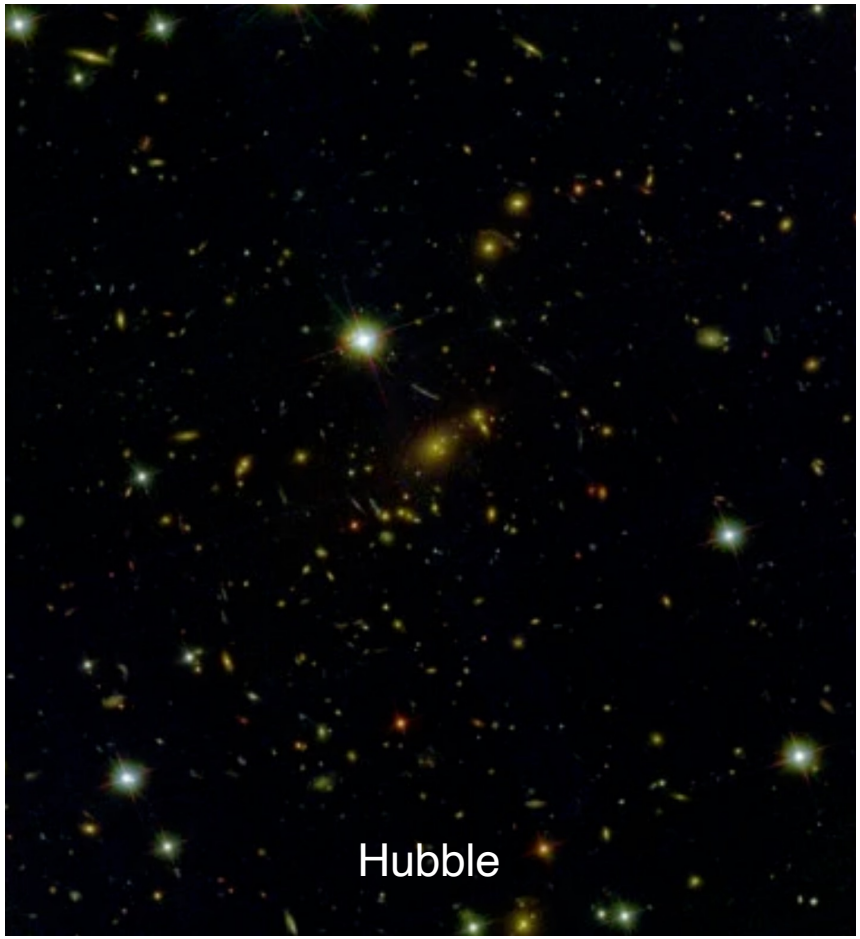
Leveraging the new Carterra LSA^{XT} for challenging HT-SPR applications

carterra®

LSA accomplishments in just 5 years

- Redefined the scale of biotherapeutic characterization
- Core technology at 19 of top 20 pharma
- Published in high impact journals including Science, Nature, and Cell
- > 120 units deployed across the globe
- Part of Lilly's incredible 90 days to FIH
- Key for emerging AI/ML discovery strategies


Hubble

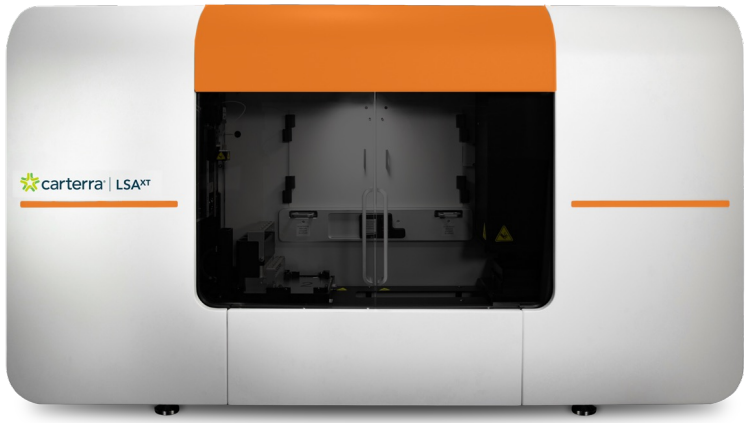

Webb

Meet the Carterra LSA^{XT}

Launched SLAS ’23
San Diego

Expanding on the LSA platform: Carterra LSA^{XT}

100x the data | 10% the time | 1% the sample, *plus*:



- Enhanced signal-to-noise
- Increased signal uniformity
- Faster data collection rate

Application	LSA	LSA^{XT}
Purified or crude antibody kinetics/affinity	✓	✓
Purified or crude epitope binning	✓	✓
Peptide mapping	✓	✓
Mutant mapping	✓	✓
Quantitation	✓	✓
General multiplexed binding	✓	✓
Blockade assays	✓	✓
DEL compounds	✓	✓
Membrane proteins	✓	✓
Peptides (analytes)		✓
Cell therapy, e.g., TCRs		✓
FcγRs		✓
Cytokines		✓
PROTACs/molecular glues		✓
Kinase inhibitors		✓
Thermodynamics		✓
Protein:protein inhibition		✓

LSA^{XT}: Exploring smaller analyte formats using protein kinase inhibitors

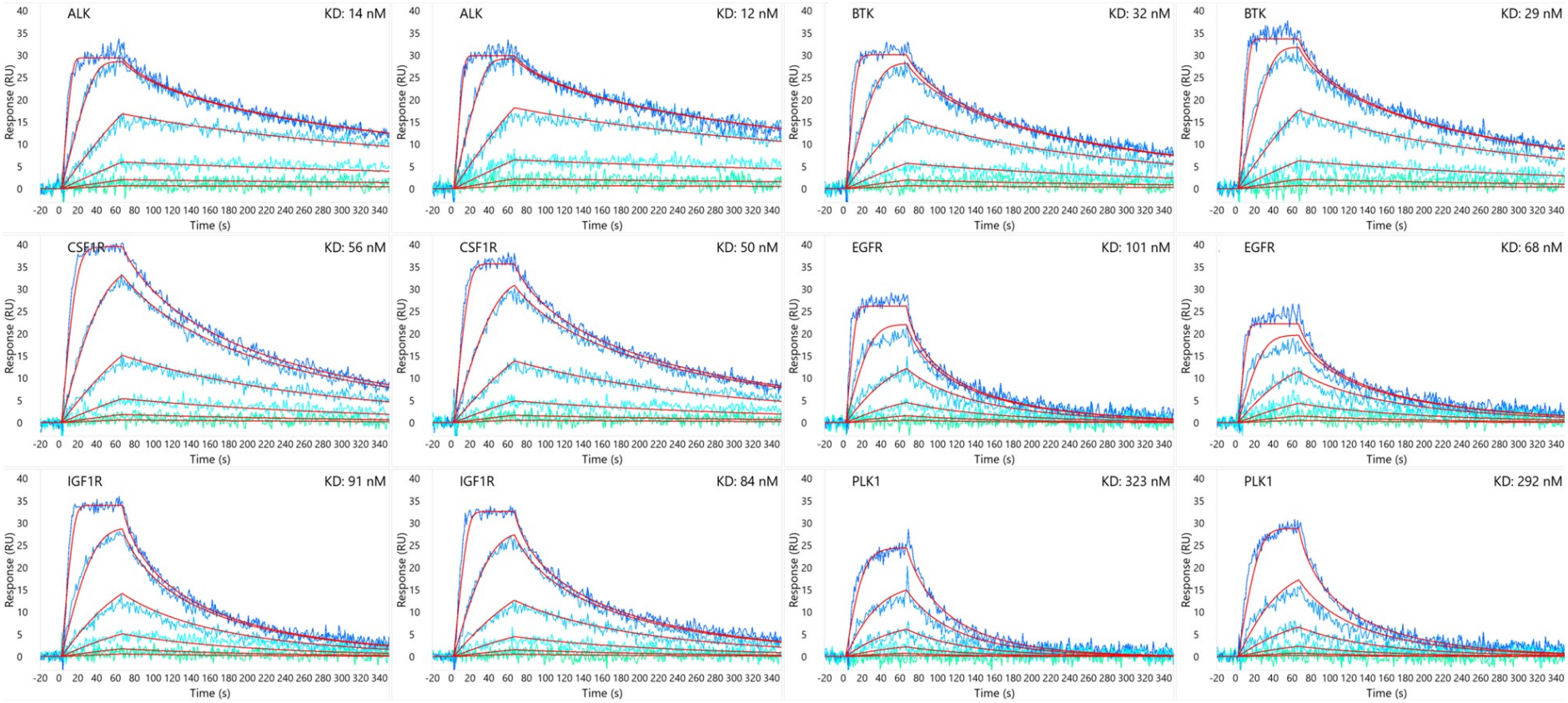
carterra

Assay conditions

- Six biotinylated kinases captured on SAD200M sensor chip in duplicate
- Injections of kinase antagonists prepared as three-fold dilutions starting from 3uM
- Assay buffer: HBS + 0.005% Tween20, 10 mM $MgCl_2$, 3% DMSO, pH 7.4
- Temp: 15˚C (increase protein life on the surface)

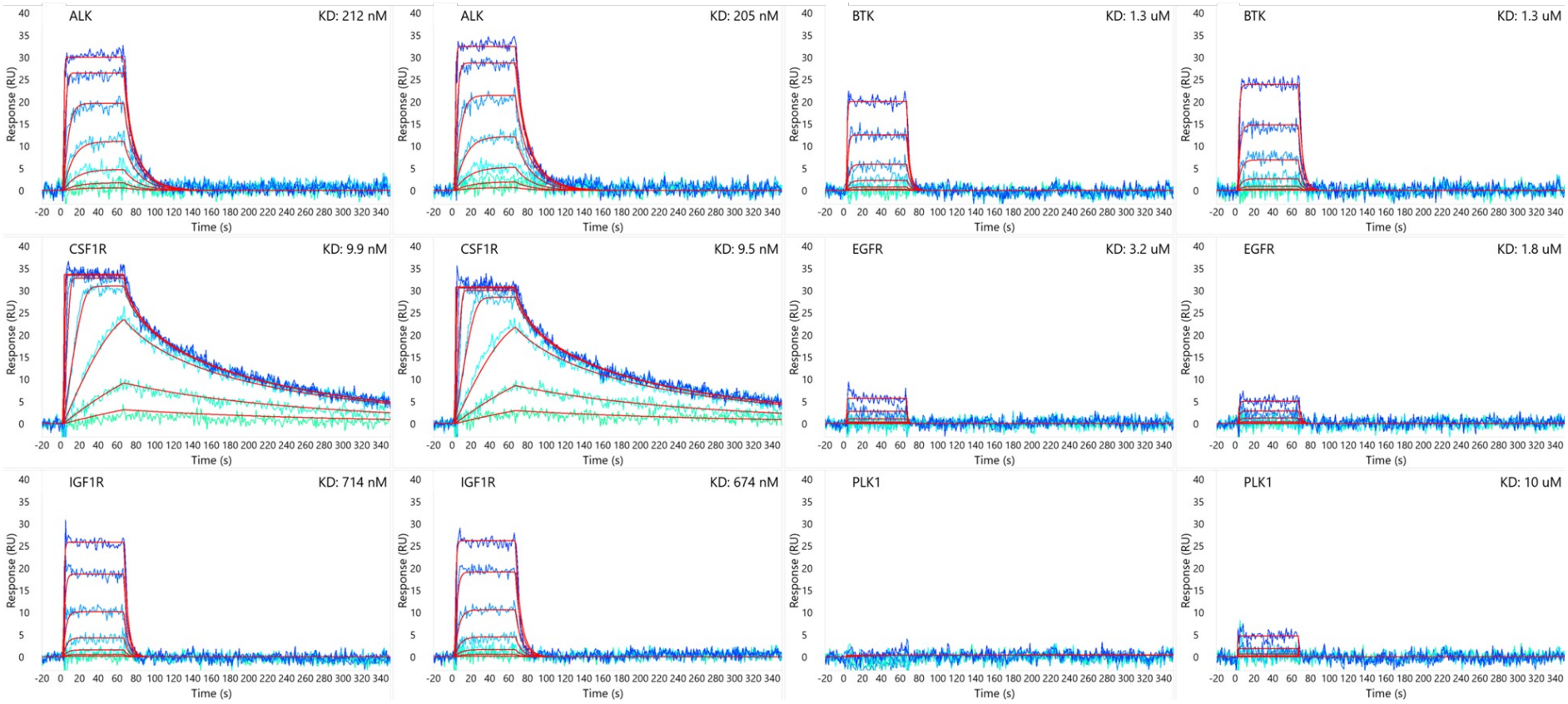
Staurosporine: Expected profile of higher affinity but low selectivity

466.54 Da compound



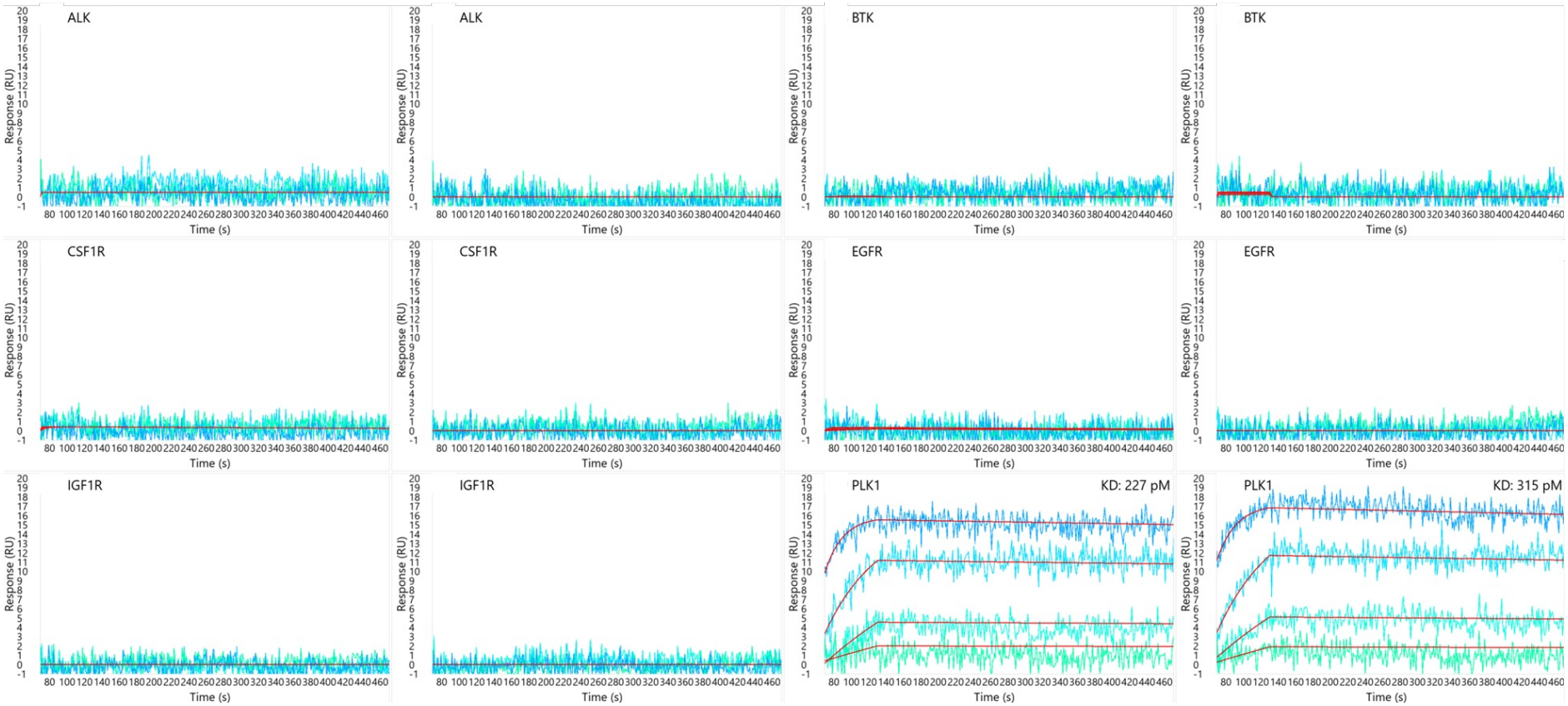
Sunitinib: Increased selectivity for CSF1R but still many off-target interactions

532.56 Da compound



GSK-461364: Highly selective, high affinity PLK1 inhibitor

543.60 Da compound



Value of LSA^{XT} for affinity and selectivity screening

- Easily measure compounds down to at least 500 Da
- High capacity of array allows for a broad panel of targets and off-targets
- Both weak and high affinity interactions are readily quantitated

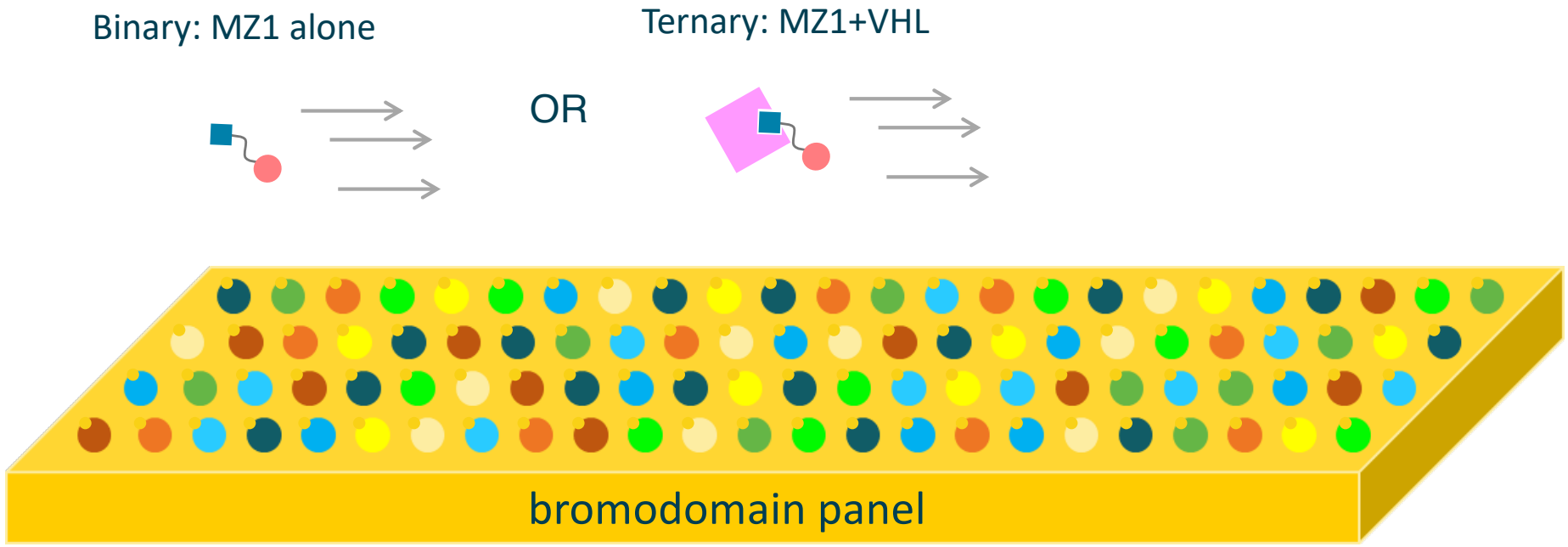
Carterra LSA^{XT}: PROTAC® characterization

carterra®

PROTAC assay design

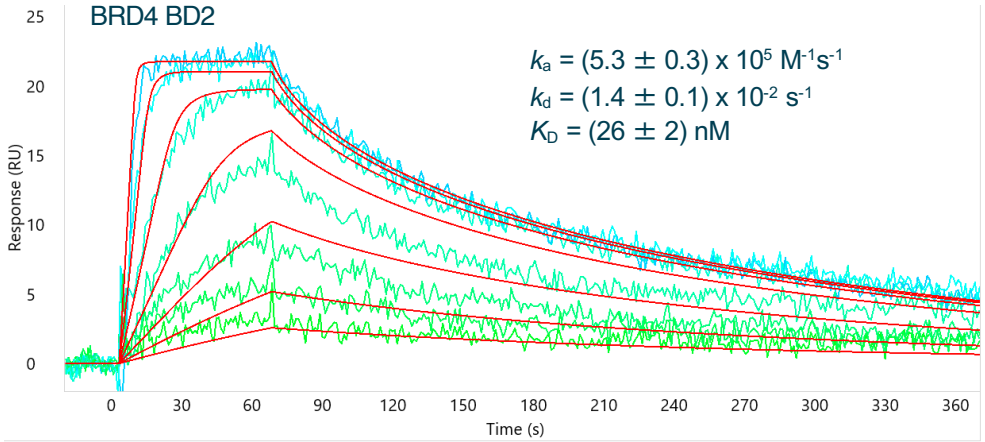
- Running buffer: HBS + 0.005% Tween-20, 5% glycerol, 1 mM TCEP, 1% DMSO, pH 7.4
- Temp: 15˚C (increase protein life on surface)
- Binary kinetics
  - His-bromodomain proteins and His-E3 ligases captured on a NiHC200M sensor chip, between 1 to 0.05ug/ml depending on the protein
  - MZ1 titration injected across array from 0.004 to 3uM
- Ternary kinetics
  - His-bromodomain proteins amine coupled to CMDP sensor chip
  - MZ1 titration injected in presence of ≥ 20-fold molar excess of VHL across the array

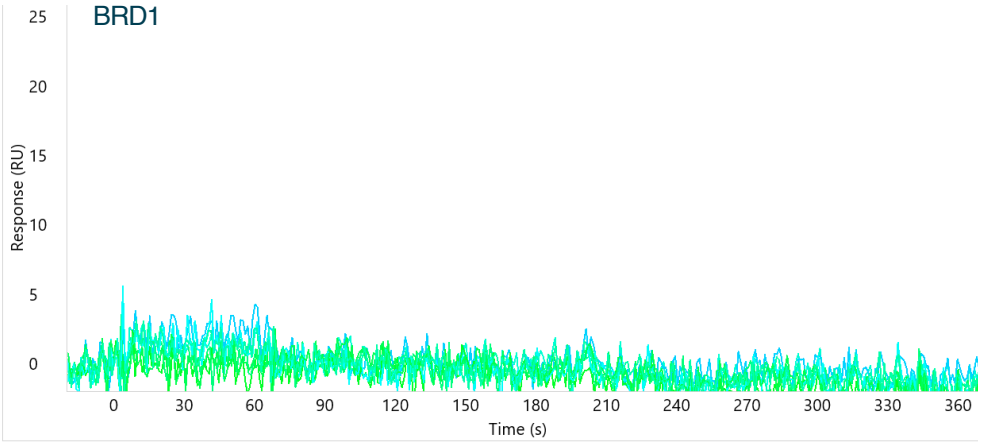
Assay format: Titrations of MZ1 +/- VHL against array of bromodomain proteins

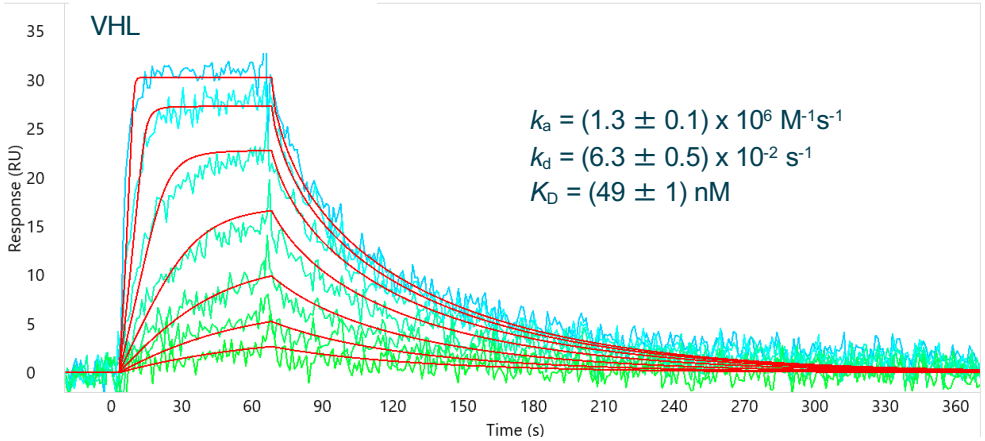


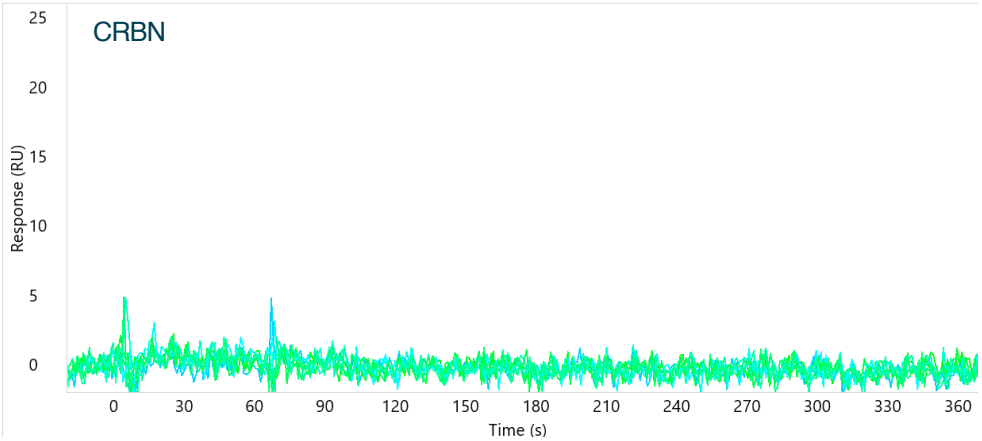
carterra

Example of MZ1 binary kinetics including negative controls



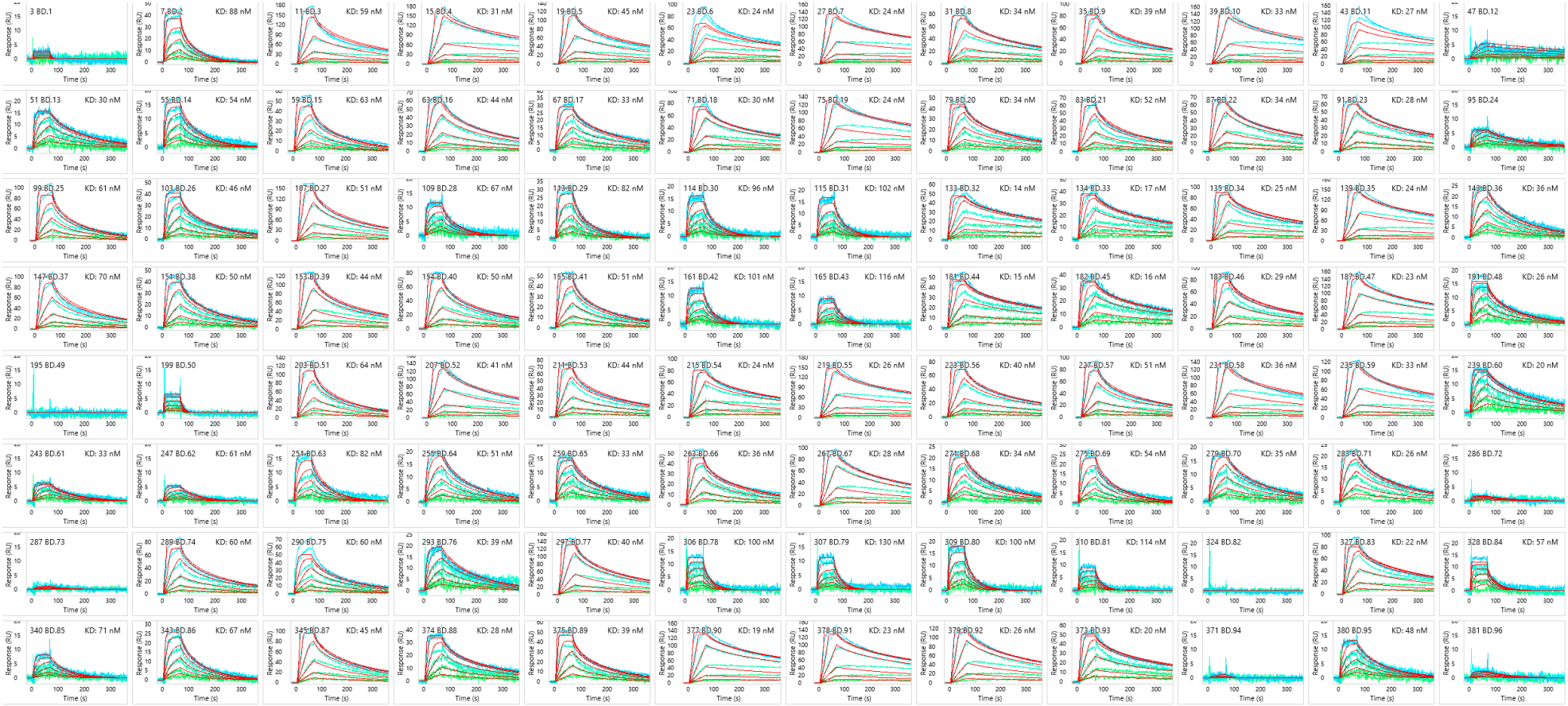






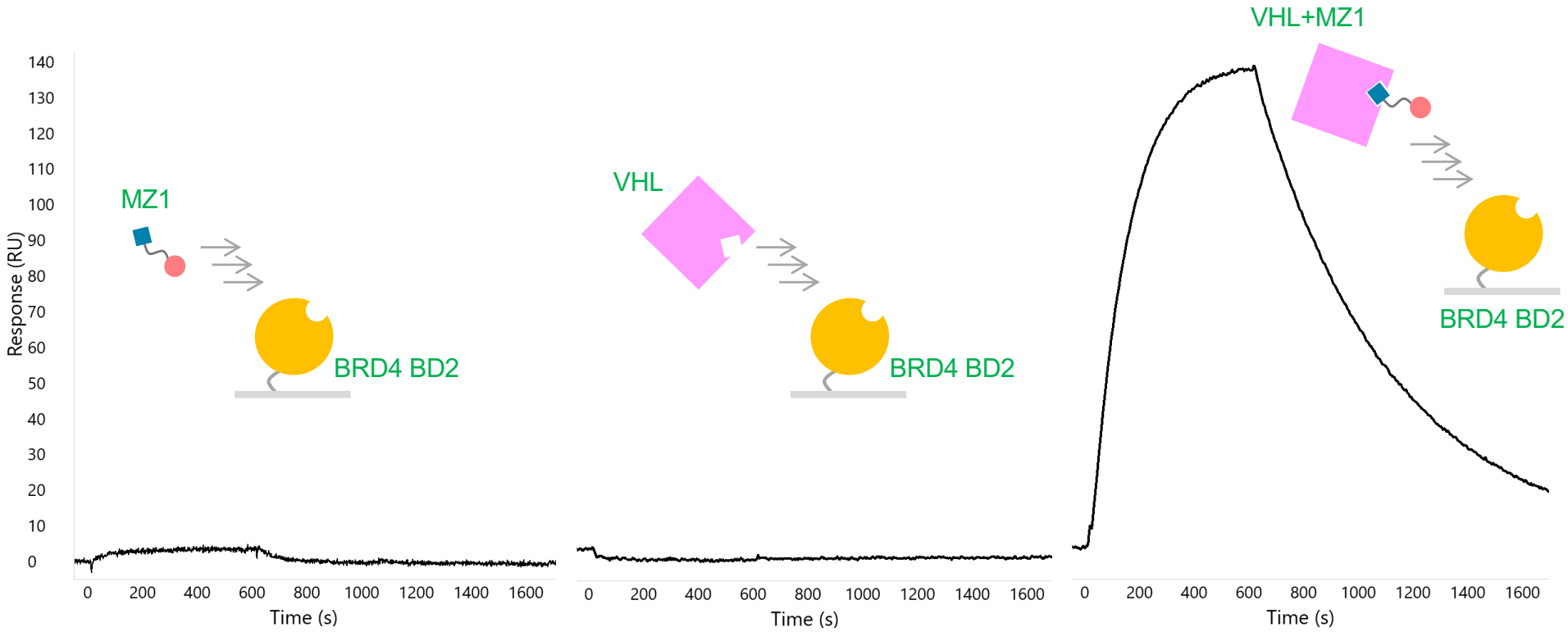
carterra

MZ1 binary kinetics against panel of bromodomain proteins



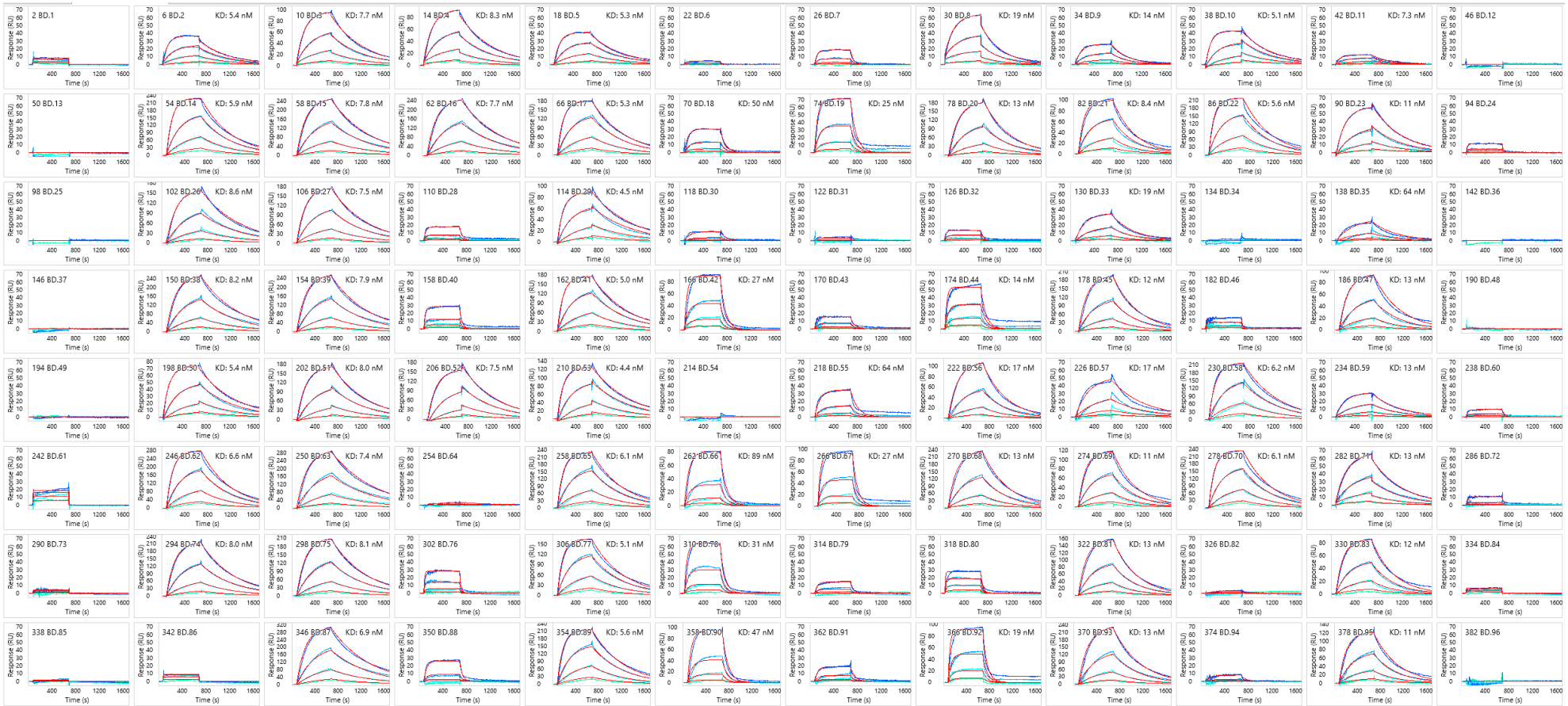
carterra®

Expected profiles for ternary complex formation



carterra

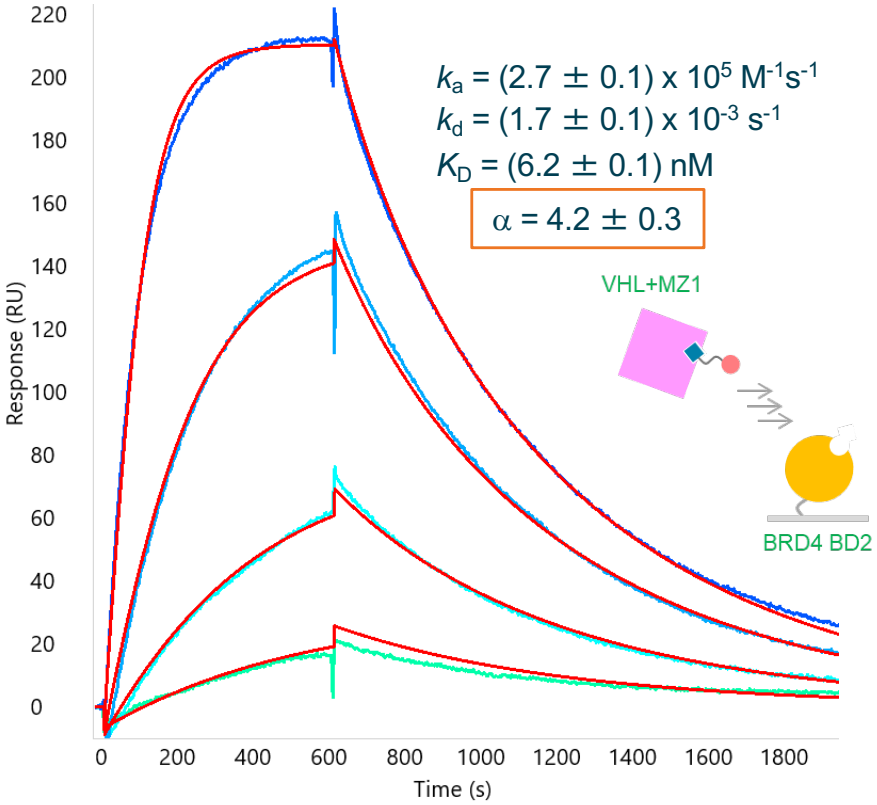
MZ1/VHL ternary kinetics against array of bromodomain proteins



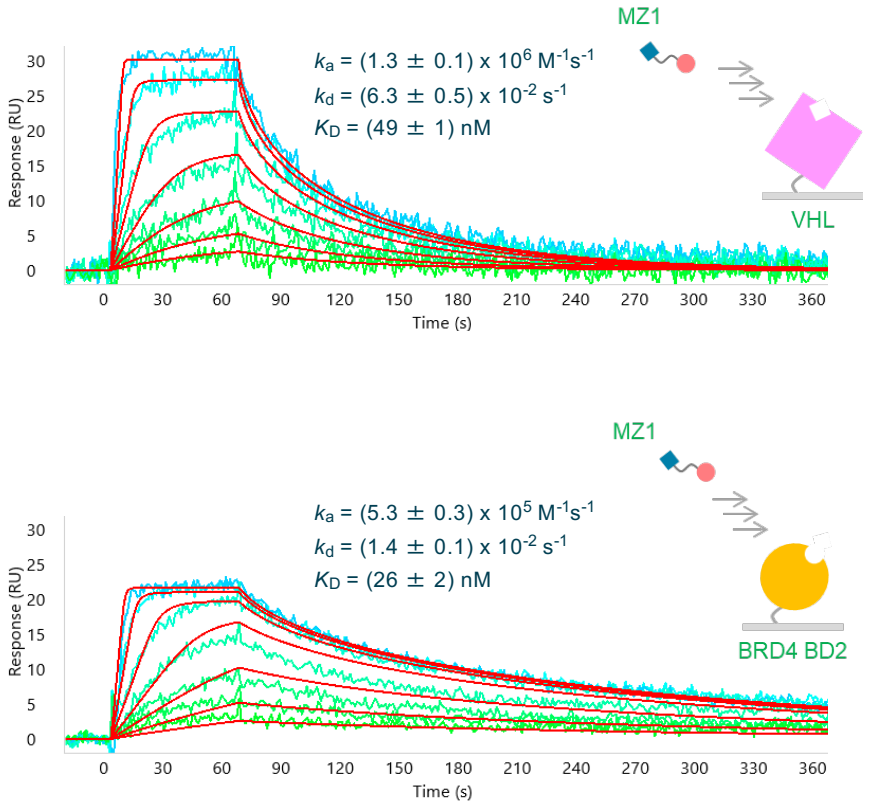
carterra®

Example of MZ1/VHL cooperativity towards BRD4 BD2

ternary interaction



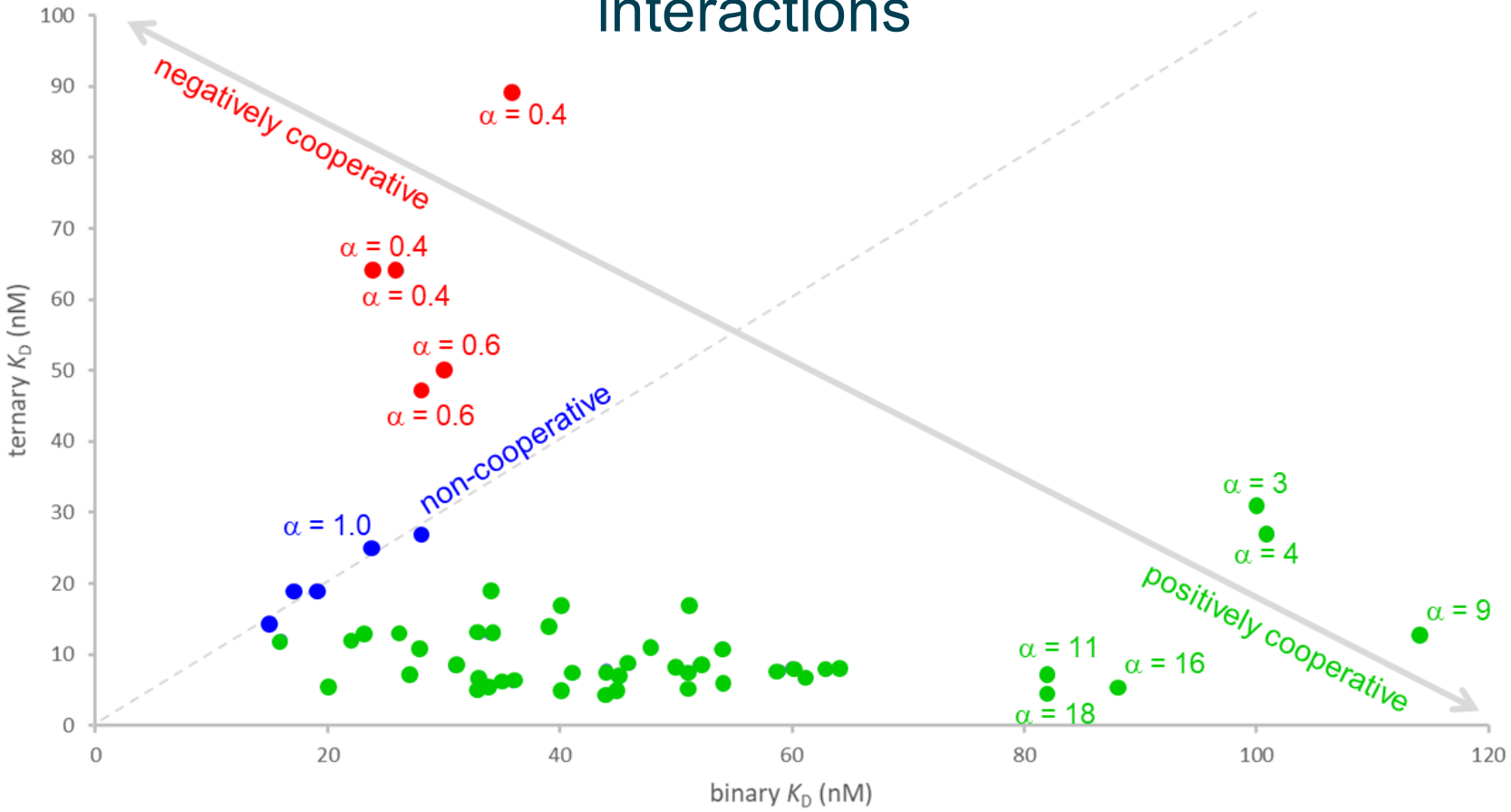
binary interactions



carterra

cooperativity = α = (K_D^{binary} / $K_D^{ternary}$)

Mapping cooperativity of >50 VHL/MZ1/bromodomain protein interactions



carterra

cooperativity = α = (K_D^{binary} / $K_D^{ternary}$)

Key benefits of LSA^{XT} for PROTAC characterization

- No labeling of reagents to confound binding interactions
- Direct and quantitative measurement of detailed kinetics for up to hundreds of interactions in parallel
- Flexibility to include controls, replicates, or different conditions all in a single run
- Minimal analyte sample requirements from the one-on-many fluidic design
- Bidirectional flow allows for extended injection contact times

Takeaways

- Researchers now have two HT-SPR options depending on workflows and project needs
- LSA^{XT} enables researchers to further expand the capabilities of HT-SPR
  - Rapid kinetics
  - Small analytes
  - Low signals

Thank You!

carterra