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

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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 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. Over 80,000 binding interactions were measured during a 3-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 enabling thoughtful discovery of safe and efficacious drug candidates.

METHODS AND MATERIALS

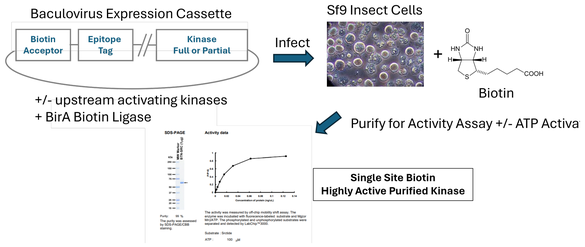

Figure 1A. Production Schema for Highly Validated Single Site Biotinylated Active Kinases from Carna Biosciences. Target kinases are cloned and expressed from baculovirus following infection of insect Sf9 cells. Activated, single site biotinylated kinases are purified using an epitope tag. Purity and 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.

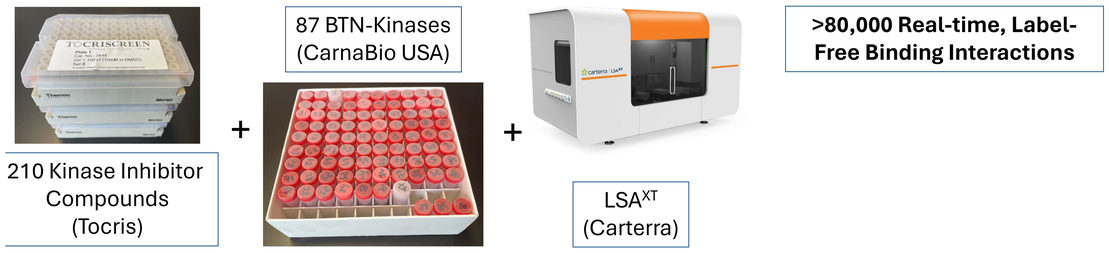

Figure 1B. Kinetics Workflow for Inhibitor Compounds Binding to a Kinase Array. Binding studies were performed at 15°C using the Carterra LSA^{XT} HT-SPR biosensor. Multiple densities of each kinase and off-target proteins (in HBS, 0.005% Tween-20, 5% glycerol, 0.5 mg/mL BSA, pH 7.4) were captured at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

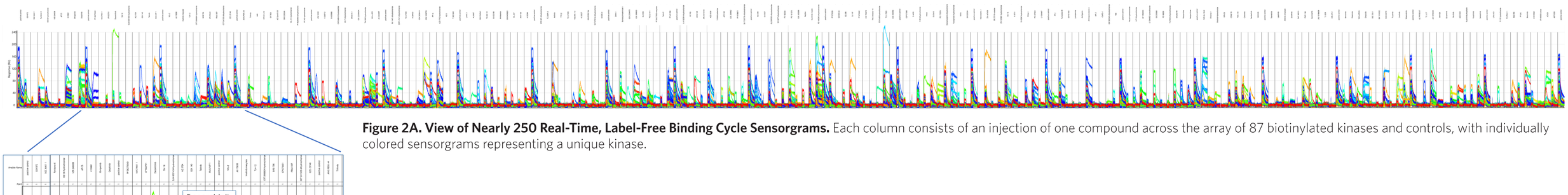

Figure 2A. View of Nearly 250 Real-Time, Label-Free Binding Cycle Sensorgrams. Each column consists of an injection of one compound across the array of 87 biotinylated kinases and controls, with individually colored sensorgrams representing a unique kinase.

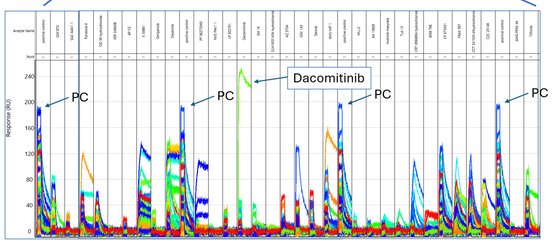

Figure 2B. Zoomed View of Select Binding Cycles. Positive control (PC) compound staurosporine was injected every 10 cycles to monitor activity across the array of biotinylated-Kinases. While many compounds displayed poor selectivity, dacomitinib is highlighted as an example of a compound shown to specifically bind with high affinity to a single kinase.

RESULTS

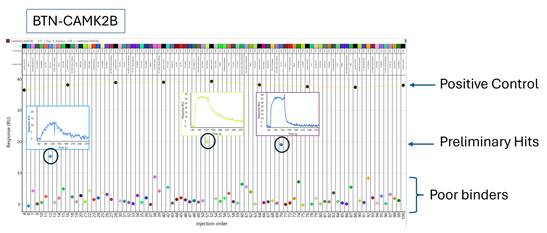

Figure 3. Carterra Kinetics Software Plot of Binding Responses for Compounds Against CAMK2B. The control compound staurosporine was injected at 10 uM while test compounds were screened at 1 uM with a 2 min association and 5 min dissociation. Repetition of the control compound shows consistency and durability of CAMK2B activity over time. Kinetic profiles for three compounds are highlighted as potential hits that may be valuable for further characterization.

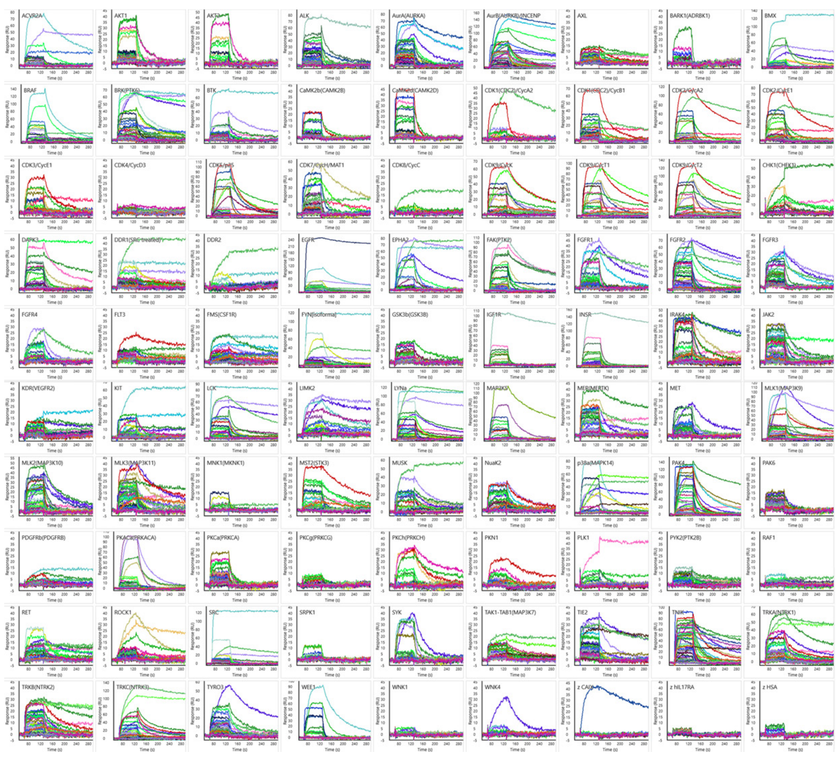

Figure 4. Tile Overlay View of Compounds Binding to 87 Kinases. Each tile plot corresponds to a single kinase, with each colored curve representing a distinct compound binding profile. A diverse range of rapid to very stable interactions are demonstrated. Three negative control protein surfaces are included in the bottom right. Binding to the negative control CAIX by a sulfonamide is consistent with known targeting of CAIX by this family of compounds.

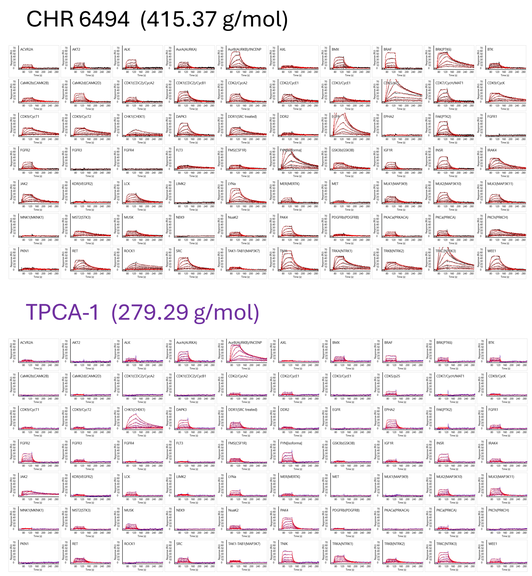

Figure 5. Detailed Kinetic Fingerprints of CHR 6494 and TPCA-1 Against Select Kinases. CHR 6494 binds across many kinases while TPCA-1 is much more selective in the kinases it recognizes. Beyond presence/absence of binding, the data describe the discrete kinetics of each interaction.

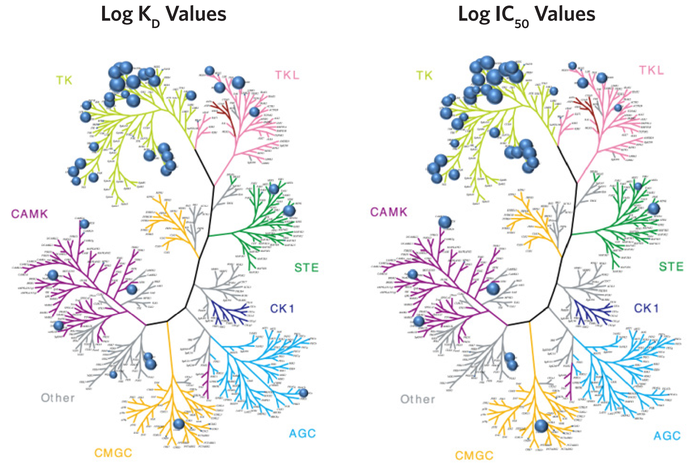

Figure 6. Orthogonal validation of K_D data from Carterra LSA^{XT} and IC_{50} values from Mobility Shift Assay with Sunitinib (SUTENT) treatment. Carterra kinetics at 1 uM (expressed as Log K_D) against CarnaBio BTN-kinases were compared to data from CarnaBio kinase profiling of Sunitinib using mobility 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 multitargeted tyrosine kinase inhibitor, and used in the clinic to inhibit PDGFR, FGFRs, VEGFR and PDGFR. The correlation of K_D determined on Carterra 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 on the LSA^{XT}
- 210 compounds were screened against 104 kinases over 3 days, of which 87 kinases showed activity against a library of kinase inhibitors and/or positive control staurosporine.
- 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 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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