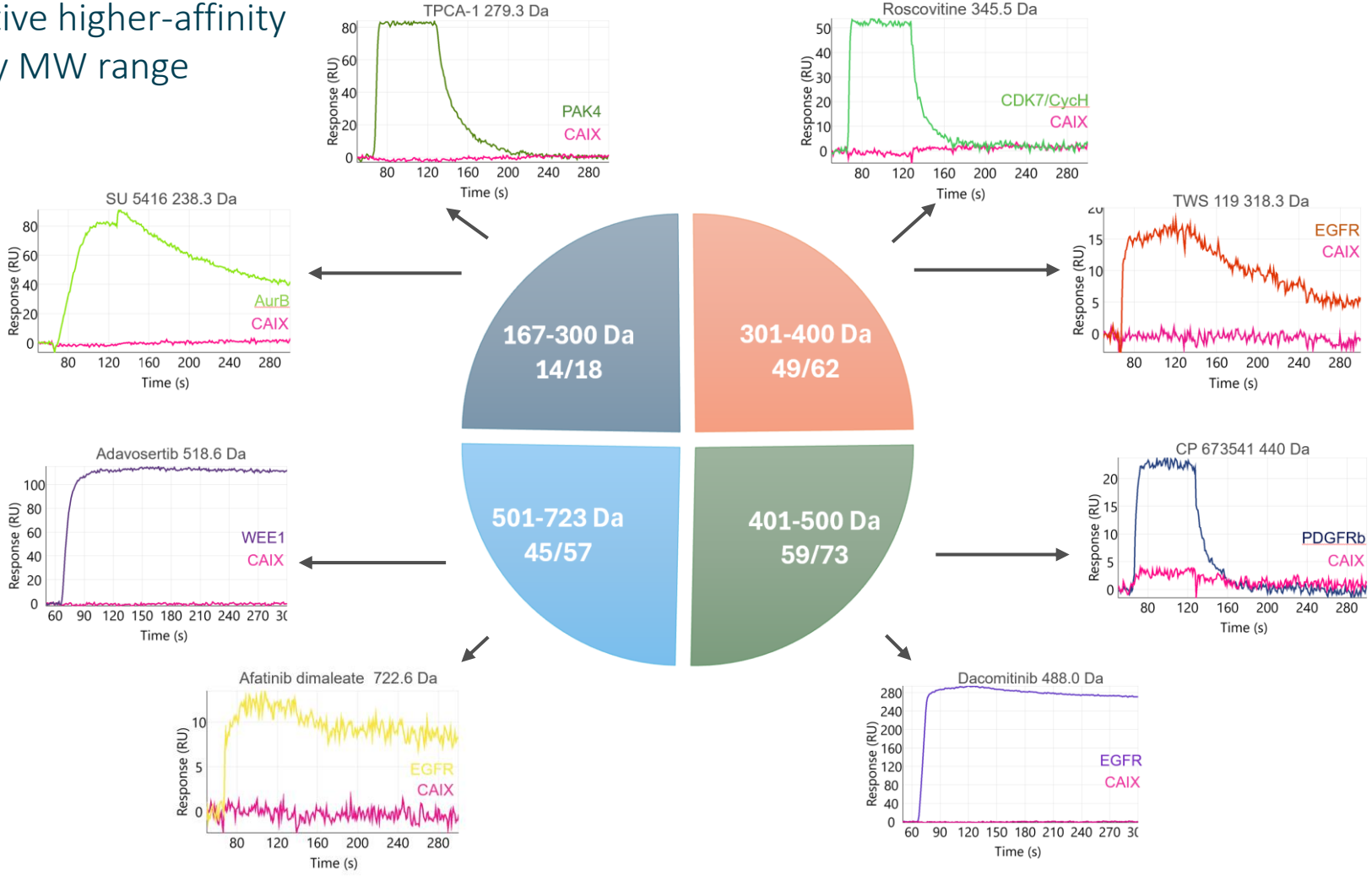
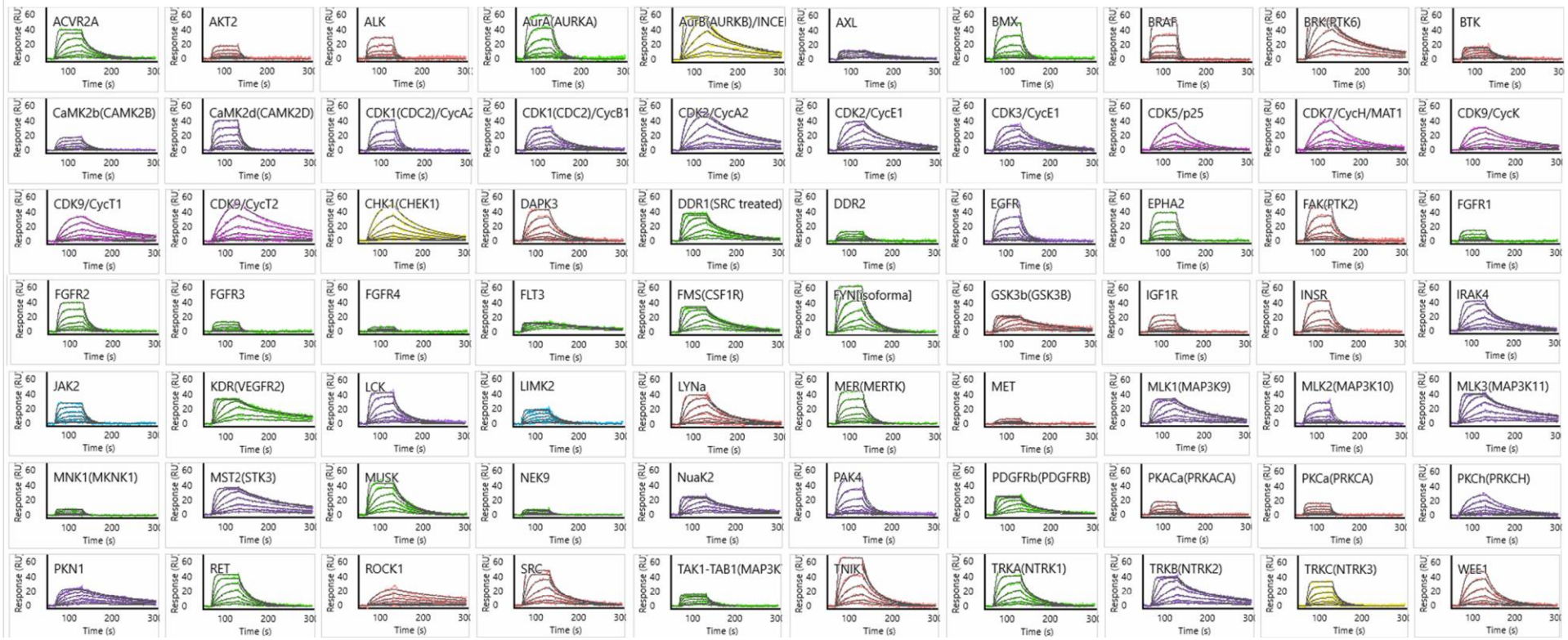
Representative higher-affinity hits by MW range



Hit follow up: detailed kinetics

SR 7826 (387.4 Da)

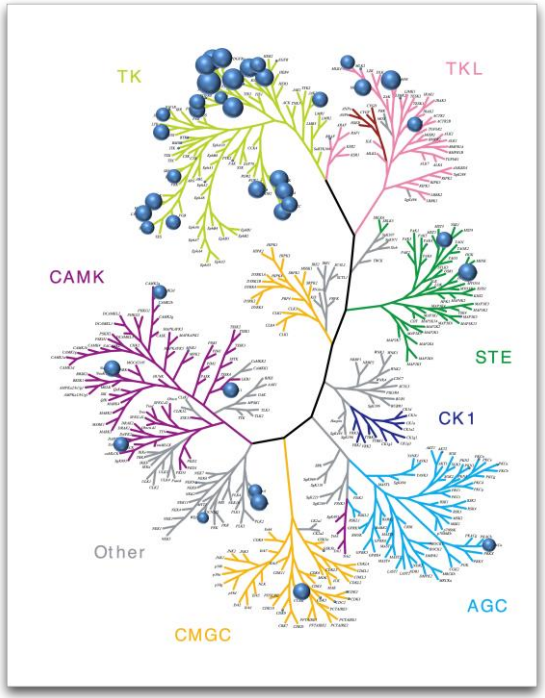
CHR 6494 415.4 Da Dorsomorphin 499.4 Da CP 673451 440.0 Da CRT 0066854 497.5 Da TPCA-1 279.3 Da



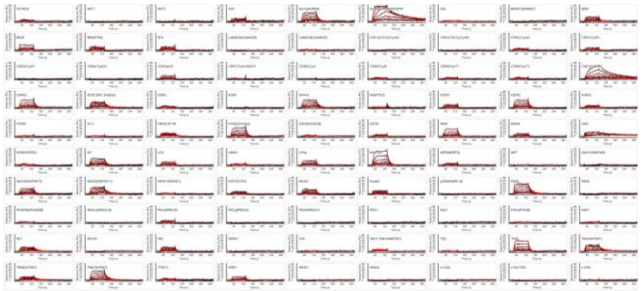
A new paradigm for small molecule drug discovery

- Deep mining of binding profiles across target families as well as off-targets in a single experiment
- These assays are resource prohibitive on any other platform

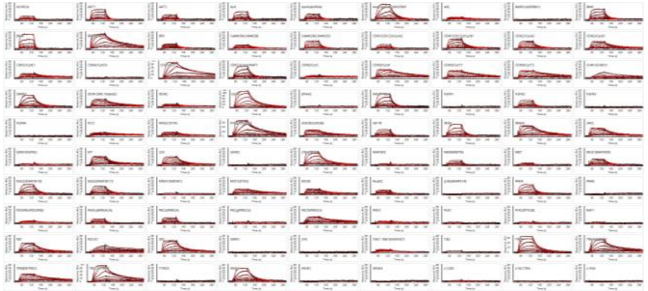
Kinome Mapping

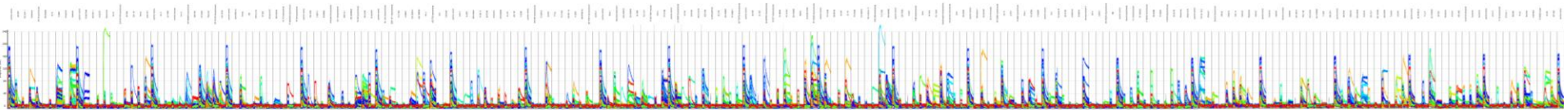


TPCA-1 279 Da



CHR 6494 415 Da



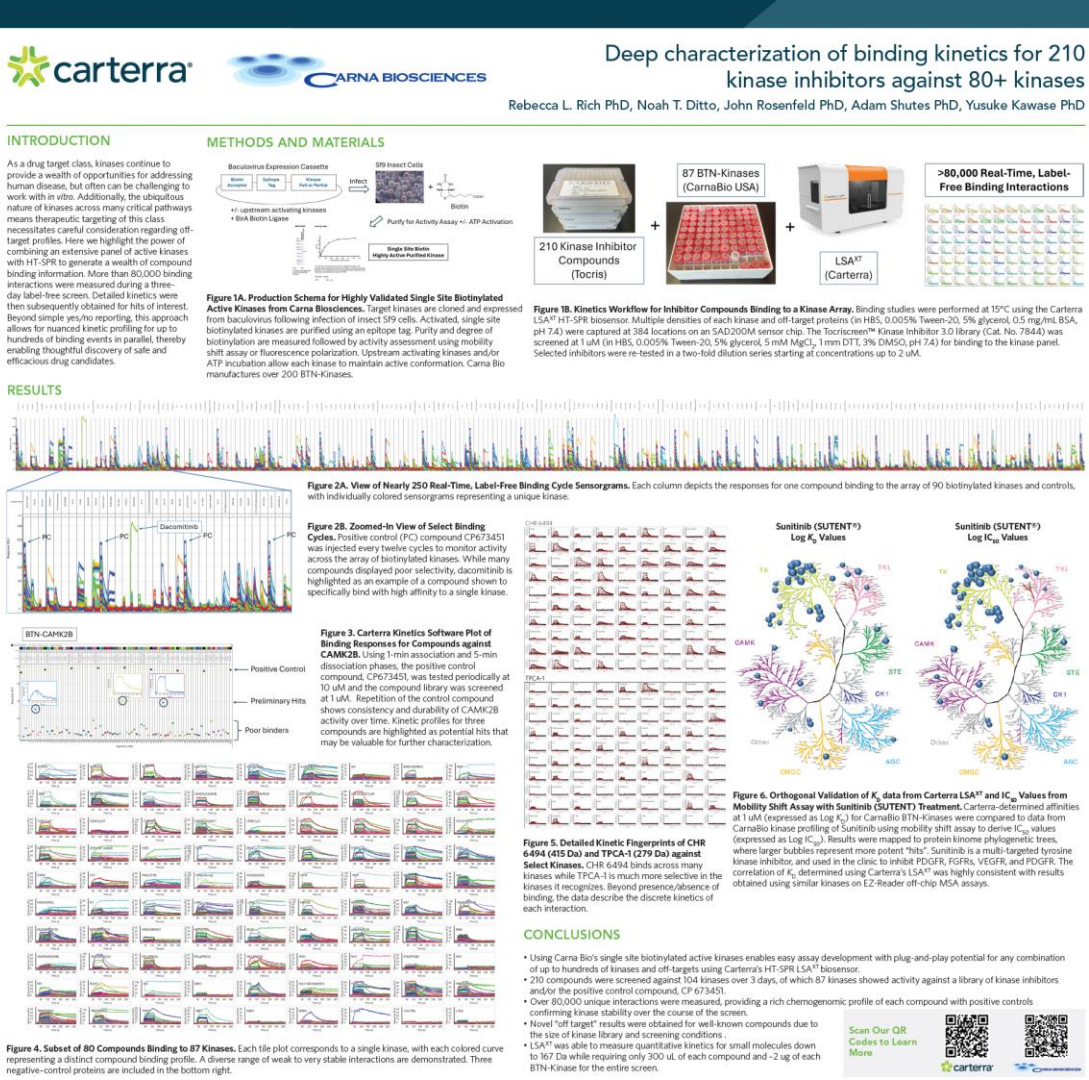


Check out our **kinase inhibitor poster** for more details:

https://carterra-bio.com/resource-category/posters/

or

Grab a hard copy at today's symposium!



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CARNA BIOSCIENCES

Deep characterization of binding kinetics for 210 kinase inhibitors against 80+ kinases

Rebecca L. Rich PhD, Noah T. Ditto, John Rosenfeld PhD, Adam Shutes PhD, Yusuke Kawase PhD

INTRODUCTION

As a drug target class, kinases continue to provide a wealth of opportunities for addressing human disease, but often can be challenging to work with *in vitro*. Additionally, the ubiquitous nature of kinases across many critical pathways means therapeutic targeting of this class necessitates careful consideration regarding off-target profiles. Here we highlight the power of combining an extensive panel of active kinases with HT-SPR to generate a wealth of compound binding information. More than 80,000 binding interactions were measured during a three-day label-free screen. Detailed kinetics were then subsequently obtained for hits of interest. Beyond simple yes/no reporting, this approach allows for nuanced kinetic profiling for up to hundreds of binding events in parallel, thereby enabling thoughtful discovery of safe and efficacious drug candidates.

METHODS AND MATERIALS

Figure 1A. Production Schema for Highly Validated Single Site Biotinylated Active Kinases from Carna Biosciences. Target kinases are cloned and expressed from baculovirus following infection of insect Sf9 cells. Activated, single site biotinylated kinases are purified using an epitope tag. Purity and degree of biotinylation are measured followed by activity assessment using mobility shift assay or fluorescence polarization. Upstream activating kinases and/or ATP incubation allow each kinase to maintain active conformation. Carna Bio manufactures over 200 BTN-Kinases.

Figure 1B. Kinetics Workflow for Inhibitor Compounds Binding to a Kinase Array. Binding studies were performed at 15°C using the Carterra LSA^XT HT-SPR biosensor. Multiple densities of each kinase and off-target proteins (in HBS, 0.005% Tween-20, 5% glycerol, 0.5 mg/mL BSA, pH 7.4) were captured at 384 locations on an SAD200M sensor chip. The Tocriscreen™ Kinase Inhibitor 3.0 library (Cat. No. 7844) was screened at 1 uM (in HBS, 0.005% Tween-20, 5% glycerol, 5 mM $MgCl_2$, 1 mm DTT, 3% DMSO, pH 7.4) for binding to the kinase panel. Selected inhibitors were re-tested in a two-fold dilution series starting at concentrations up to 2 uM.

RESULTS

Figure 2A. View of Nearly 250 Real-Time, Label-Free Binding Cycle Sensorgrams. Each column depicts the responses for one compound binding to the array of 90 biotinylated kinases and controls, with individually colored sensorgrams representing a unique kinase.

Figure 2B. Zoomed-In View of Select Binding Cycles. Positive control (PC) compound CP673451 was injected every twelve cycles to monitor activity across the array of biotinylated kinases. While many compounds displayed poor selectivity, dacomitinib is highlighted as an example of a compound shown to specifically bind with high affinity to a single kinase.

Figure 3. Carterra Kinetics Software Plot of Binding Responses for Compounds against CAMK2B. Using 1-min association and 5-min dissociation phases, the positive control compound, CP673451, was tested periodically at 10 uM and the compound library was screened at 1 uM. Repetition of the control compound shows consistency and durability of CAMK2B activity over time. Kinetic profiles for three compounds are highlighted as potential hits that may be valuable for further characterization.

Figure 4. Subset of 80 Compounds Binding to 87 Kinases. Each tile plot corresponds to a single kinase, with each colored curve representing a distinct compound binding profile. A diverse range of weak to very stable interactions are demonstrated. Three negative-control proteins are included in the bottom right.

Figure 5. Detailed Kinetic Fingerprints of CHR 6494 (415 Da) and TPCA-1 (279 Da) against Select Kinases. CHR 6494 binds across many kinases while TPCA-1 is much more selective in the kinases it recognizes. Beyond presence/absence of binding, the data describe the discrete kinetics of each interaction.

Figure 6. Orthogonal Validation of K_D data from Carterra LSA^XT and IC_{50} Values from Mobility Shift Assay with Sunitinib (SUTENT) Treatment. Carterra-determined affinities at 1 uM (expressed as Log K_D) for CarnaBio BTN-Kinases were compared to data from CarnaBio kinase profiling of Sunitinib using mobility shift assay to derive IC_{50} values (expressed as Log IC_{50}). Results were mapped to protein kinome phylogenetic trees, where larger bubbles represent more potent "hits". Sunitinib is a multi-targeted tyrosine kinase inhibitor, and used in the clinic to inhibit PDGFR, FGFRs, VEGFR, and PDGFR. The correlation of K_D determined using Carterra's LSA^XT was highly consistent with results obtained using similar kinases on EZ-Reader off-chip MSA assays.

CONCLUSIONS

- Using Carna Bio's single site biotinylated active kinases enables easy assay development with plug-and-play potential for any combination of up to hundreds of kinases and off-targets using Carterra's HT-SPR LSA^XT biosensor.
- 210 compounds were screened against 104 kinases over 3 days, of which 87 kinases showed activity against a library of kinase inhibitors and/or the positive control compound, CP 673451.
- Over 80,000 unique interactions were measured, providing a rich chemogenomic profile of each compound with positive controls confirming kinase stability over the course of the screen.
- Novel "off target" results were obtained for well-known compounds due to the size of kinase library and screening conditions .
- LSA^XT was able to measure quantitative kinetics for small molecules down to 167 Da while requiring only 300 uL of each compound and ~2 ug of each BTN-Kinase for the entire screen.

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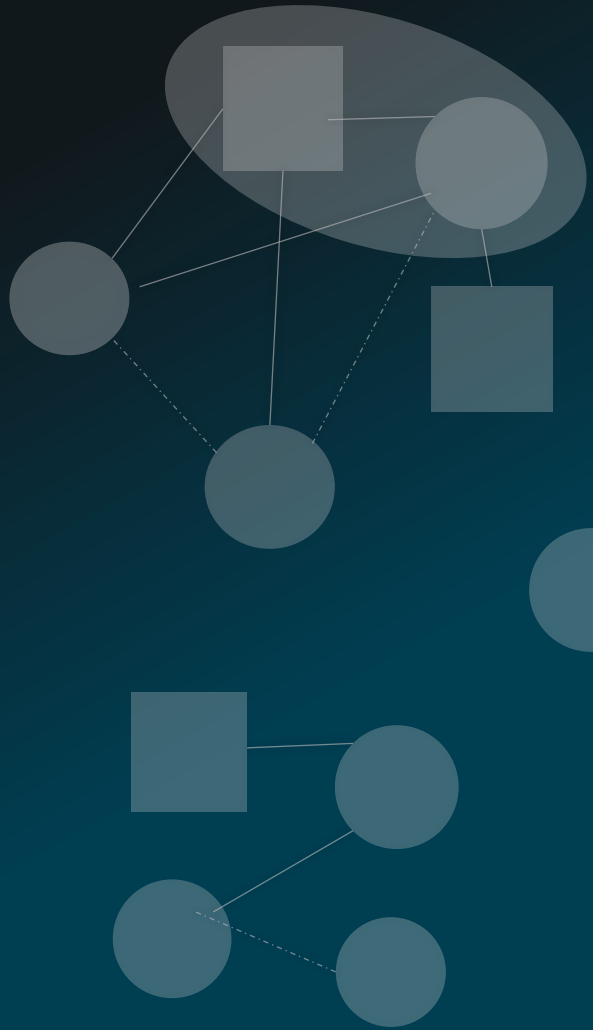
CARNA BIOSCIENCES

Artificial Intelligence/Machine Learning



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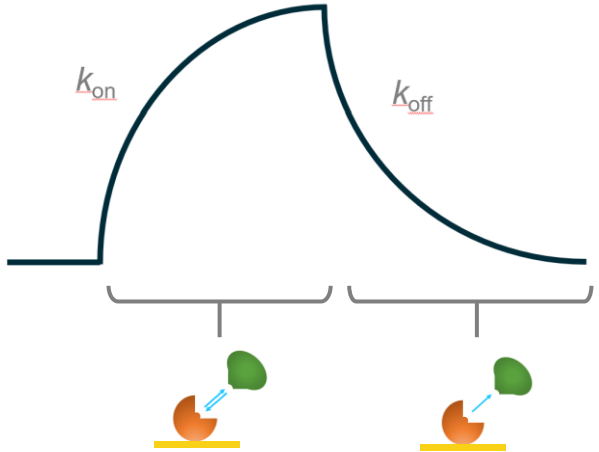
What makes HT-SPR attractive for AI/ML?

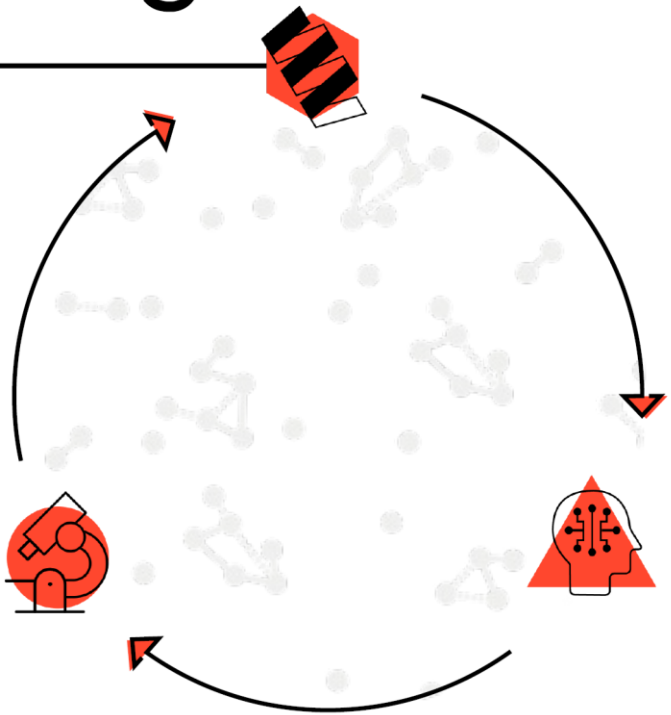
- Important aspect of ML methods is they need to be trained on a set of data called learning set,
- Result of ML campaign certainly depends on quality of learning set.
- With LSA products can capture up to 384 ligands & via 384 well analyte plate can produce 384 x 384 = 147,456 interactions to feed into learning models.



Absci is leading the way with our Integrated Drug Creation™ platform

DATA TO TRAIN

Proprietary wet-lab assays capable of generating billions of protein-protein interactions a week for ML training



AI TO CREATE

Generative AI engine to create new antibodies and next-gen biologics

WET LAB TO VALIDATE

Scalable wet-lab infrastructure capable of validating 2.8 million unique AI-generated designs a week

Publications over past year highlighting Carterra's HT-SPR technology

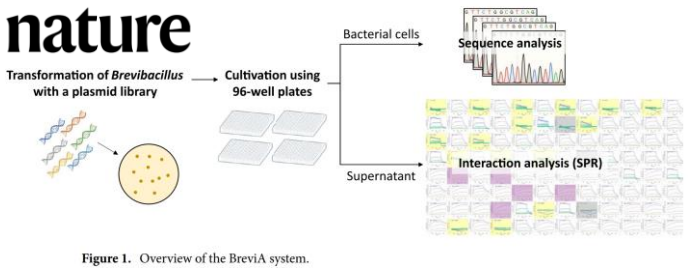
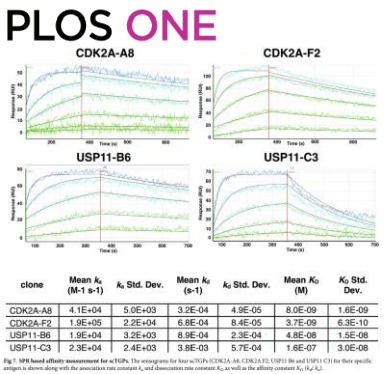


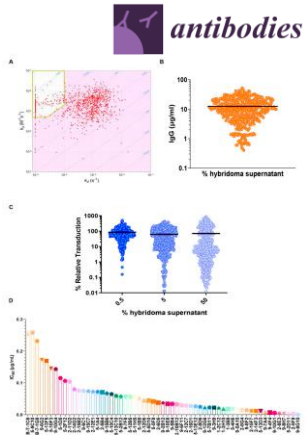
Figure 1. Overview of the BreviA system.

Matsunaga R et al. High-throughput analysis system of interaction kinetics for data-driven antibody design. Sci Rep. 2023 Nov 21;13(1):19417.



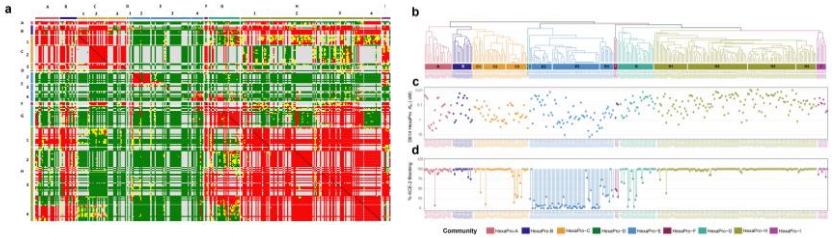
clone	Mean k_a (M-1 s-1)	k_a Std. Dev.	Mean k_d (s-1)	k_d Std. Dev.	Mean K_D (M)	K_D Std. Dev.
CDK2A-A8	4.1E+04	5.0E+03	3.2E-04	4.9E-05	8.0E-09	1.6E-09
CDK2A-F2	1.9E+05	2.2E+04	6.8E-04	8.4E-05	3.7E-09	6.3E-10
USP11-B6	1.9E+04	3.2E+03	8.9E-04	2.3E-04	4.8E-08	1.5E-08
USP11-C3	2.3E+04	2.4E+03	3.8E-03	5.7E-04	1.6E-07	3.0E-08

Velappan N et al. Direct selection of functional fluorescent-protein antibody fusions by yeast display. PLoS One. 2023 Feb 24;18(2):e0280930.



D'Acunto E et al. Isolation and Characterization of Neutralizing Monoclonal Antibodies from a Large Panel of Murine Antibodies against RBD of the SARS-CoV-2 Spike Protein. Antibodies (Basel). 2024 Jan 5;13(1):5.

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology



Li K et al. Cryptic-site-specific antibodies to the SARS-CoV-2 receptor binding domain can retain functional binding affinity to spike variants. J Virol. 2023 Dec 21;97(12):e0107023.

Cell Reports

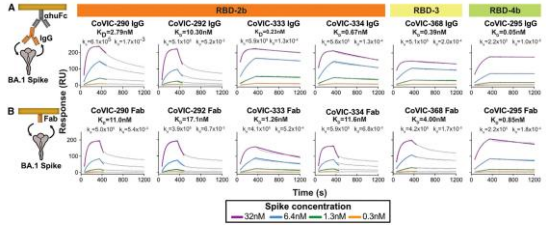


Figure 5. Binding kinetics of BA.1 spike ectodomains to IgG and Fabs

Surface plasmon resonance experiments comparing the binding kinetics of IgG (A) and Fab (B) to Omicron BA.1 spike. For each sample, experimental data (gray line) and 1:1 fitted curve (colored lines) are shown. Spike concentrations range from 0.3–32 nM. K_D, k_{on}, and k_d values for each interaction are indicated. See also Figure S4.

Callaway HM et al. Bivalent intra-spike binding provides durability against emergent Omicron lineages: Results from a global consortium. Cell Rep. 2023 Jan 31;42(1):112014.

Using the LSA to create learning set to feed into ML

Humanisation of Ab derived from animals often results in lower affinities than those of original Abs so an approach is taken to optimise sequence of obtained Ab to improve its affinity.

Conventional approach is to randomise certain residues & obtain higher affinity clones using Ab display technology such as phage & yeast display.

Other approaches are based upon CAD, introducing mutations in silico to Ab residues at interaction interface based upon Ag-Ab complex structure, with candidate mutant residues with improved affinities identified by comparing interaction energy scores before & after mutation.

Whilst several successful examples have been reported, these approaches require expt. evaluation of many clones as predictive performance is not usually uniformly high.

This creates a bottleneck as most affinity measurement systems are low throughput.

Using LSA to create learning set, Bacterial expression system, BreviaA

In this paper users reported HT-SPR using a bacterial expression system named BreviA in conjunction with the LSA to capture 384 Fab in 96 well plate format from supernatants.

Plasmids extracted from Brevibacillus can be sequenced in same manner as E coli, so simultaneously expressed Fabs expressed can be captured to chip surface and SPR performed with antigen, constructing a data set consisting both (i) sequence (ii) interaction parameters.

Described here is mutational analysis to optimise an anti hPD-1 Ab, immune checkpoint inhibitor, to cross-react with mPD-1.

Using LSA to create learning set, Bacterial expression system, BreviaA

Mutant Ab library created that contained all possible single mutants in which CDR residues were mutated to Ala or Tyr.

Ala mutants designed to shave off side chains that would interfere with interaction interface whilst Tyr mutants designed to create new interactions at Ag Ab interface.

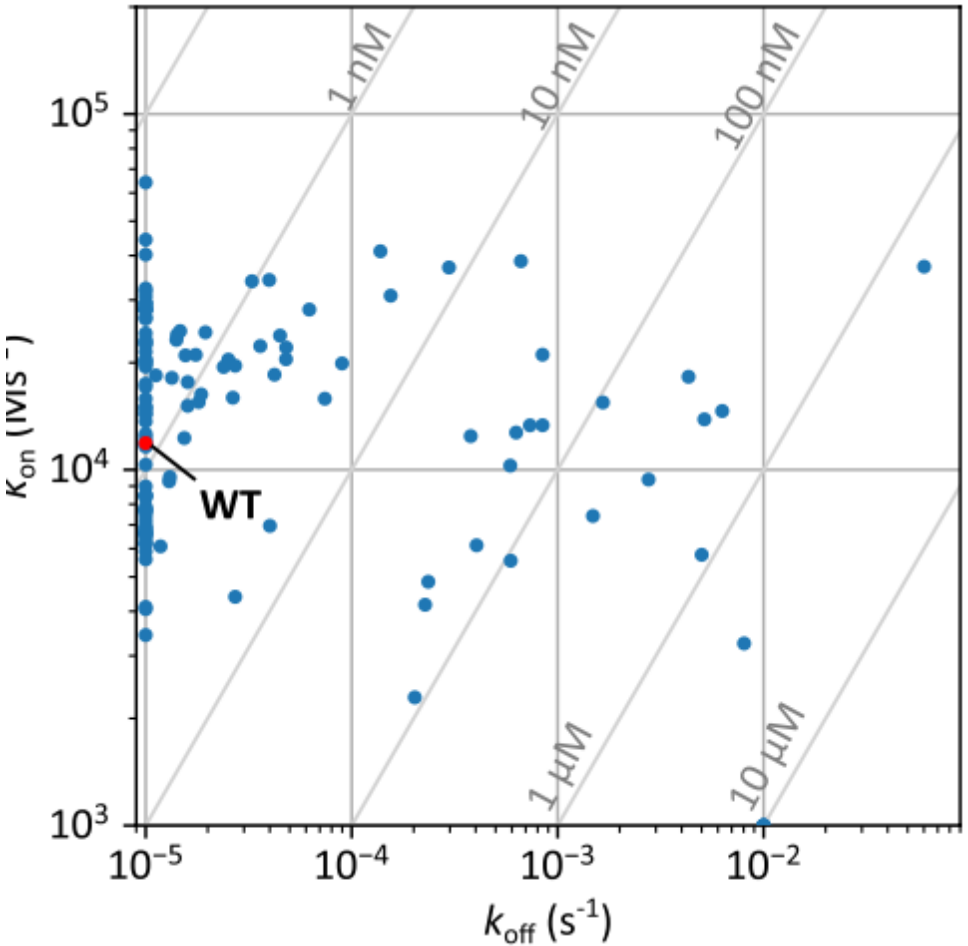
Plasmids for expression of Fabs with hexa HIS tag on L chain were created & resulting colonies incubated for 60h. Supernatants treated with essential ammonium sulphate precipitation, diluted 10x, then captured on LSA sensor surface functionalised with Ni-NTA groups.

Kinetic parameter measurement with Ag performed at 5 concentrations via a 4 fold dilution series and affinity values obtained via non regenerative kinetics protocol.

Kinetic parameters for Ala and Tyr mutants as measured on the LSA.

Many constructs, including WT had k_d slower than can be reported by conditions used in this assay (5 mins dissociation)

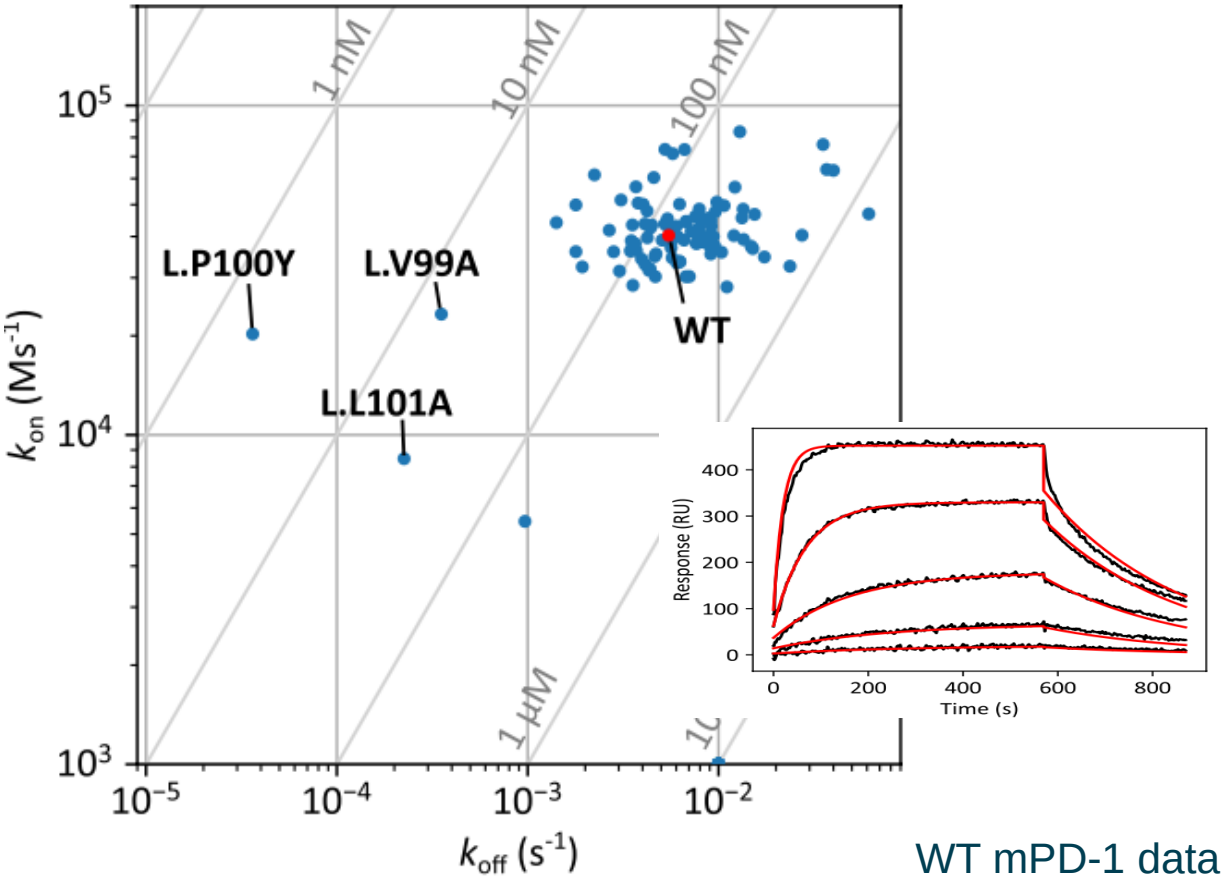
Some mutations had particularly increased k_d, indicating they are hotspot residues.



Kinetic parameters for Ala and Tyr mutants as measured on the LSA.

SPR analysis on LSA showed that against mPD-1-Fc, most mutations had little change from WT, but 3, at 99, 100 and 101, showed markedly increased affinity.

These 3 residues also identified as hotspot residues in hPD-1



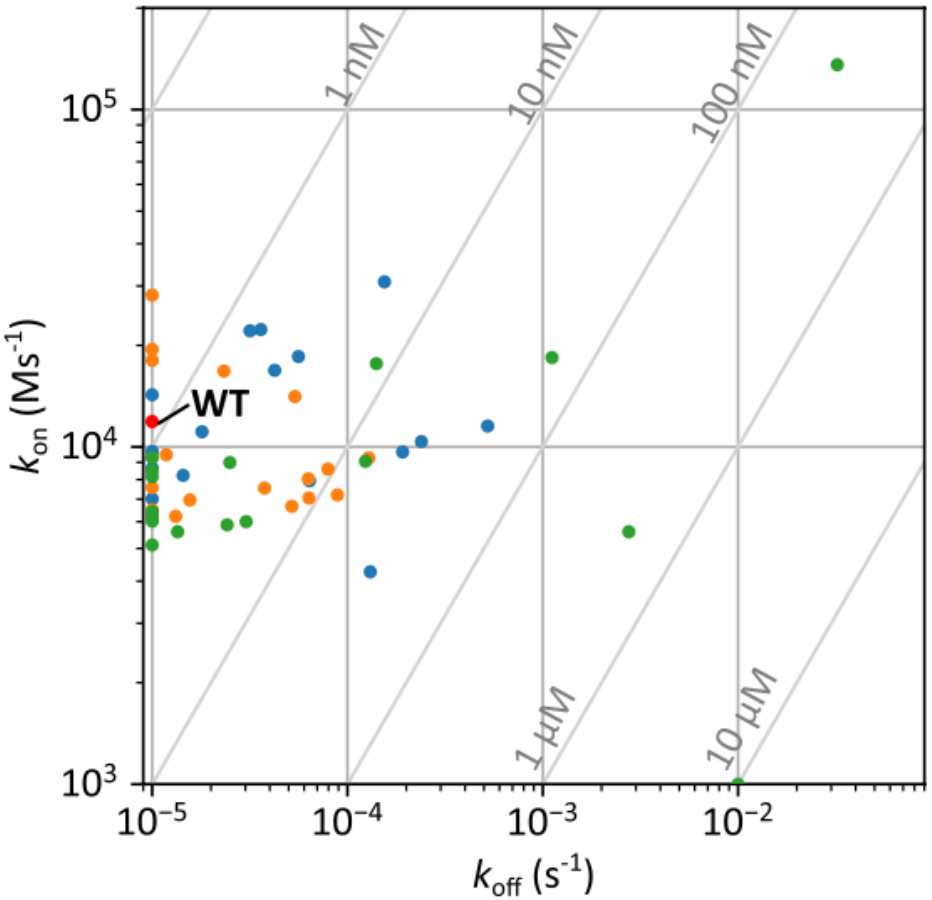
WT mPD-1 data

Deep mutational scanning at V99, P100 and L101.

As these 3 regulate affinity to mPD-1, authors generated further library containing mutants for other 17 amino acids except Ala, Try and Cys.

For human hPD-1 some clones showed decreased affinity and some lost affinity completely.

Blue = V99, orange = P100, green = L101

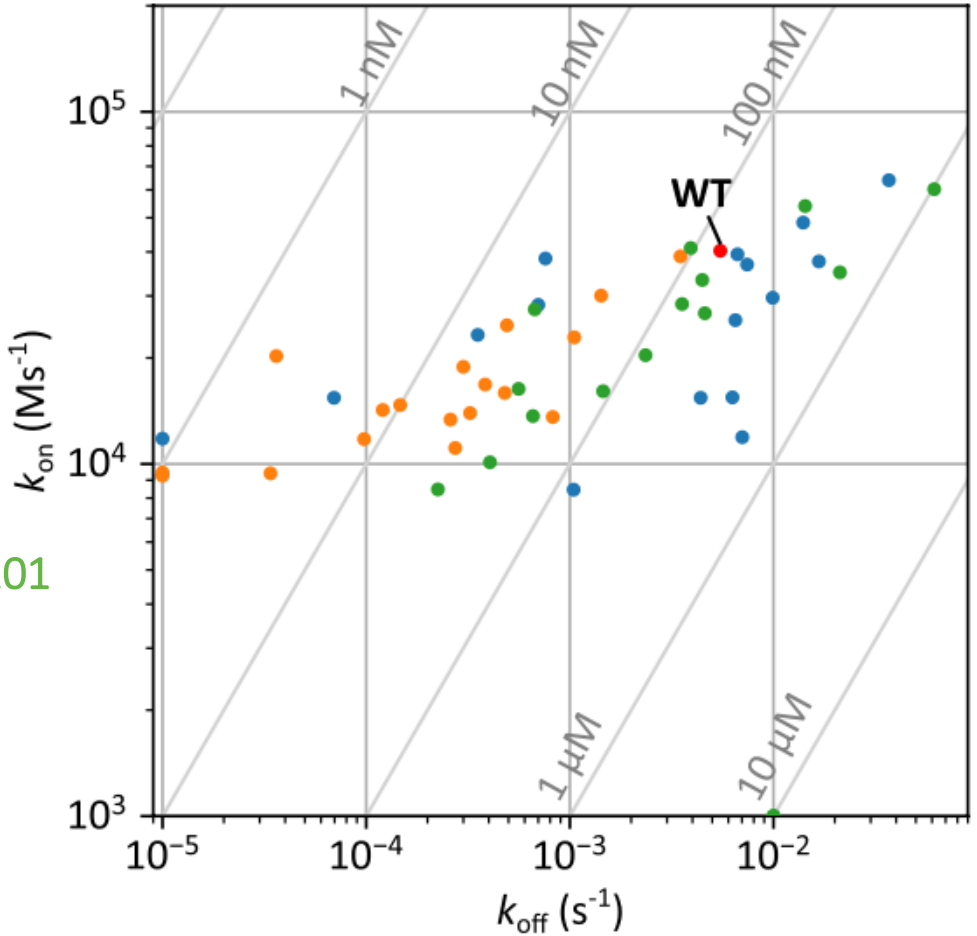


Deep mutational scanning at V99, P100 and L101.

For mouse a wide range of interaction properties were obtained, compared with Ala/Tyr scanning of all CDR residues.

Mutations V99G & P100H showed more than 100 fold increased affinity for mouse.

Blue = V99, orange = P100, green = L101



Using LSA to create learning set, Bacterial expression system, BreviaA

Based upon experimental data discovered that residues 99 to 101 play pivotal role in acquisition of cross reactivity to mPD1-Fc.

Use of traditional mutational scanning with HT-SPR, feeding into deep mutational scanning with SPR is an essential tool for creating learning tools for ML driven approaches.

BreviaA can be expanded into kinetic interaction collection system, generating the few 1000 interaction parameters needed for ML learning data sets.

Sensor chip updates

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New sensor chip offerings

NiCMDP

Planar NTA derivatized carboxymethyldextran, < 5nm coating thickness

Low density capture of His-tagged ligands

PAGP

Planar protein A/G derivatized carboxymethyldextran, < 5nm coating thickness

Great choice for Fc-formatted ligands at low densities

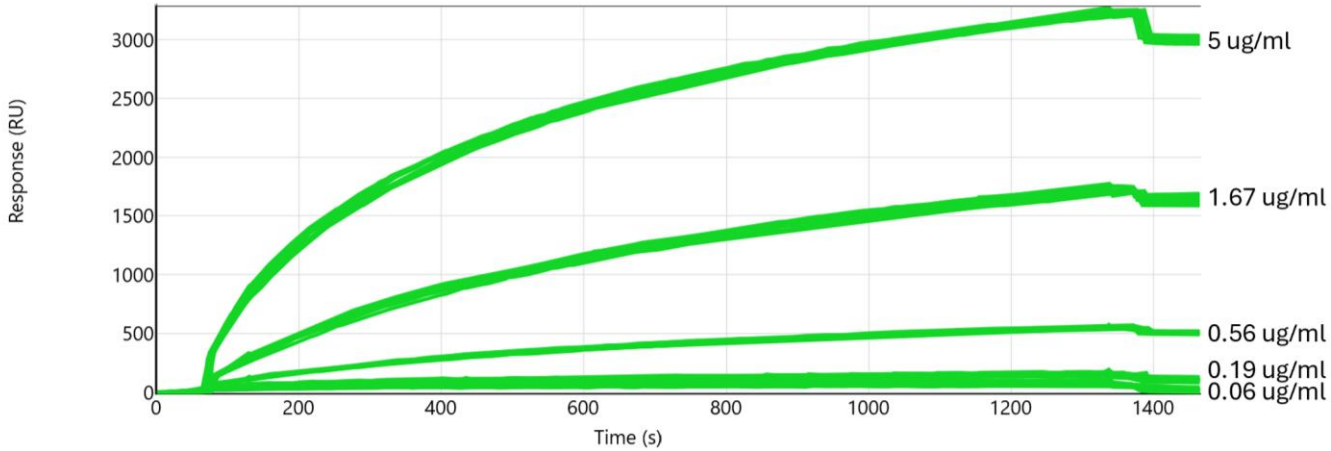
RSA200M KIT

Oligonucleotide derivatized carboxymethyldextran for reversible immobilization of biotinylated ligands, 200nm coating thickness. RSA reagent included.

Enables creation of streptavidin coated chip surface up to 15 times to increase unattended throughout and maximise data per chip

NiCMDP Binding to His tagged PRGN protein

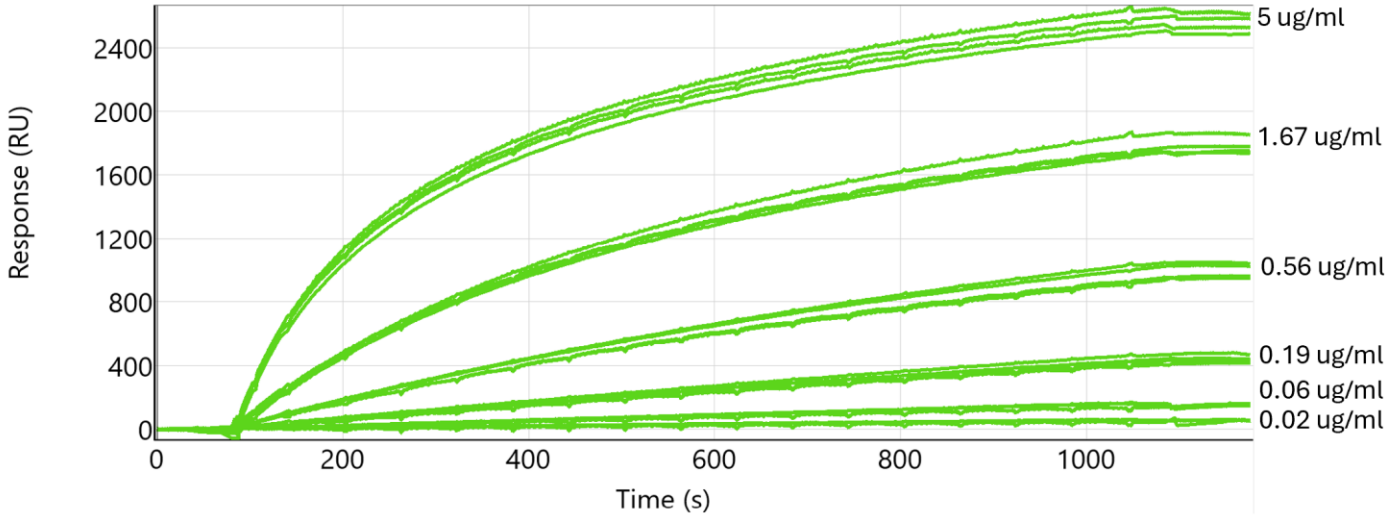
- Planar CMD based NTA Surface
- When charged with Ni^{2+} will bind polyhistidine tagged proteins
- Compared to NiHC200M offers lower surface capacity & lower surface complexity
- May be more appropriate for many kinetic applications



Surface Prep Array capture of human progranulin-His (Sino Bio #10826-H08H) at 5, 1.67, 0.56, 0.19, and 0.06 ug/ml in HBST buffer on NiCMDP sensor chip at 25°C.

PAGP- Binding Rabbit IgG

- Planar dextran (CMDP) based chimeric protein A/G capture surface
- Protein A/G binds to human, monkey, rat, mouse, and rabbit IgG Fc domains
- New planar variant captures with lower capacity than other A/G offerings
- Lower density is preferable for larger analytes & provide capture capacities optimal for kinetic analysis

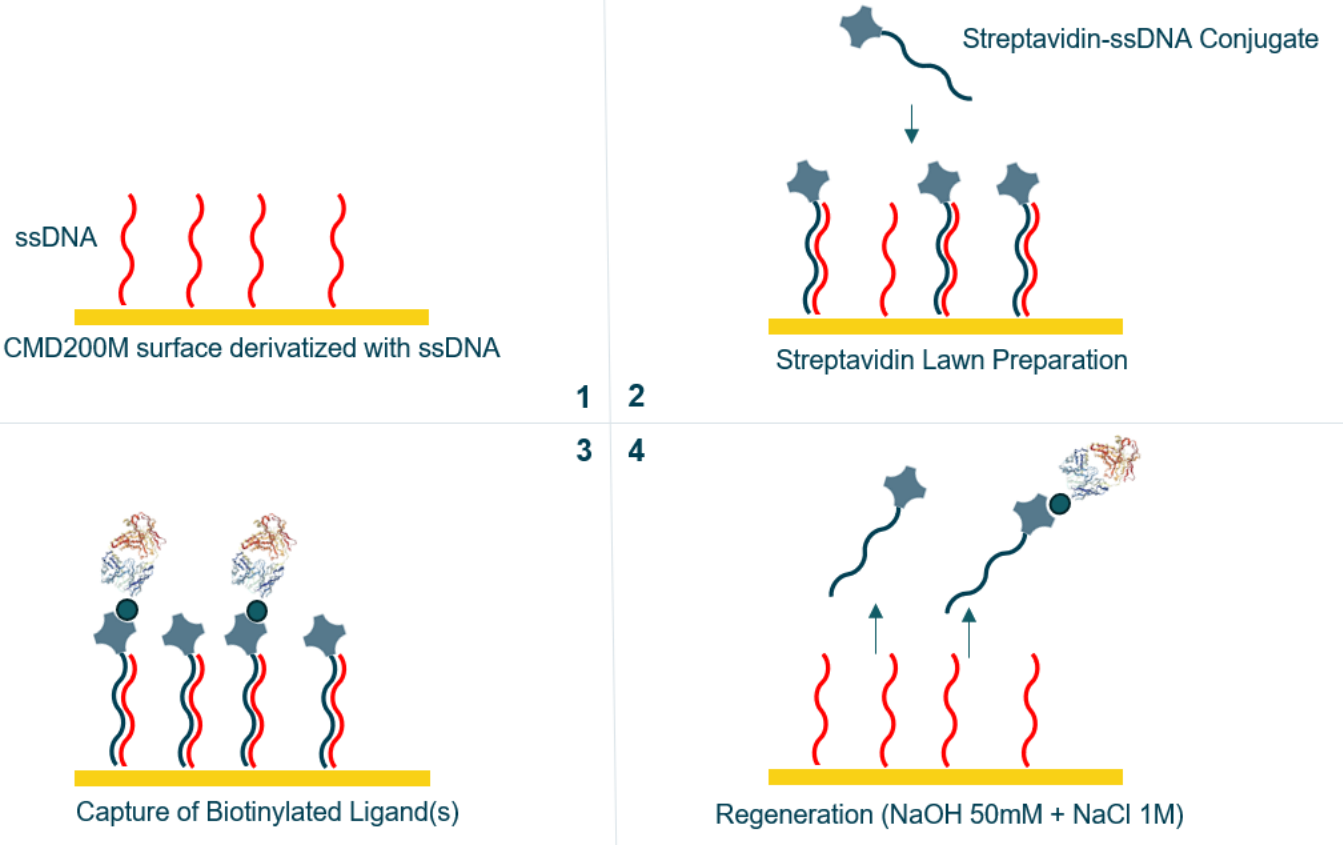


Surface Prep Array capture of anti-progranulin rabbit IgG mAb (Sino Bio #50396-R019) at 5, 1.67, 0.56, 0.19, 0.06, and 0.02 ug/ml in HBSTE buffer on PAGP sensor chip at 25°C.

RSA200M Kit: Reversible capture of biotinylated ligands

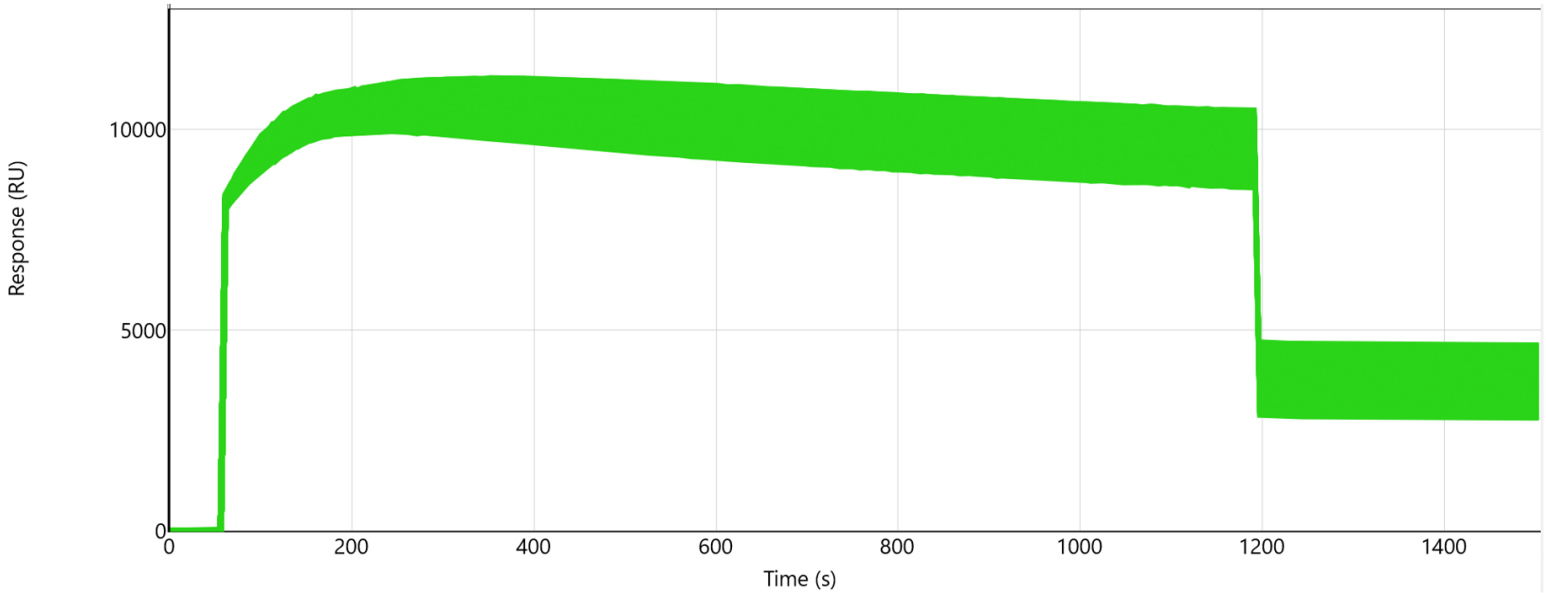
Kit contains SA conjugated to complementary DNA oligo (150 ul of 10 uM)

Each experimental cycle thus starts with capture of ss-SA to ss-CMD with kit containing sufficient ss-SA for 15 coatings of sensor chip.



RSA200M Kit: **Reversible capture** of biotinylated ligands

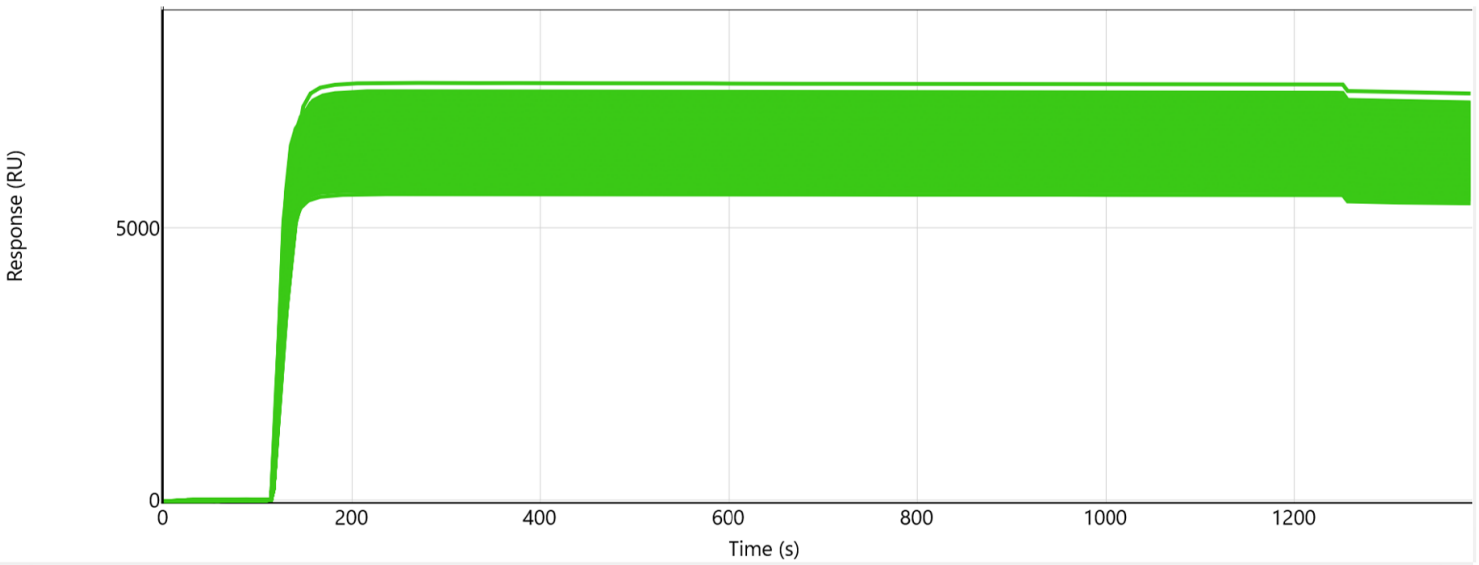
(1) Coat surface with RSA reagent (oligo-SA)



1. **RSA Reagent Capture:** 20-minute injection of 300 nM RSA Reagent prepared in HBSTE containing 1 M NaCl on RSA200M sensor chip at 25°C. Average capture was 3870 RU ± 356 RU.

RSA200M Kit: **Reversible capture** of biotinylated ligands

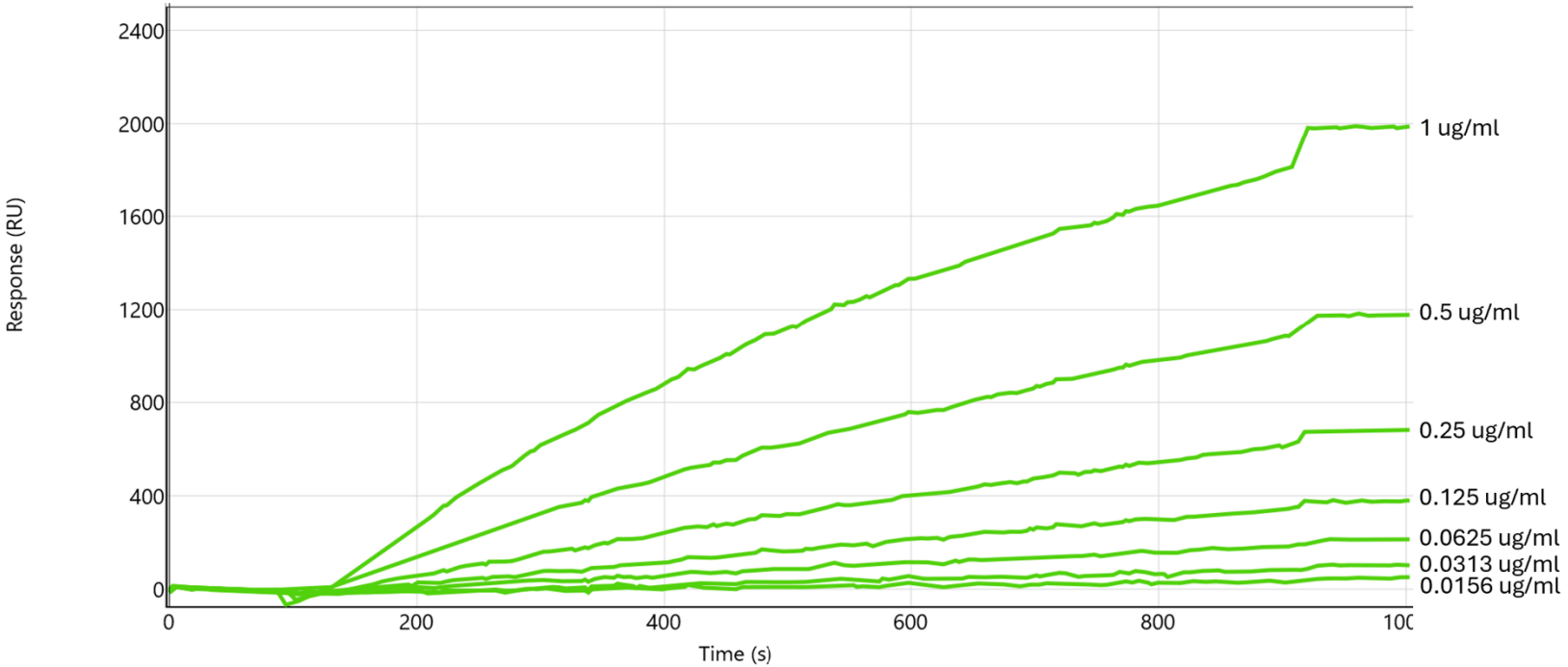
(2) Create lawn of biotinylated anti-kappa/lambda sdAbs



2. **Anti-Fab Kappa/Lambda Capture:** 20-minute injection of biotinylated anti-Fab kappa sdAb (Thermo #7103302100) + anti-Fab lambda sdAb (Thermo #7103312100), each at 50ug/ml, in HBSTE onto RSA200M surface coated with ~3870 RU RSA Reagent. Average capture was 6610 RU ± 431 RU.

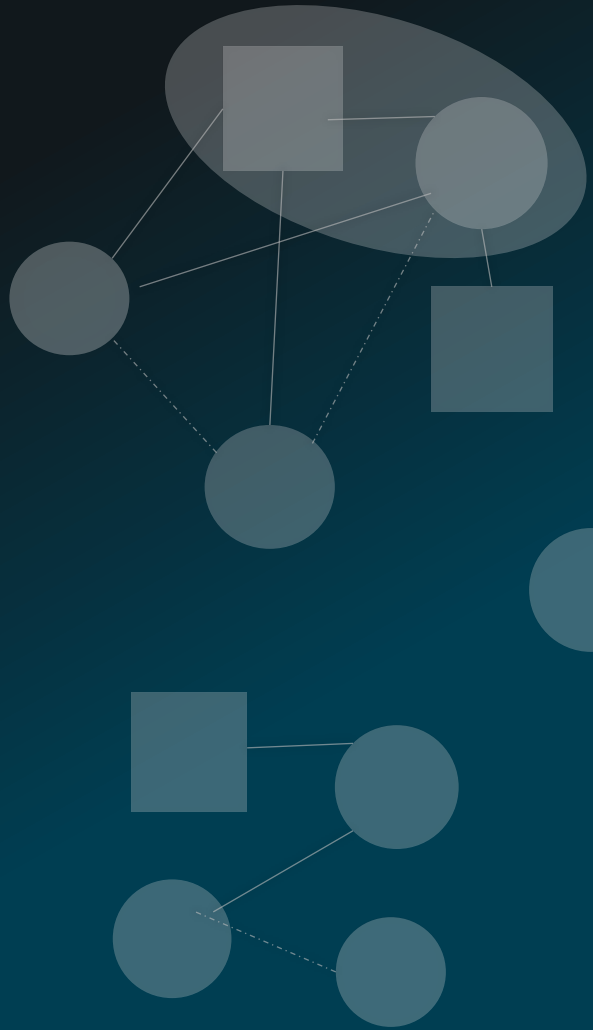
RSA200M Kit: **Reversible capture** of biotinylated ligands

(3) Capture IgGs



3. **Mab Capture:** 15-minute capture of human IgG 1 mAb in HBSTE on anti-Fab kappa/lambda surface at multiple concentrations.

Carterra SPR Camp



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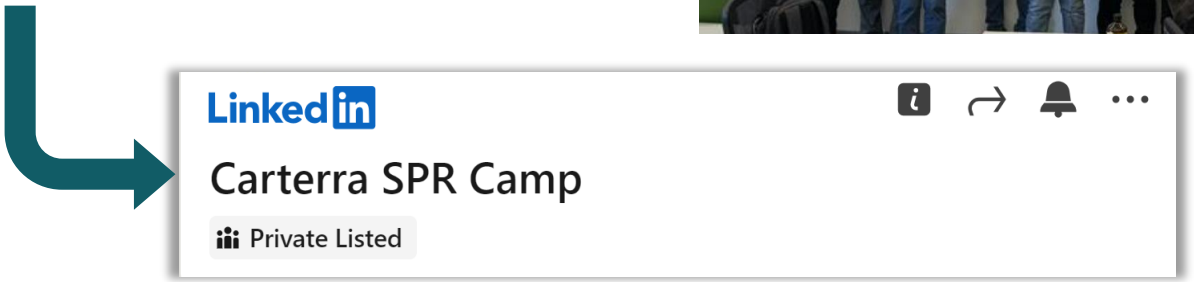
2024 SPR Camp

- 2-day personalised, hands-on training with Carterra's FAS Team
- Open to all skill levels
- Alumni can access the exclusive Carterra SPR Camp LinkedIn User Group



2024 Carterra **SPR Camp Schedule**

- **Spring 2024**
  - Boston - April 16/17 **DONE**
  - Munich – May 7/8 **DONE**
  - Walnut Creek – May 29/30 **DONE**

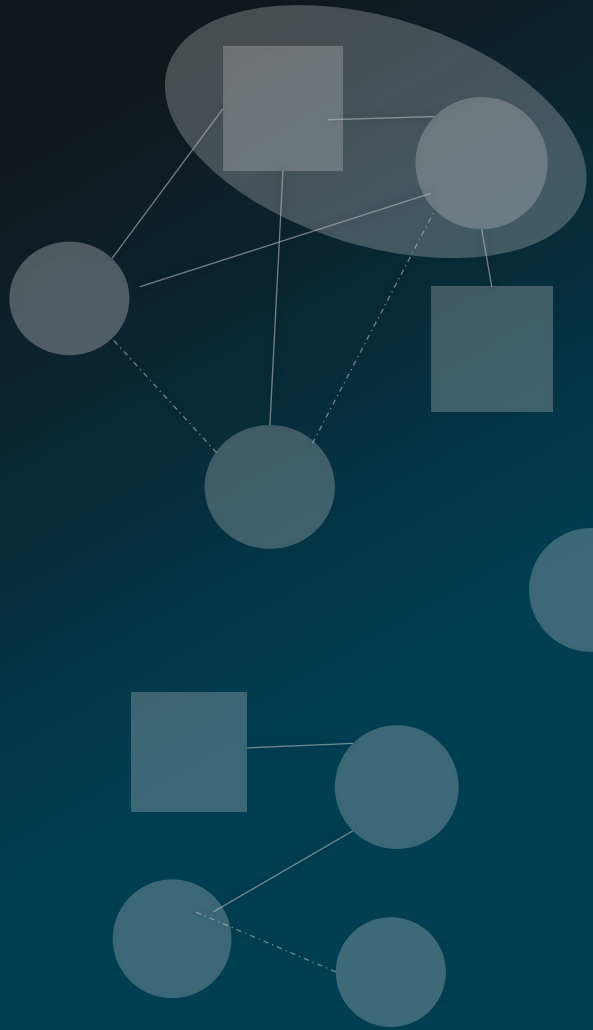
- **Autumn 2024**
  - US – Boston – date TBD
  - Europe – UK – date TBD

Early Bird Discounts Available

Contact Your Local Carterra Rep to Secure A Spot!

Thank You!

Questions?



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