



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.



Biotin Acceptor

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.

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

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



>80,000 Real-time, Label-Free Binding Interactions