# Applications from mAbs to Fragment Screening

## HT-SPR Platforms Enable Highly Parallel Analysis Advancing All Drug Discovery Modalities

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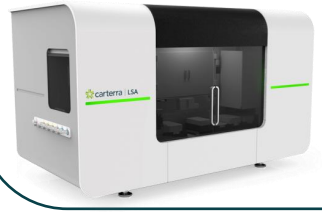
Senior Manager, Field Applications Science

carterra®

# Complete HT-SPR Solutions

Ultra

LSA$^{XT}$



LSA

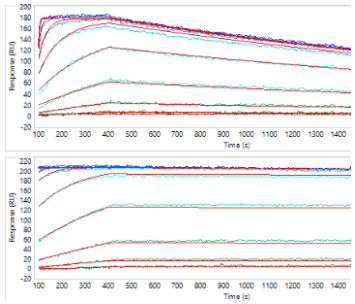
Instrumentation

Control and analysis software

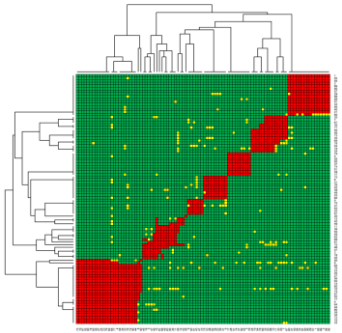

Biosensor chips and consumables

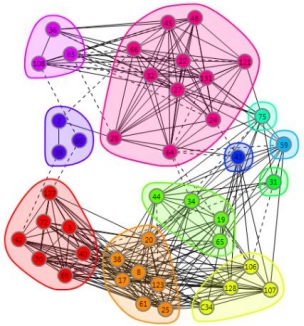
# LSA's core applications

## Kinetics/Affinity

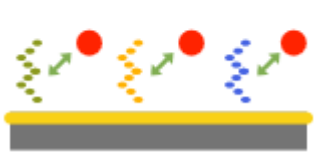


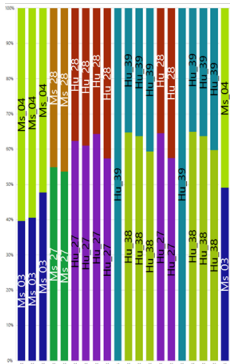

## Epitope Binning



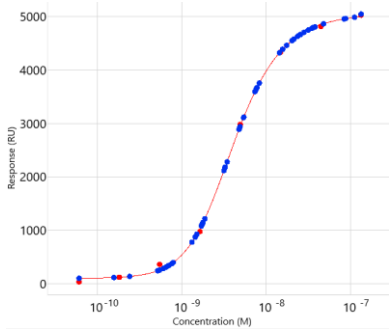


## Mapping






## Quantitation



# Application Examples

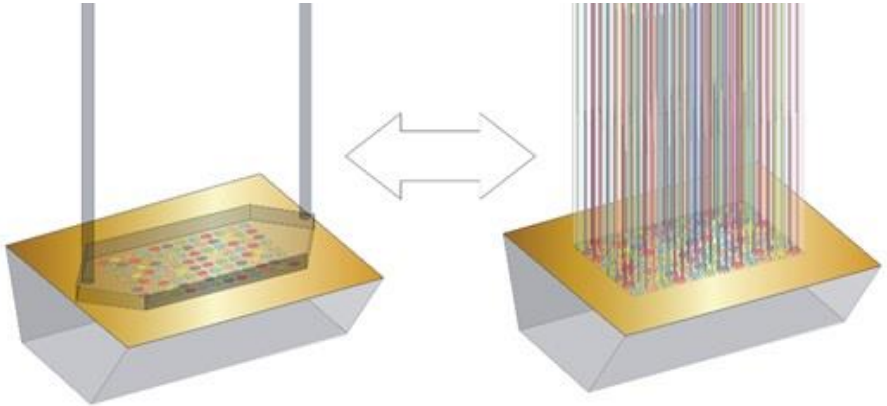
- The Carterra LSA$^{XT}$ provides a highly scalable and data rich approach to characterizing binding kinetics of Fab candidates directly from crude PPE sources
  - Full kinetics ($k_a$, $k_d$, and $K_D$) for thousands of Fabs can be measured in a single week, whilst eliminating the need for purification
- Kinetic and Affinity characterization of TCR binding to pMHC Panels
  - Enables an understanding of specificity in TCR-T Cell therapy and T-cell engagers
- Carterra Ultra enables binding screens of small molecule and fragment libraries to many targets simultaneously
  - Small molecule binding to whole protein classes, panels of family members, and off-target, enables a new paradigm in high-throughput screening and affinity characterization

# Novel microfluidics transform SPR

**Single-channel mode**
**(single flow cell)**

**Multi-channel mode**
**(96-channel printhead)**

*Minimal analyte consumption via "one-on-many" assay format*

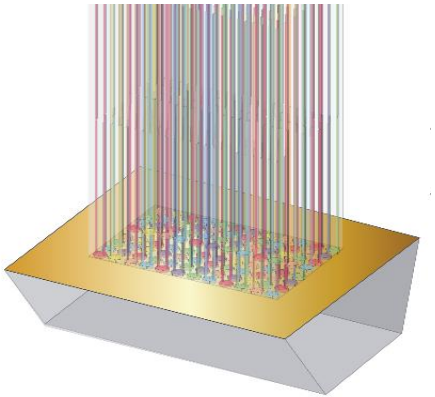


*Serially print at 4 nested locations to create a 384-array*

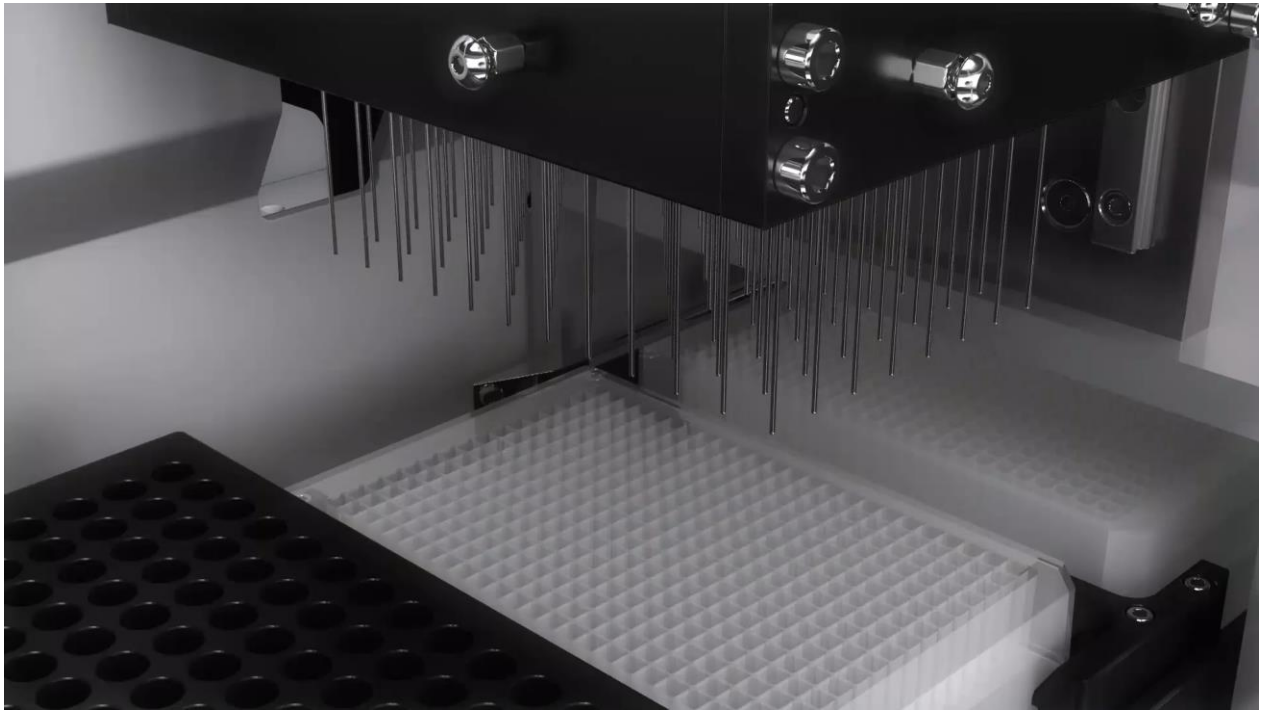
- Automated flow cell switching between multi- and single-channel modes
- 384 reaction spots + reference interspots per array
- In-line reloading of array
- Supports capture formats and standard amine coupling

# Array up to 384 ligands

## 96-Channel Printhead (96PH)



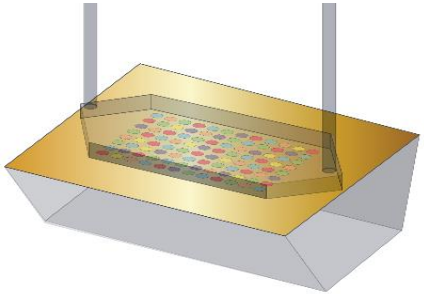
Create 384-ligand array via 4 serial docks of 96PH



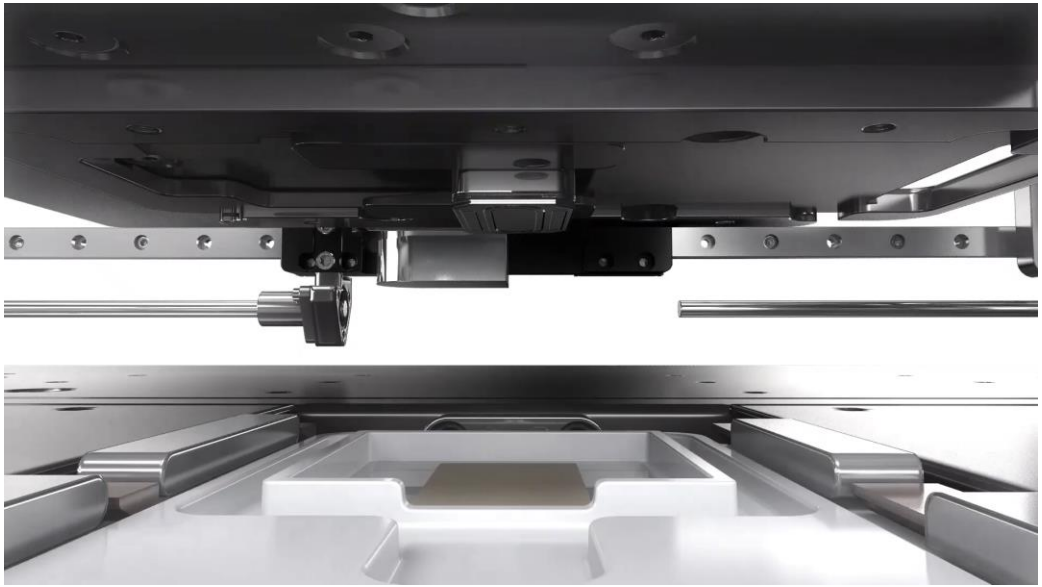
Sample deck holds 3x 384well plates (1152 ligands)

# Screen one analyte over many ligands with the SFC

## Single Flow Cell (SFC)



Inject 270 analyte over entire array in a “1-on-384” analyte-on-ligand mode



Sample deck holds a variety of tubes (50ml, 15ml, 1.5ml) and a 96-well (or 384-well) plate

# Full Kinetics of Fab binding from crude PPEs

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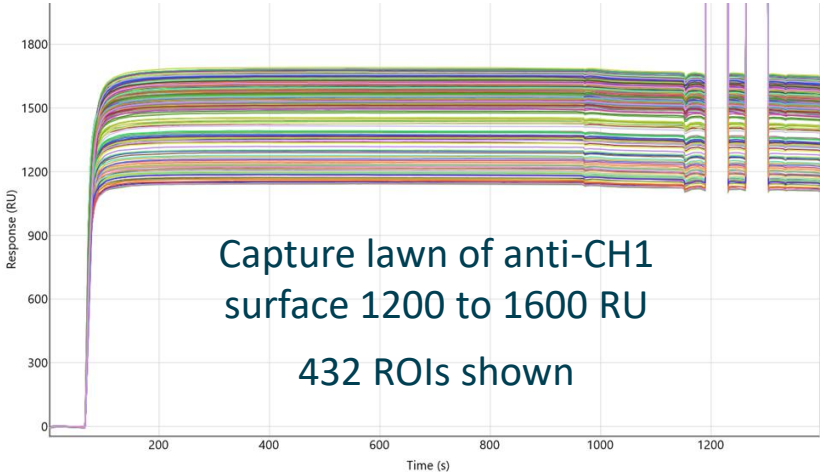
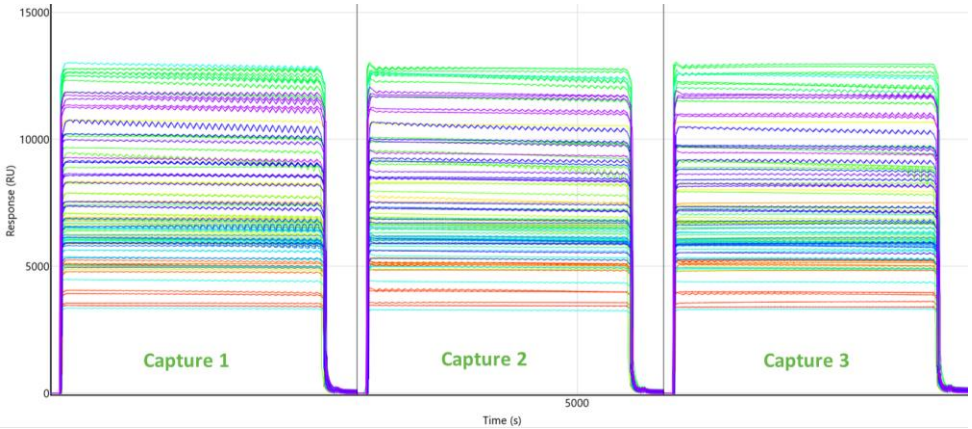
# Surface Preparation and Capture of Fabs From PPEs

- A pre-functionalized streptavidin sensor chip (SAHC30M) was used
- Capture lawn prepared with biotinylated anti-CH1 VHH (Thermo CaptureSelect) at 25 µg/mL



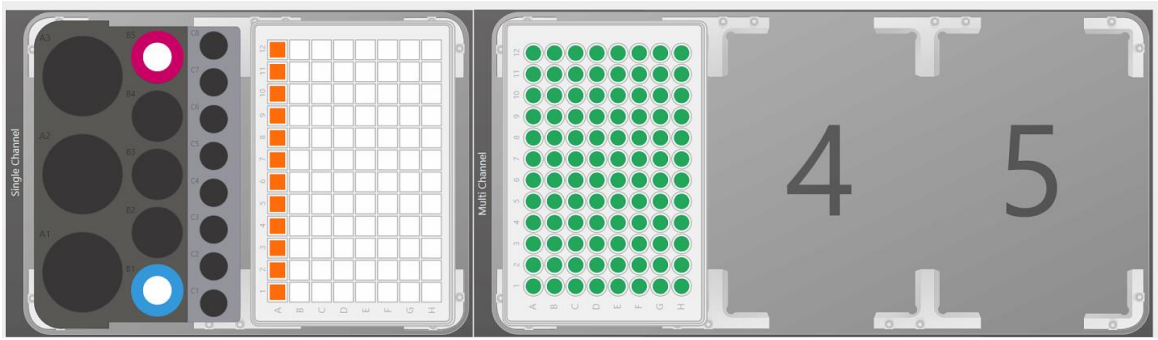

- Next a 96-well plate of 2x diluted Fab PPEs were captured for 40 min, in triplicate for a total 288 unique positions on the sensor surface
  - 3 consecutive captures at different locations on the chip via the 96-needle flow cell system, with samples returned to the plate following each capture
- Average captured levels of Fabs were approximately 75 RU per spot on the chip surface
  - Samples are low concentration, long capture time enables enrichment and kinetics analysis

# Antigen Titration Kinetics

- Following Fab capture, the single flow cell (SFC) was used to inject a concentration series to establish binding kinetics
  - 10 cycles of running buffer (HBST+BSA) to stabilize
  - The 39 kDa protein antigen was injected from 1.95 nM up to 500 nM in a five-point, four-fold titration as a non-regenerative kinetic series
  - Cycle times:  1-minute baseline, 5-min association, & 10-min dissociation
  - Regeneration after kinetic series was two 60-sec pulses of 10 mM glycine pH 2.0
    - Prepares the chip for the capture of additional Fabs
- Total time from chip conditioning through to completion of antigen injections was 10 hours - Entire workflow can be a single queued run

Simple Assay Set Up

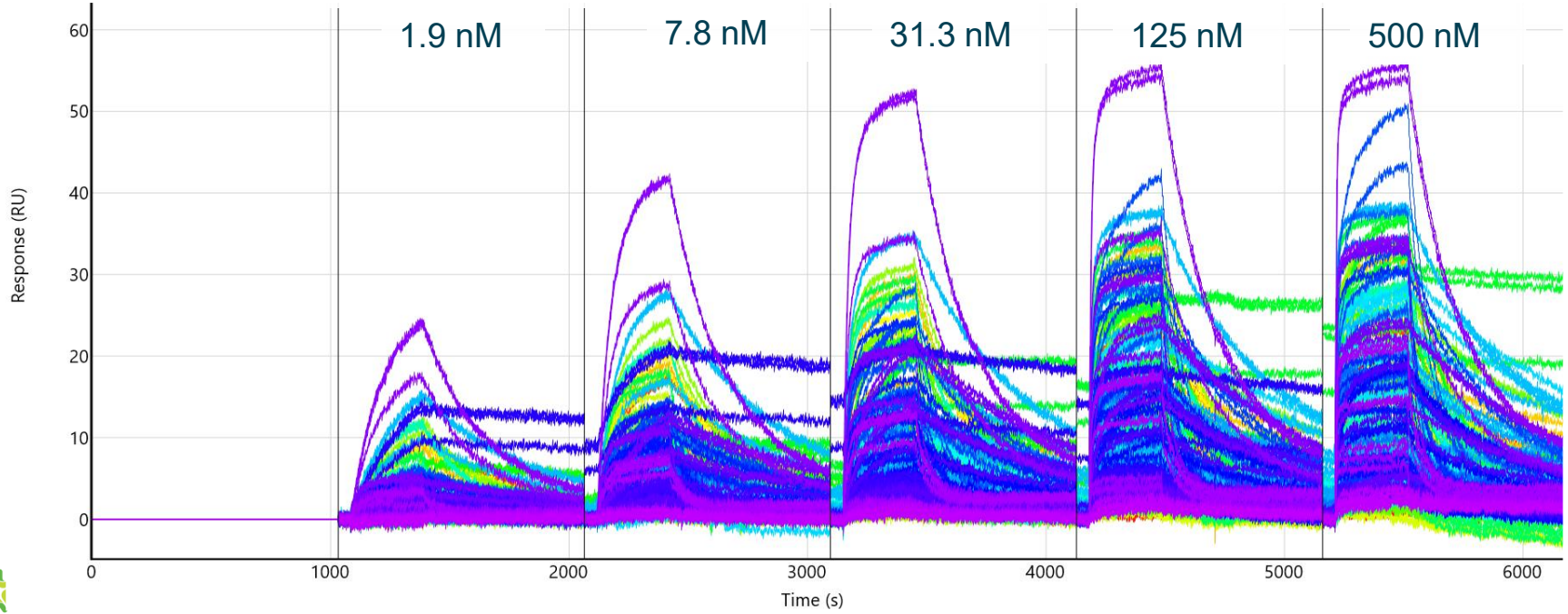
Can scale to 1152 clones/run



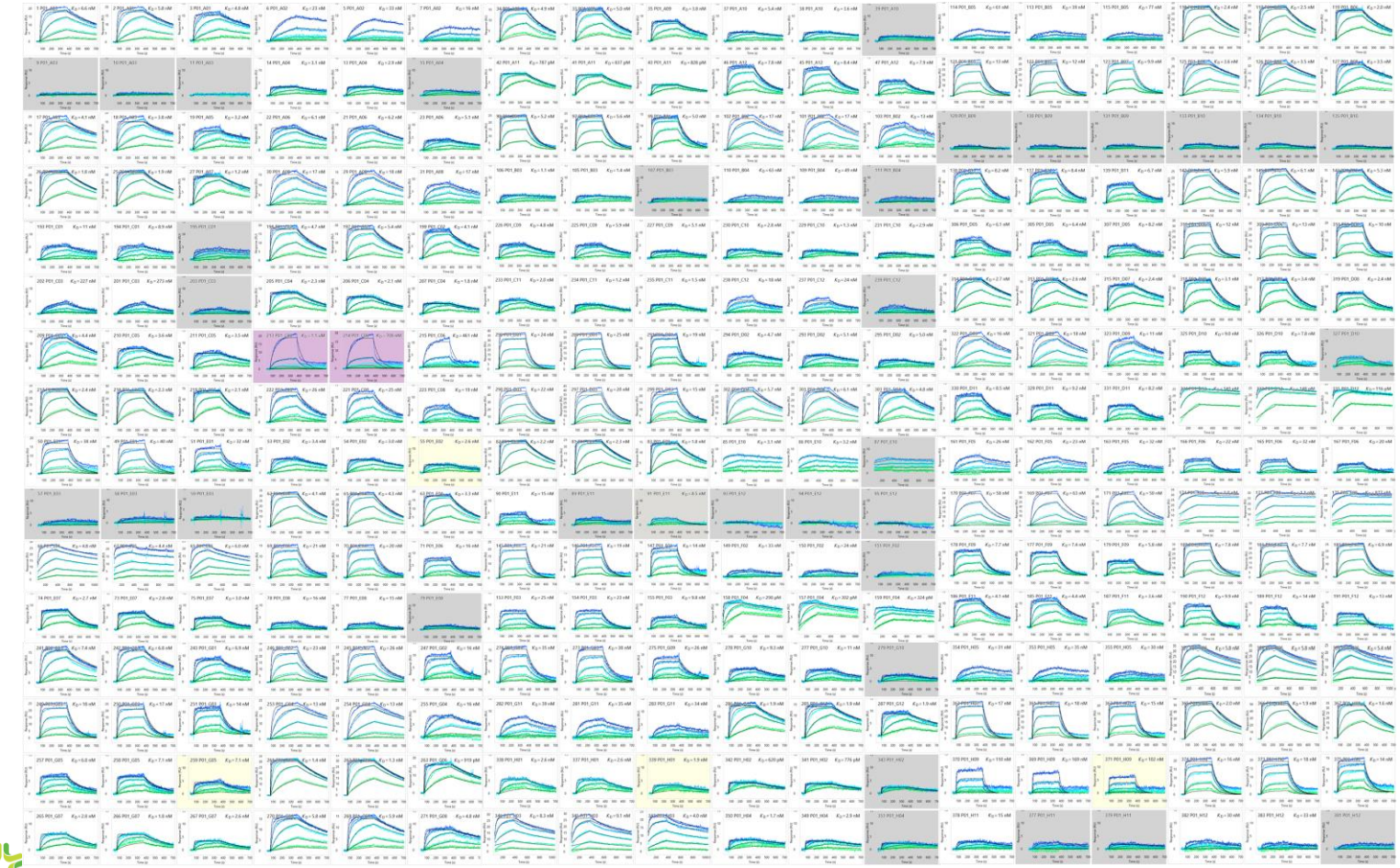
# Data Processing

- These data were double referenced by using a local anti-CH1 spot, then subtracting the leading buffer blank
- Kinetic parameters ($k_a$, $k_d$, $R_{max}$, and $K_D$) were globally fit to a 1:1 Langmuir model

**Fully processed data from non-regenerative kinetic series, 288 ROIs**



# One Run- Rich Kinetics Profiles for 288 Fab Capture Spots, Triplicates of Each



**All data collected using only 7.8 µg of Ag**

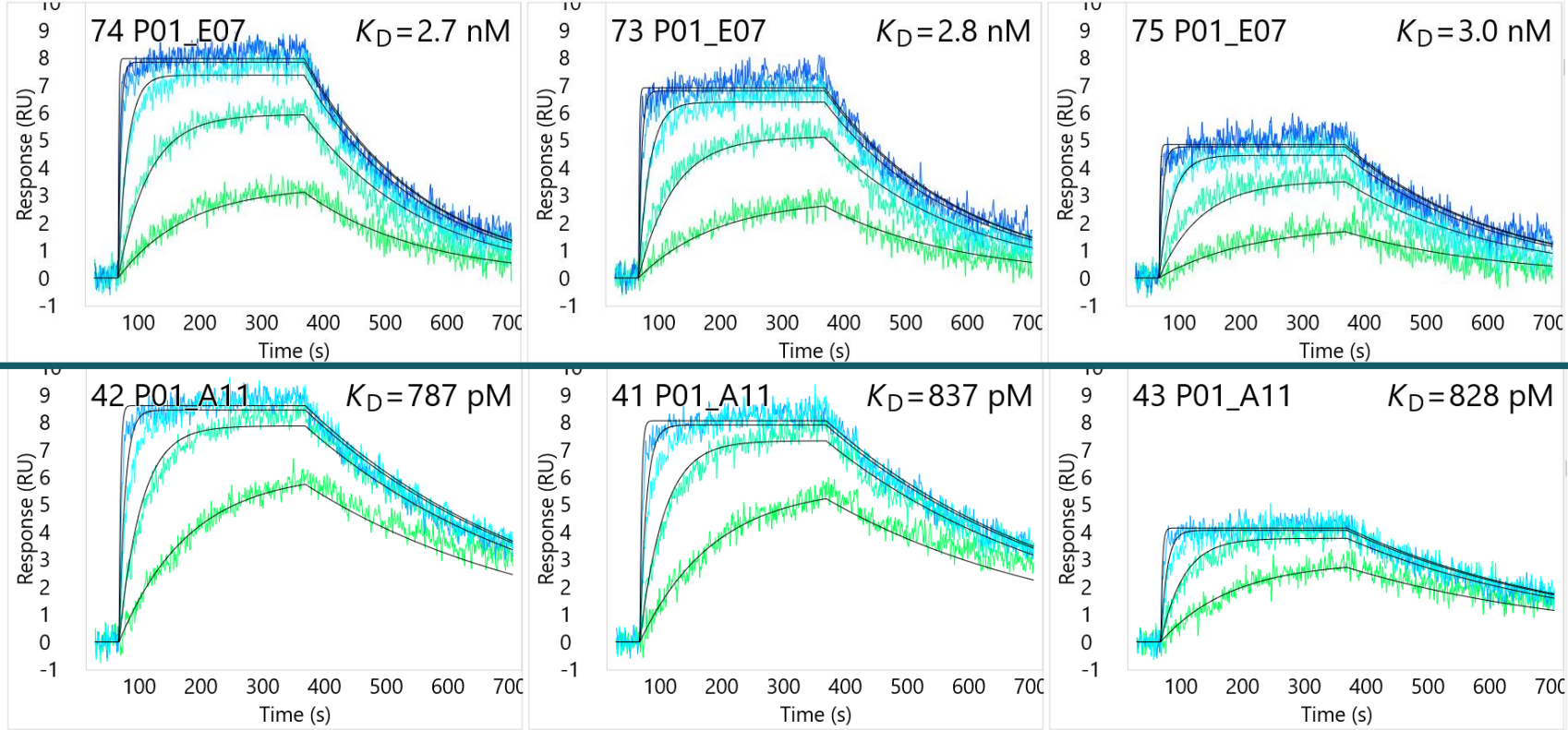
Black lines represent the 1:1 Langmuir model fits.

No/Low binding highlighted as grey

SD of residuals >10% $R_{max}$ highlighted as yellow

Indeterminate $R_{max}$ values highlighted as purple

# High Quality Kinetic Estimates From < 10 RU Responses




- Expanded view for two clones from each of 3 captures
- Both clones show well resolved kinetic profiles with $R_{max}$ values 4 to 9 RU

# Leveraging the LSA and Array SPR to characterize TCR affinity

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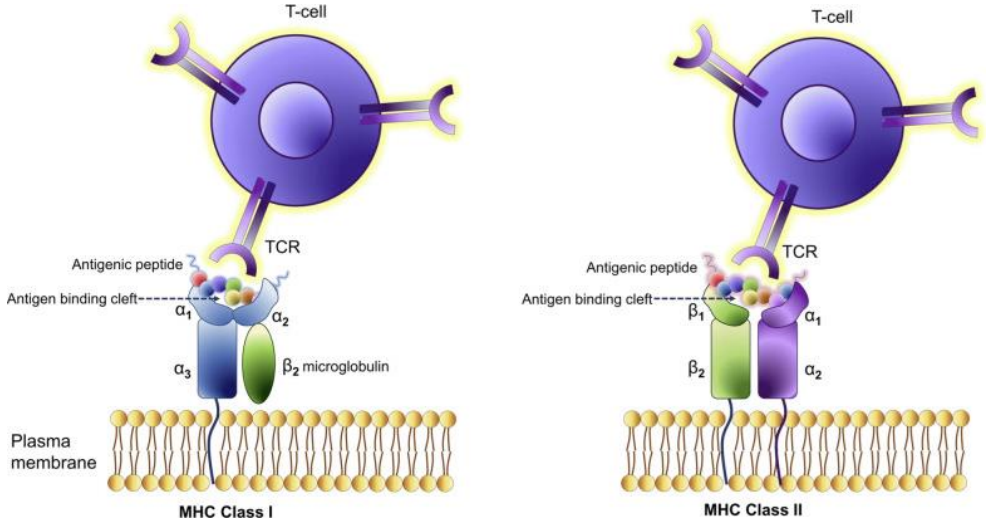
# T-cell receptor and MHC/HLA Interactions




- The T-cell receptor (TCR) is a protein complex found on the surface of T cells responsible for recognizing fragments of antigen as peptides bound to major histocompatibility complex (MHC) molecules.
  - The binding between TCR and antigen peptides is of relatively low affinity and is degenerate: that is, many TCRs recognize the same antigen peptide and many antigen peptides are recognized by the same TCR.
- TCR α-chain and β-chain each have three hypervariable or complementarity-determining regions (CDRs) and undergo somatic V(D)J recombination to create high diversity, much like antibodies but without somatic hypermutation.

Image Credit: Tsimberidou, Apostolia & Morris, Karlyle & Vo, Henry & Eck, Stephen & Lin, Yu-Feng & Rivas, Jorge & Andersson, Borje. (2021). T-cell receptor-based therapy: an innovative therapeutic approach for solid tumors. Journal of Hematology & Oncology. 14. 10.1186/s13045-021-01115-0.

# TCR Based Therapies

- TCR therapeutic Advantages
  - TCRs recognize peptide loaded MHCs, even intracellular proteins can targeted
  - Challenges are low specificity and affinity
- T-cell receptor (TCR)-based adoptive cell therapy
  - Genetically engineered human T-lymphocytes
  - Use engineered TCRs to target problem cells like cancer
- Bispecific T-cell receptor (TCR)-based T-cell engagers
  - Use engineered TCRs with additional stimulatory binding domains
  - Tebentafusp (GP100/CD3) or KIMMTRAK® is approved for metastatic Uveal Melanoma
- Any TCR based therapeutic approach benefits from understanding specificity and affinity of TCR interactions

BioRx Publication from University of Oxford doi.org/10.1101/2024.10.25.620274

# Generation of T cells with reduced off-target cross-reactivities by engineering co-signalling receptors

Jose Cabezas-Caballero[1], Anna Huhn[1], Mikhail A. Kutuzov[1], Violaine Andre[1], Alina Shomuradova[1]
P. Anton van der Merwe[1], Omer Dushek[1,¶]

[1]Sir William Dunn School of Pathology, University of Oxford, Oxford OX1 3RE, UK
[¶]Corresponding author

**One sentence summary:** Switching the CD8 for the CD4 co-receptor in cytotoxic T cells reduces the functional cross-reactivity of T cells without modifying the TCR.

Carterra White Paper

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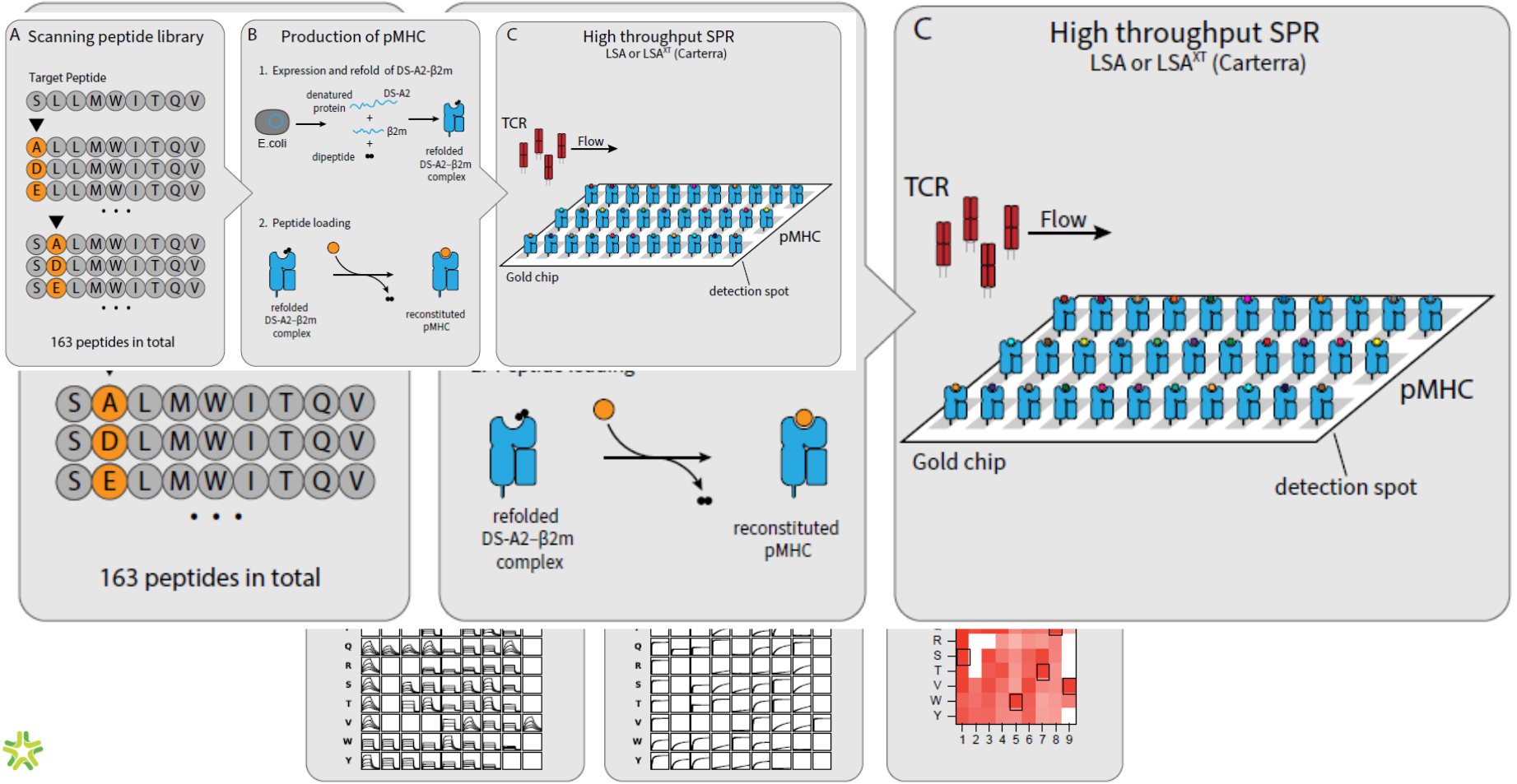
WHITE PAPER

# Parallel Measurements of Hundreds of TCR/pMHC Affinities Using The Carterra Surface Plasmon Resonance Technology

Anna Huhn[1], Mikhail A. Kutuzov[1], Jonathan F Popplewell[2], Jose Cabezas-Caballero[1], Alina Shomuradova[1], Omer Dushek[1]

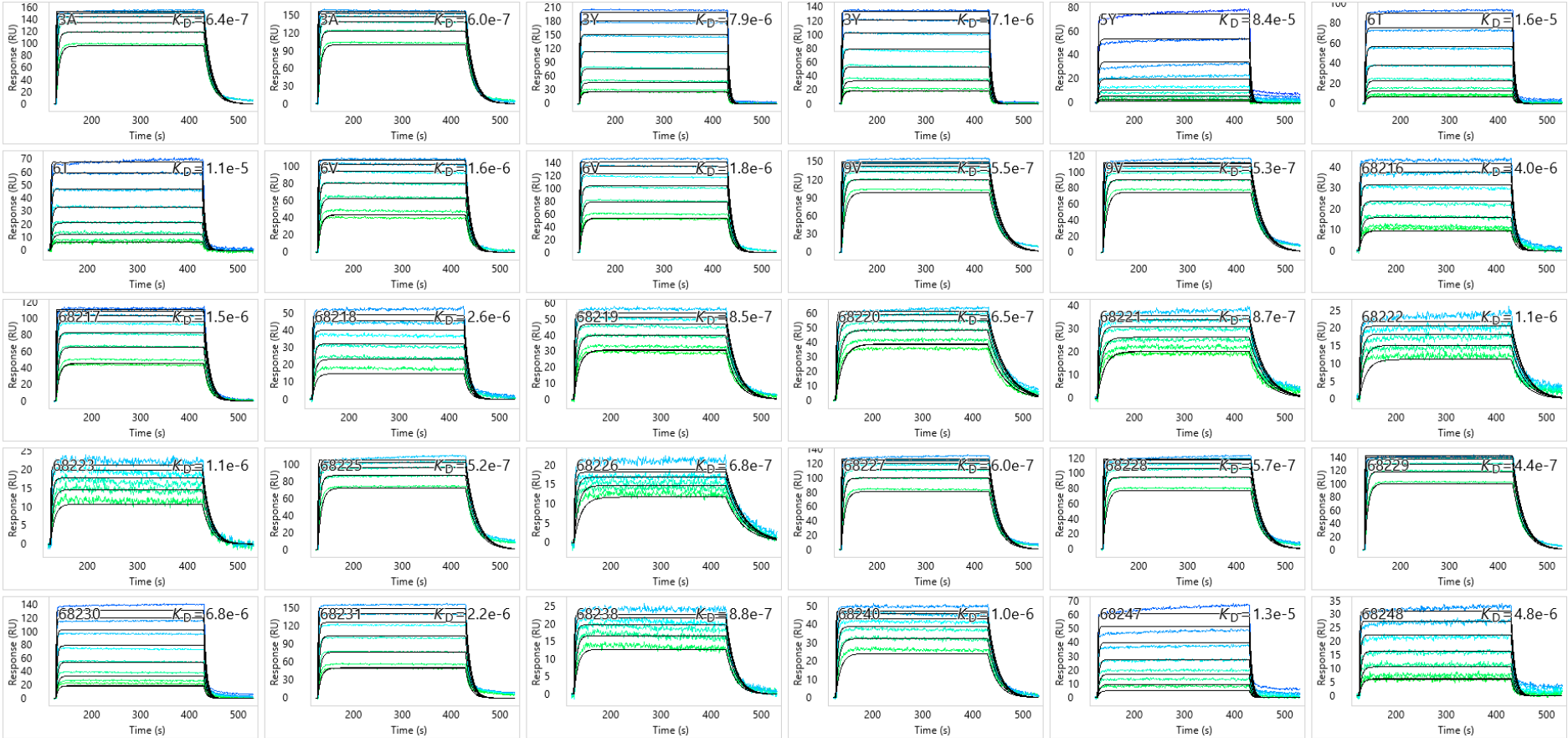
# TCR Characterization Workflow



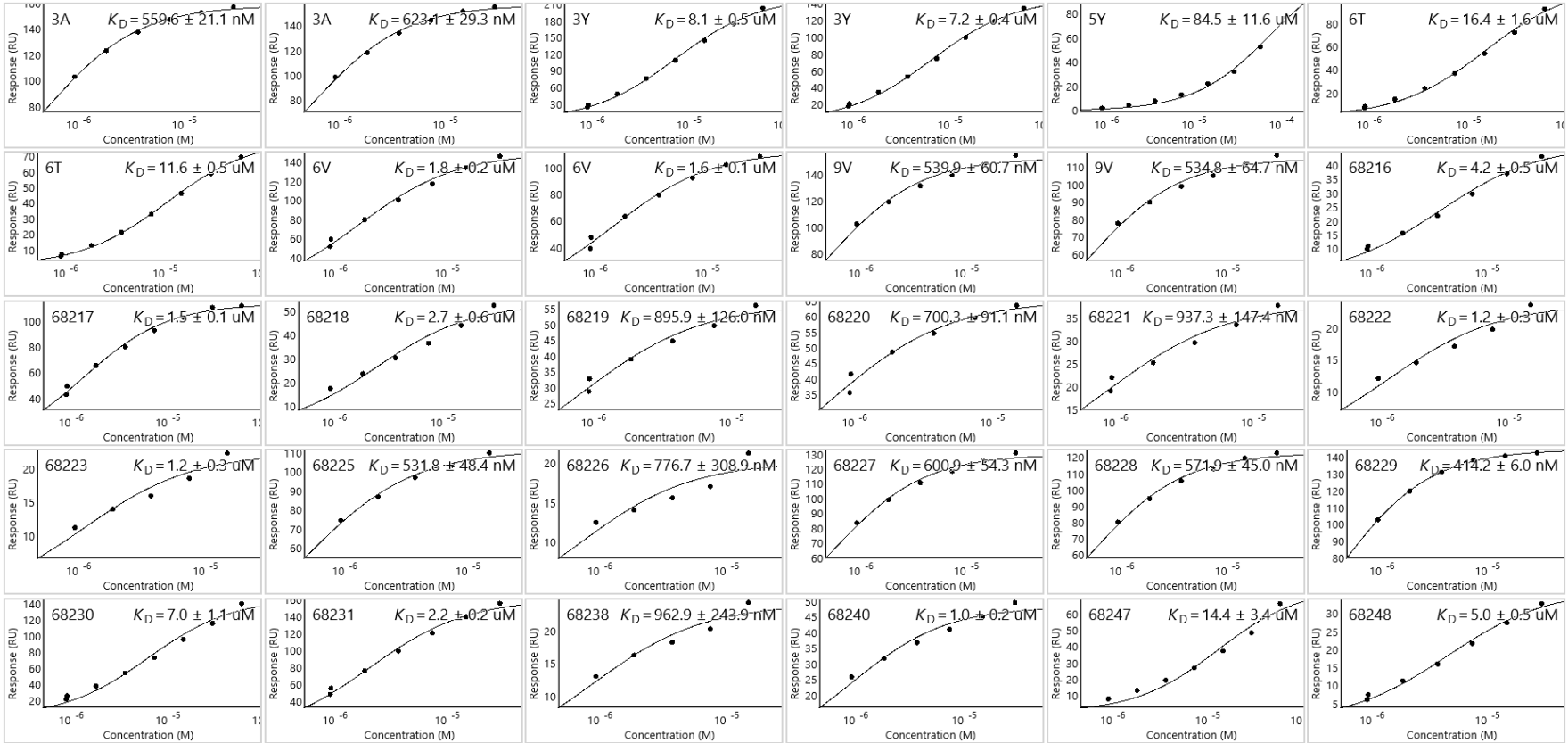
# LSA$^{XT}$ pMHC TCR study

- The binding affinity and kinetics of two T Cell Receptors (TCRs) was measured to 163 biotinylated pMHCs
- SAHC30M streptavidin chip was loaded with the pMHCs and a negative control protein (CD86) was spotted to act as a reference
- Experiment was performed at 37°C
- Two TCRs were injected in titration series up to 130 µM and analyzed by 1:1 kinetic binding and Steady State Equilibrium binding models
  - A single concentration of a conformationally sensitive mAb was injected at the end to ensure pMHCs were still peptide loaded and native
- This set up only used two thirds of the capacity of the surface array, with it possible to array three 96 well plates of pMHC to the streptavidin surface before TCR challenge
- For very rapid kinetics (TCR 2) it may be more appropriate to use an equilibrium model for affinity determination, though with good kinetic behavior the Steady State and Kinetic $K_D$s will yield the same result

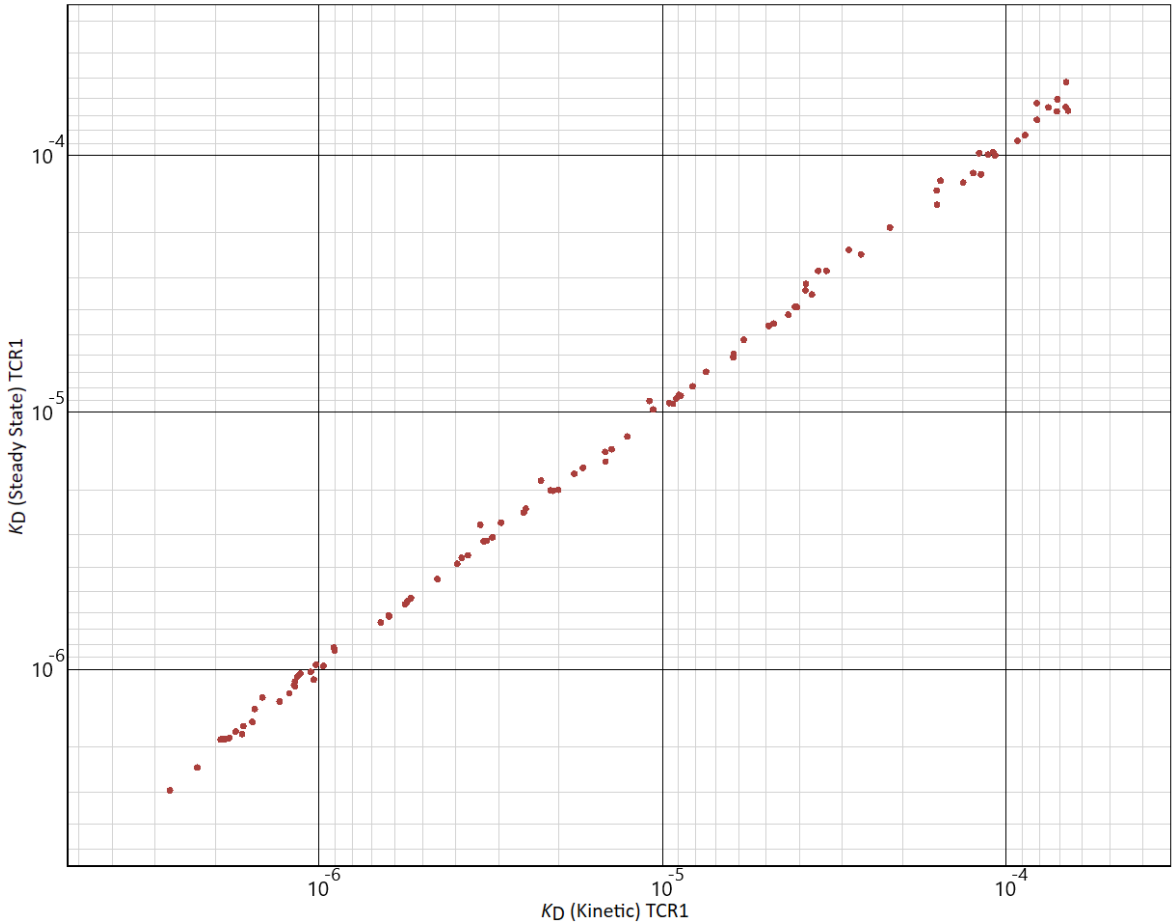
# TCR1 – Binding Kinetics Selected Examples



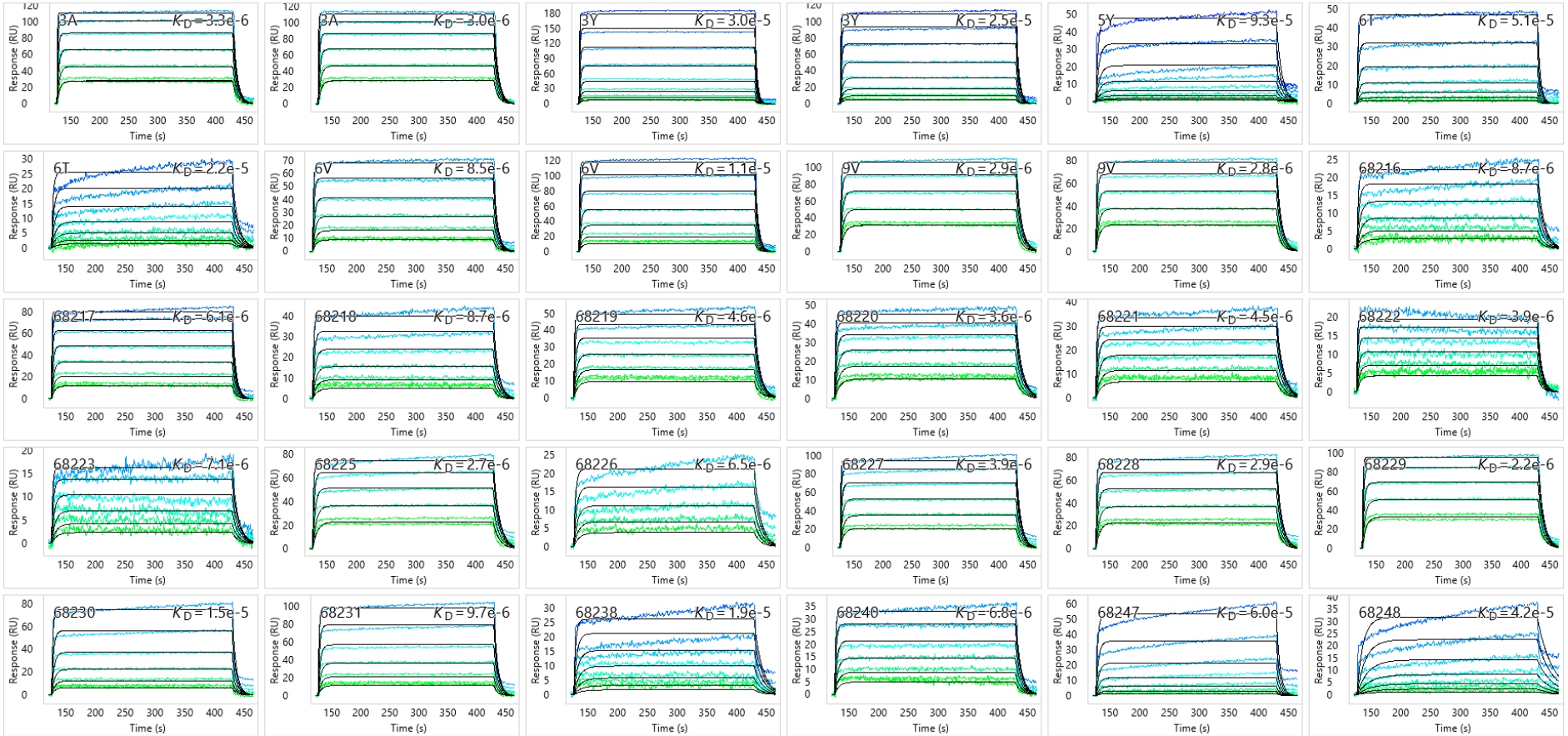
# CD86 referenced steady state data for TCR 1 – selected examples



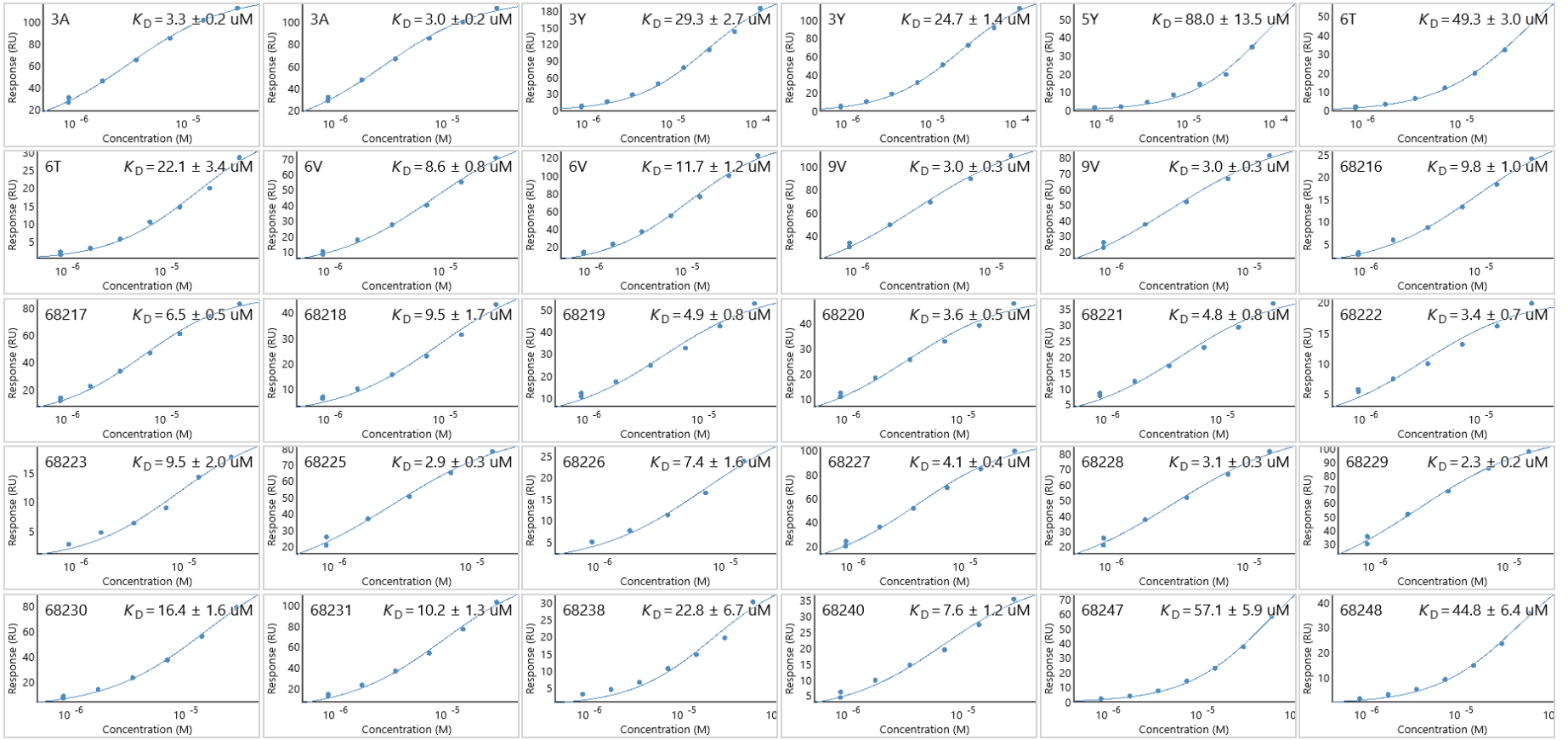
# Kinetic vs steady state $K_D$ comparison TCR 1



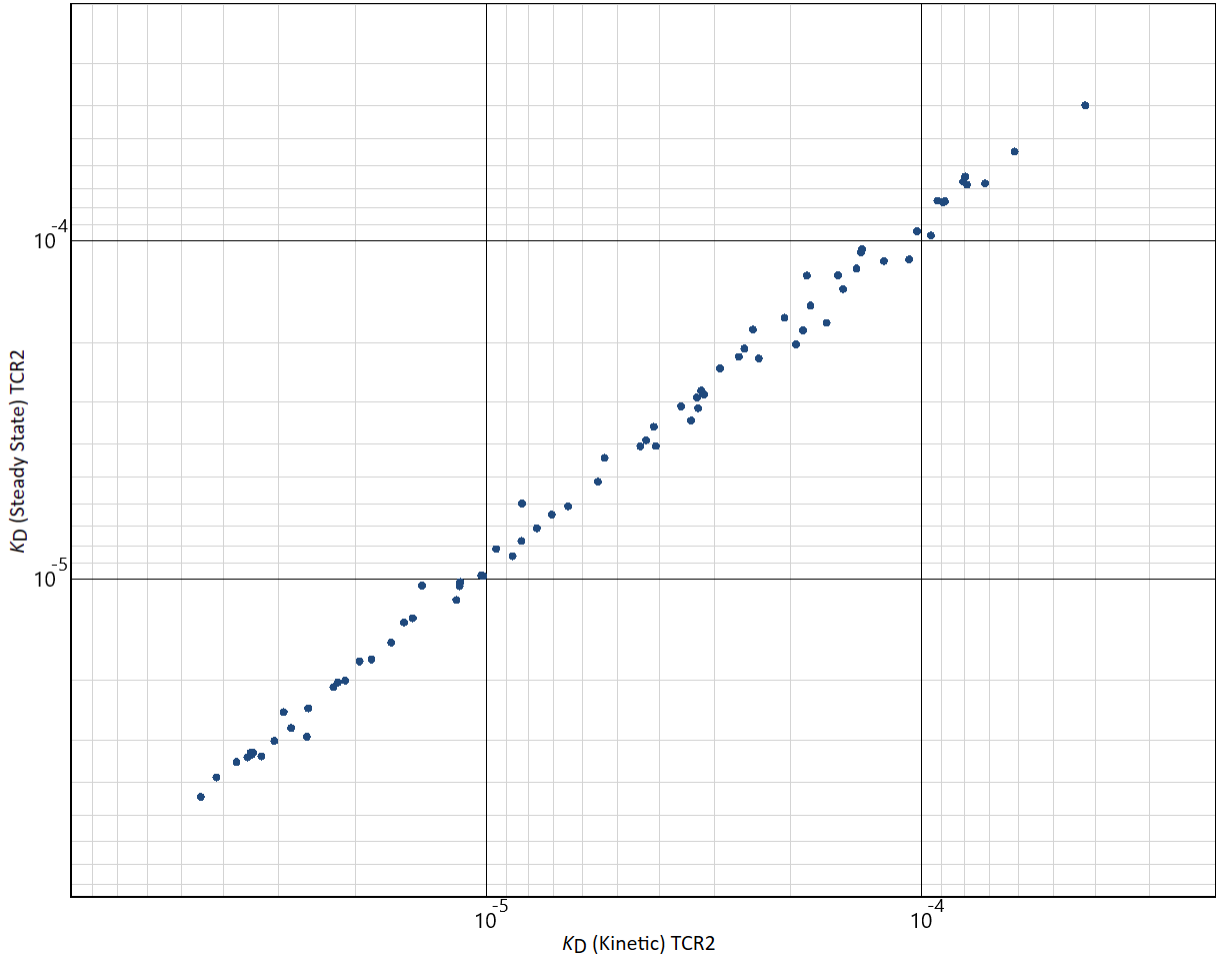
# TCR2 – Binding Kinetics Selected Examples



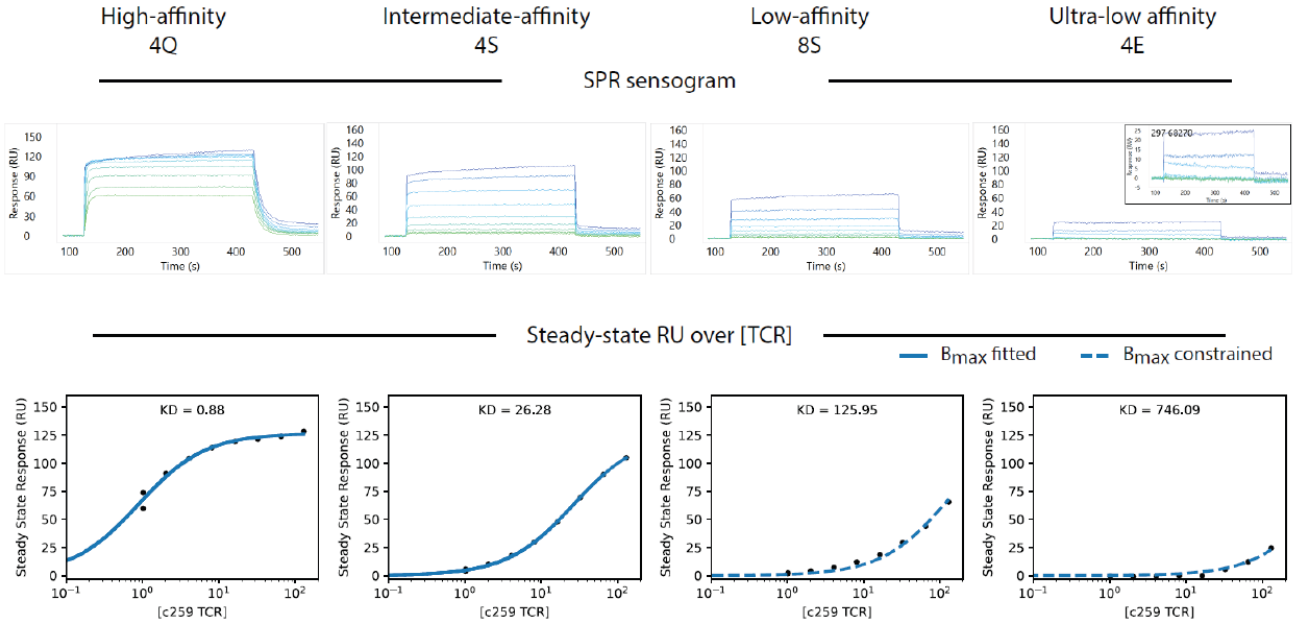
# TCR 2 steady state fit – sorted by name – selected examples



# Kinetic vs Steady State $K_D$s for TCR2

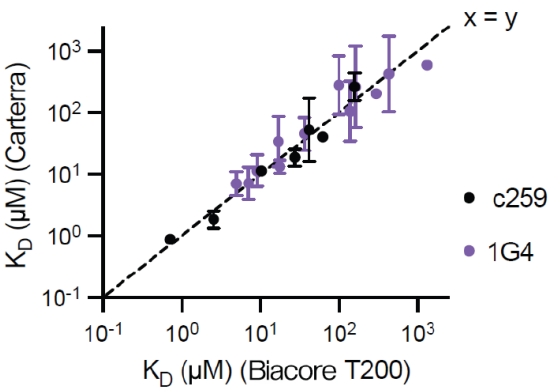


# Affinity determination into 1000 µM range




For very low affinity interaction $R_{max}$ was constrained using Ab-binding signal

$K_D$s obtained from high throughput LSA$^{XT}$ screen matched those obtained by Biacore T200 across sub- to 1000 µM range



# Carterra LSA and LSAXT enable rapid screening of TCR/pMHC interactions

- Parallel measurement of hundreds of TCR/pMHCs affinities in under a day using <30 nmols of TCR
- Accurate measurements for interaction in sub-µM to mM affinities

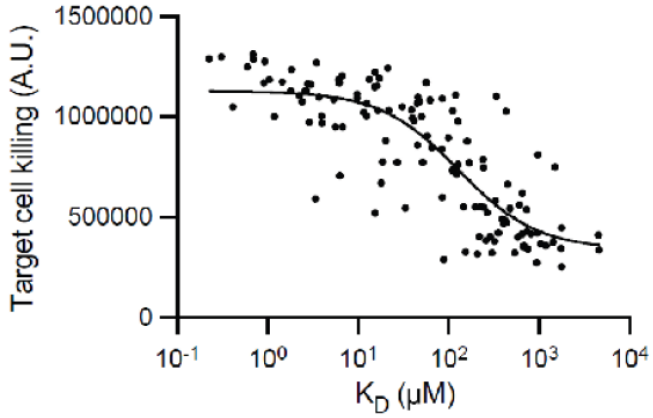
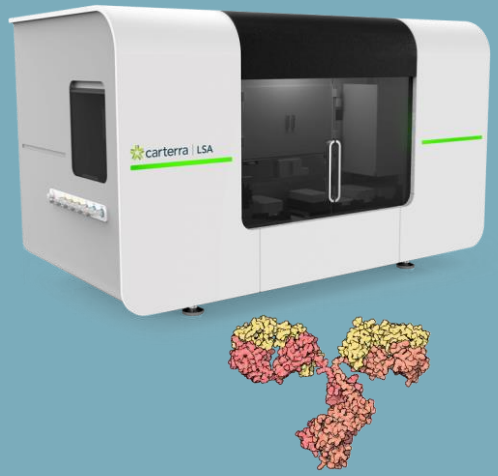



**Figure 5:** The ability of primary human CD8+ T cells transduced with the c259 TCR to kill target cells correlates with the TCR/pMHC affinity. (A) The ability of T cells to kill target cells presenting each peptide from the positional scanning library. (B) Target cell killing plotted over $K_D$.

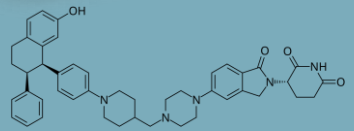
# Ultra Performance for High Sensitivity

## LSA

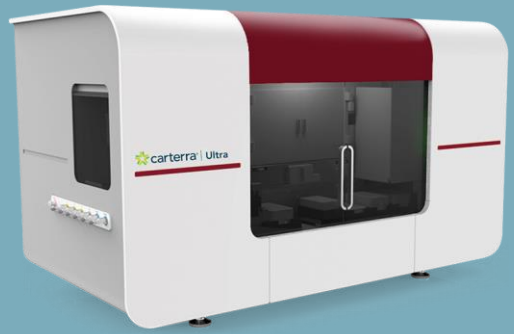


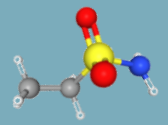



## LSA$^{XT}$


## Ultra



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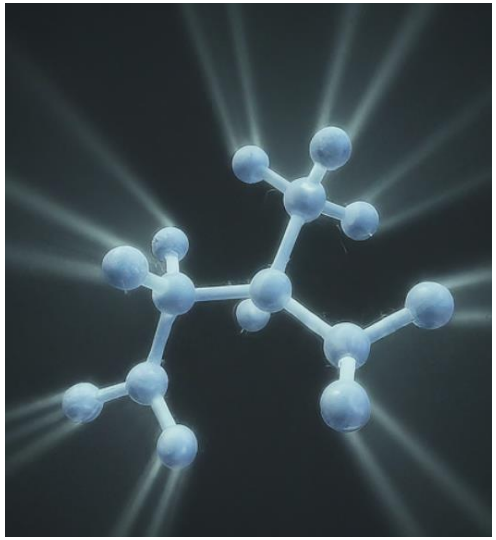
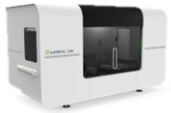
# The Carterra HT-SPR Instrument Family



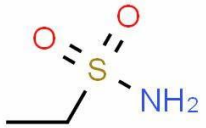


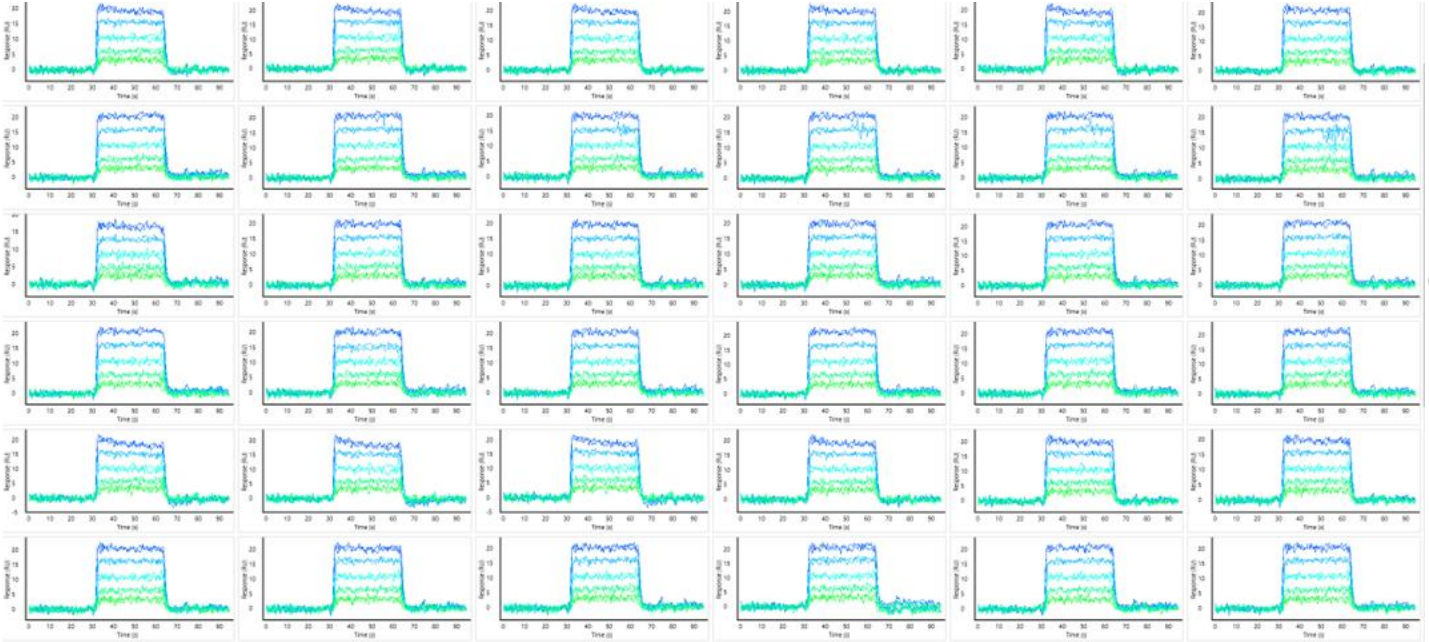
| | LSA | LSA$^{XT}$ | Ultra |
|---|---|---|---|
| Noise, RU RMS | ≤ 2 | ≤ 0.8 | ≤ 0.3 |
| Data collection rate, Hz | 0.4 | 1 | 2 |
| Unique ROIs | 432 | 432 | 192 |
| MW Range, KDa | ≥1000 Da | ≥500 Da | ≥100 Da |
| SFC Type | Standard SFC | Standard SFC | Advanced SFC™ |
| SFC Volume Injected | 270 ul | 270 ul | 180 ul |
| Max SC Injections/24 hrs | ~200 | ~200 | 384 |
| Interaction Thermals, °C | 15 to 40 | 15 to 40 | 10 to 40 |
| Sample Deck Thermals, °C | 15 to 25 | 15 to 25 | 10 to 20 |
| Max Sample Capacities | | | |
| Single Channel | 384 | 384 | 768 |
| Multi Channel | 1152 | 1152 | 1152 |

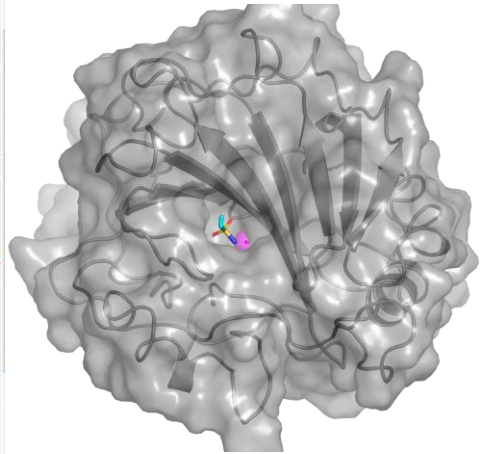
# Ultra – Low Noise, Wide Dynamic Range, Highly Reproducible

Dual replicates overlayed

Proteins captured to a NiHC200M via his tags

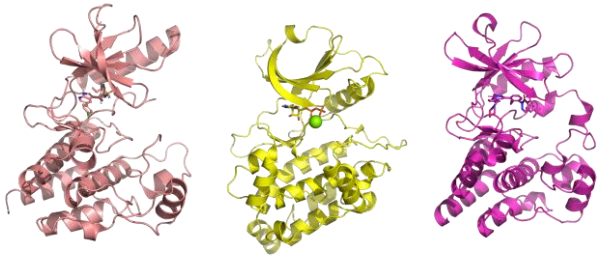




Docked structure of 109 Da ethanesulfonamide into 32,000 Da carbonic anhydrase. Excellent signal despite ~300-fold different MW

# Fragment Screening 125 Kinases

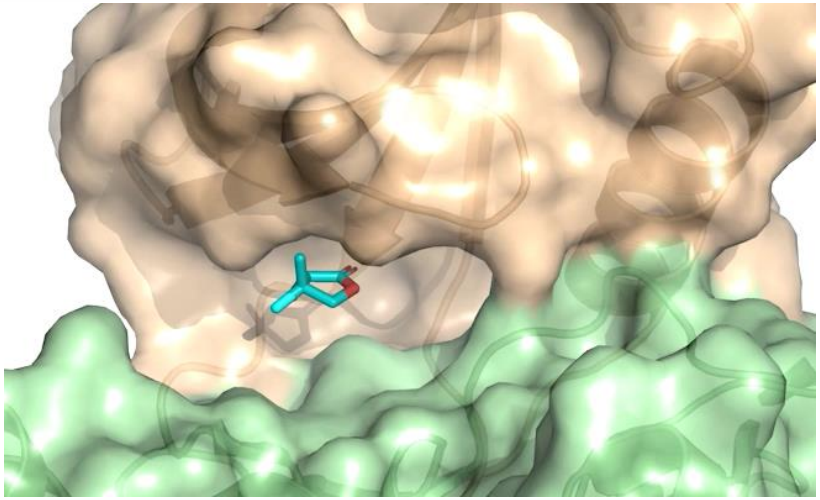


Maybridge 1000 Fragment Library

SPR Screen of 125 BTN-kinases (Carna Biosciences)

Selection of positive binders

$K_D$ and LE determination



*Real-time speed of interaction data collection (125 interactions every 3.5 minutes)*

## 125,000 Interactions

300 μM Compounds

Running Buffer:

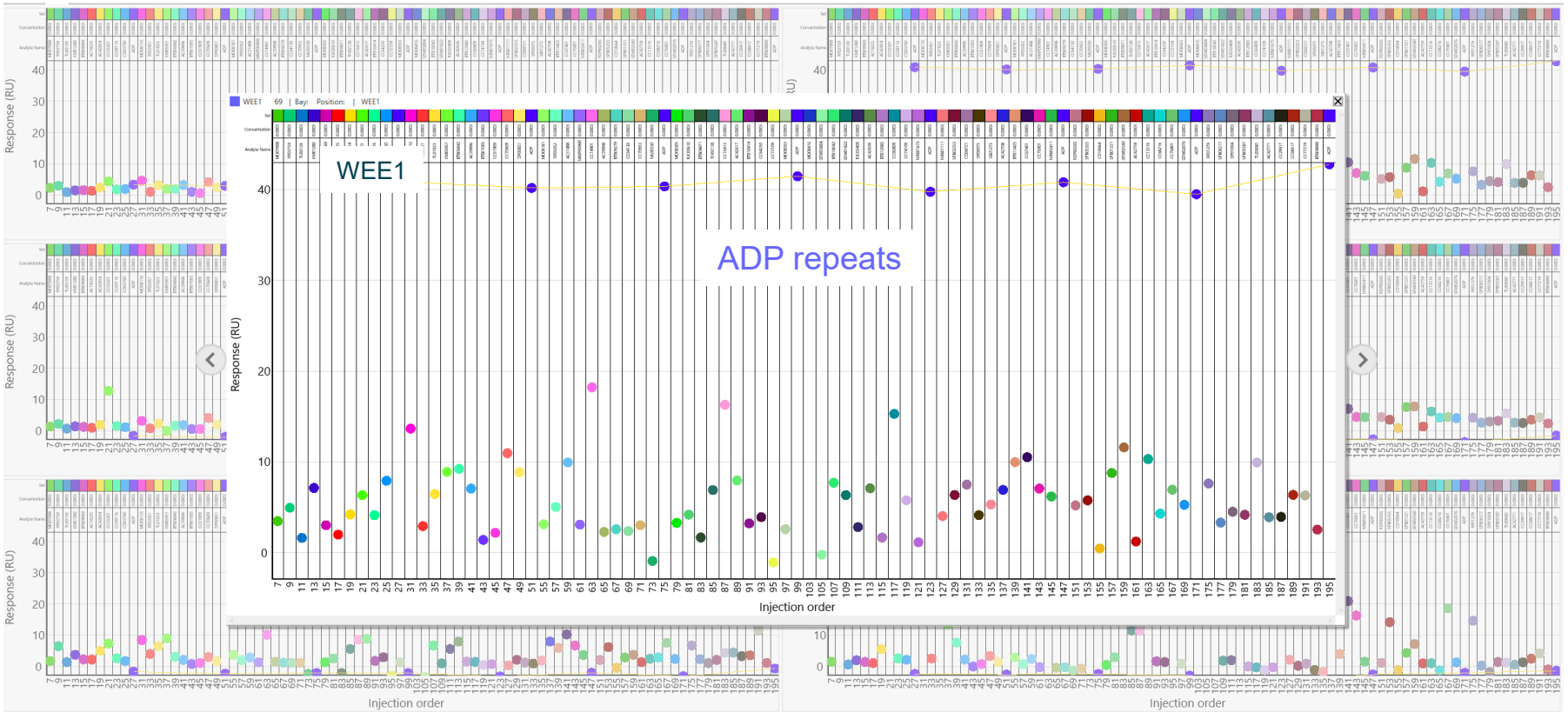
HBS, 0.005% Tween-20, 5% glycerol, 5 mM $MgCl_2$ ,1 mm DTT, 3% DMSO, pH 7.4

CARNA BIOSCIENCES

MAYBRIDGE

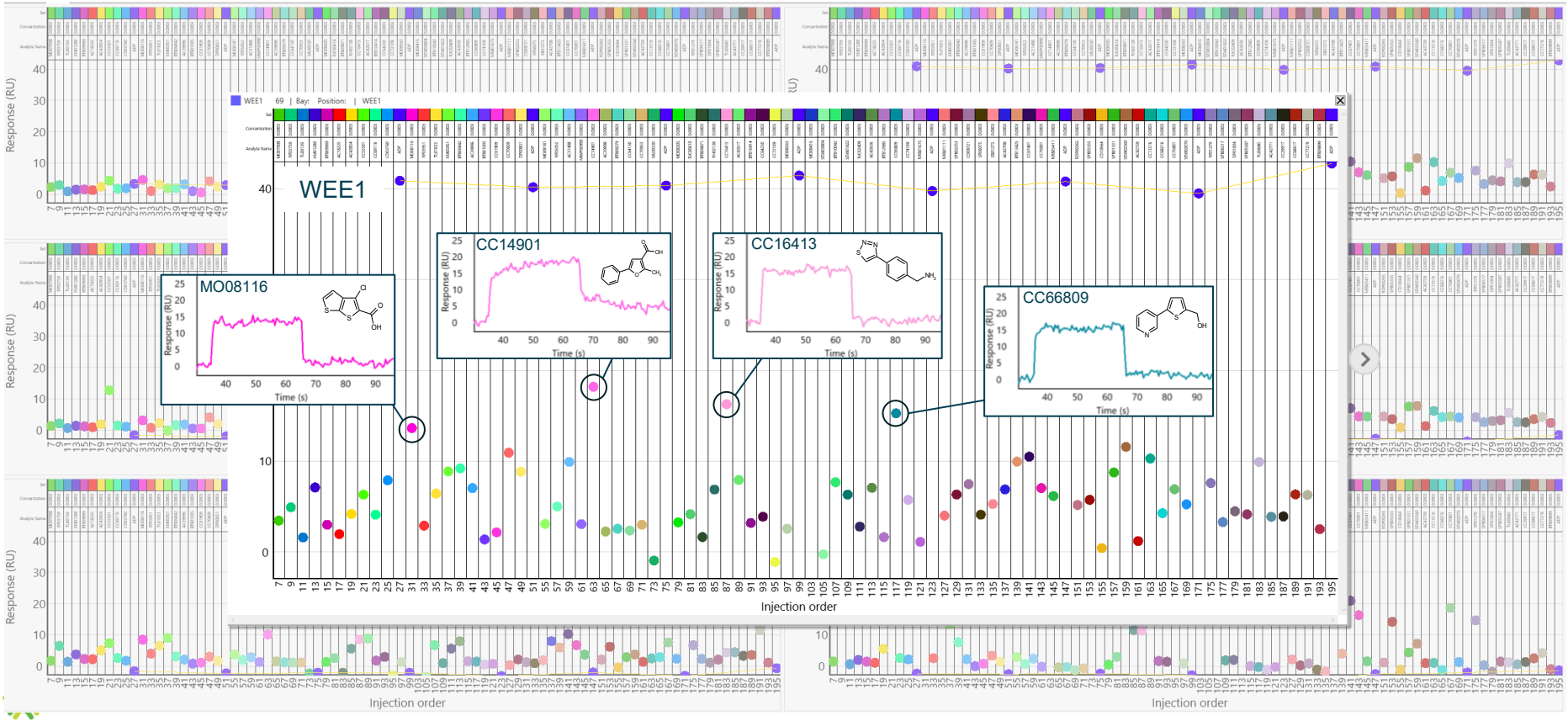
# Fragment Hit Identification

## Binding report points show kinase surface stability and identifies hits



# Fragment Hit Identification

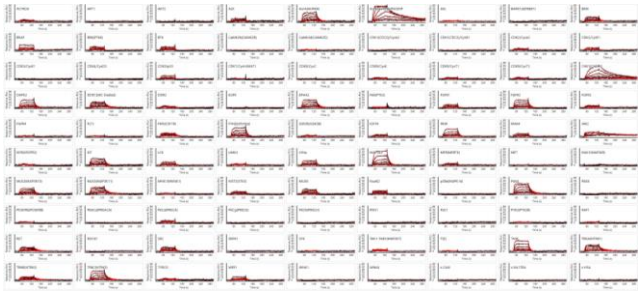
## Selected hits queued for dose-response confirmation



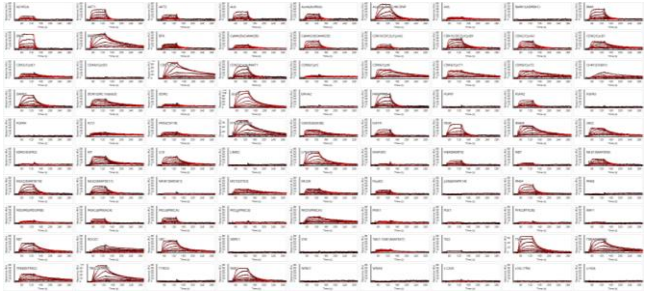
# A **new paradigm** for small molecule drug discovery

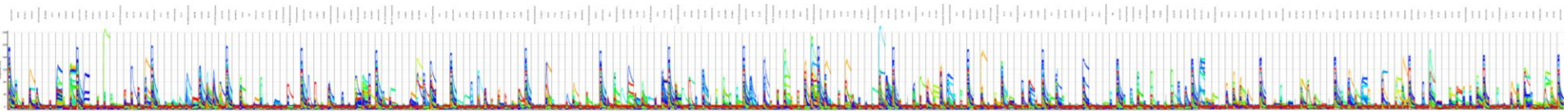
- **Deep mining** of binding profiles across target families as well as off-targets in a single experiment
- These assays are resource prohibitive on **any other platform**

TPCA-1 279 Da

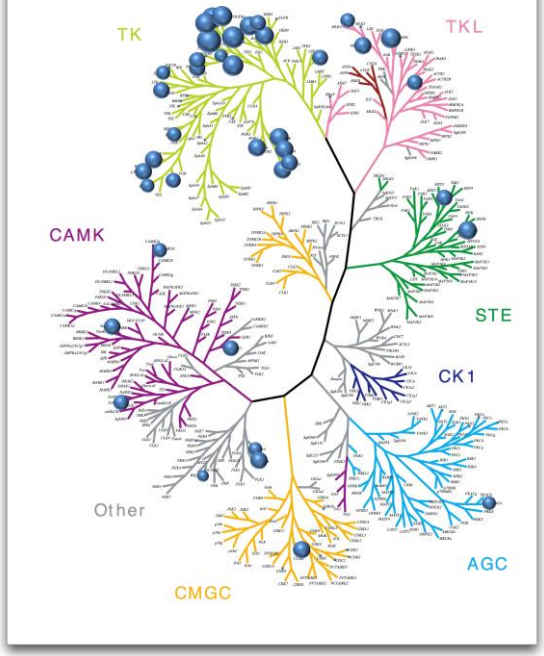


CHR 6494 415 Da





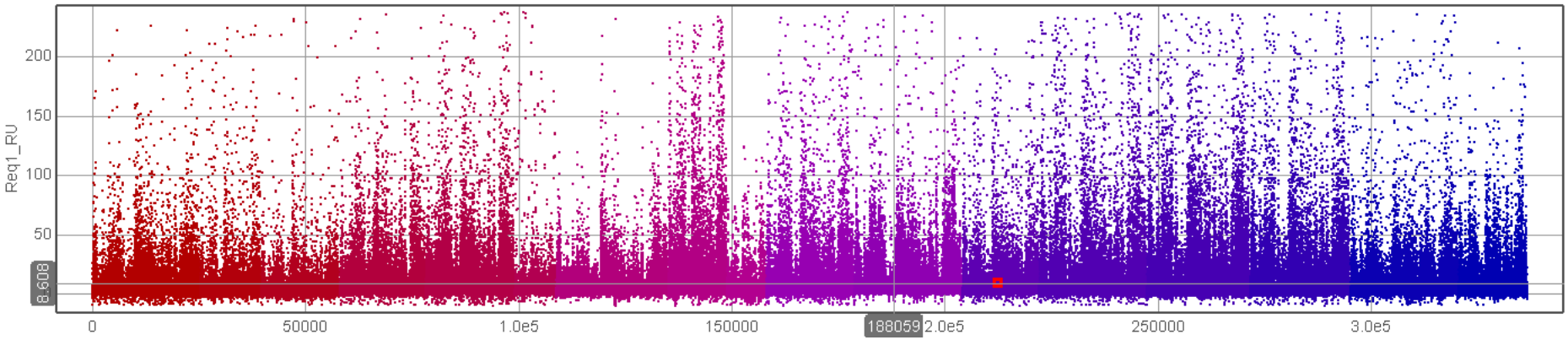
## Kinome Mapping Sunitinib (SUTENT®) $pK_D$ Values



# Even Larger Example: Fragment Screening 92 Proteins Simultaneously to 3500 Fragments!

Identifying selective binders against a broad panel of proteins (92 proteins X 3,500 compounds)

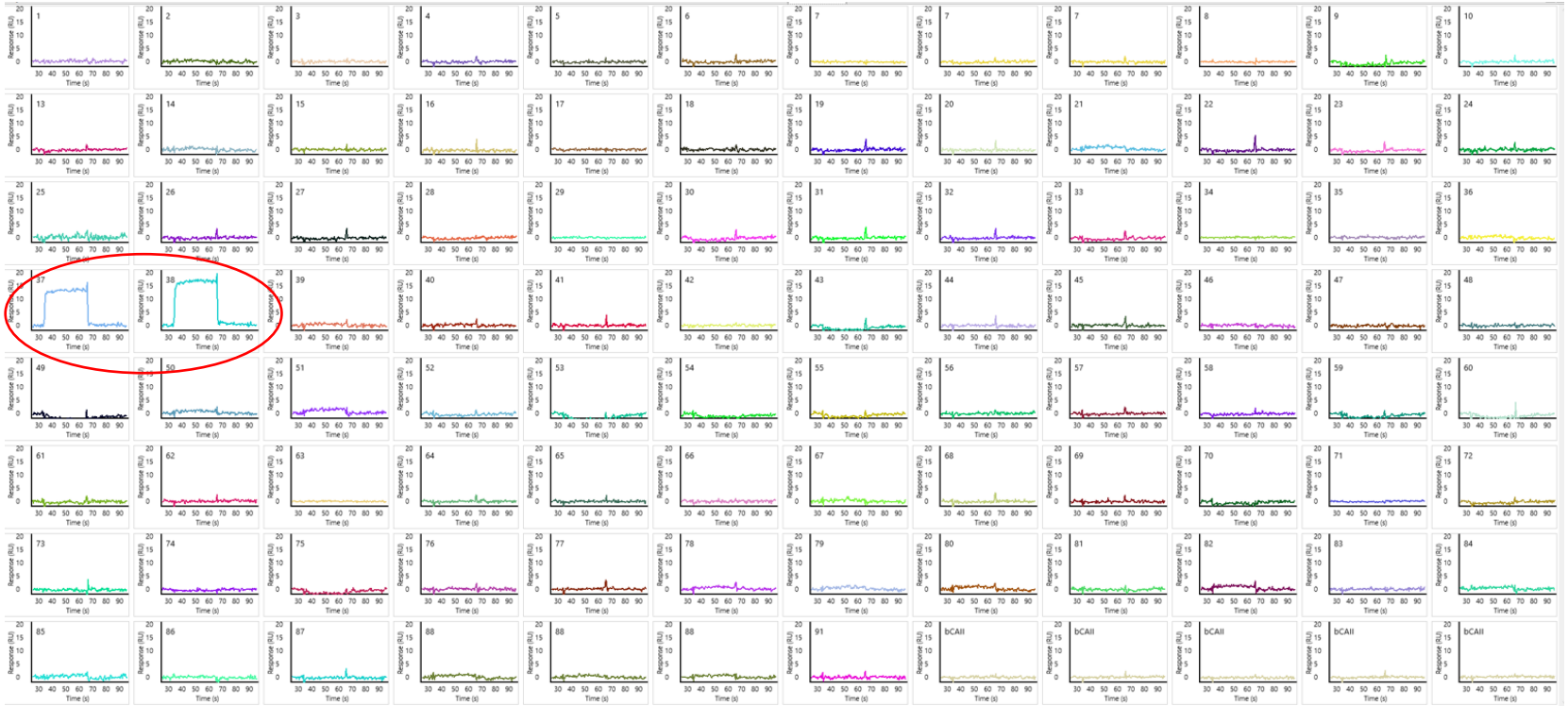
- >320,000 interactions in 11 days
- Recently presented at DDC conference




Courtesy of John Quinn, Genentech

# Fragment Screening 92 Proteins Simultaneously

Identifying selective binders against a broad panel of proteins (92 proteins X 3,500 compounds)



Courtesy of John Quinn, Genentech

# Summary

- HT-SPR is an efficient way to generate high quality kinetic data for a broad range of applications, even from crude samples
  - mAbs and other Biologics
  - TCRs and peptide MHCs
  - DELs, PROTACS, Peptides
- Ultra™ enables fragment and small molecule drug discovery
  - High-sensitivity through advanced microfluidics, optics, and enhanced thermal performance
  - Flexibility to handle nearly any sample type, from fragments to antibodies
  - Advanced hardware to decrease experimental runtimes and reduce sample consumption
  - Software tools to manage high volume of data and support small molecule screening and hit selection

# Acknowledgements


**Carterra**

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- Kristoffer Brannstrom

**Roche Genentech**

- John Quinn

**Carna Bio**

- Adam Schutes



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