From Fragments to Molecules to Approved Drugs: How the Carterra Ultra HT-SPR and CarnaBio's biotinylated kinome make kinase inhibitor discovery and optimization easy

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1. Abstract

Kinases remain an attractive drug discovery target class across many therapeutic areas. In order to dig deeper into this target class, various inhibitory mechanisms beyond ATP-competition are actively under investigation including covalency, allosteric inhibition, protein degradation, or partners for molecular glues.

Contrasting with simple activity assays, direct label-free approaches, such as SPR, can easily provide the affinity and intrinsic kinetics of interactions. These kinetic data may help to differentiate compounds and compound series, which may appear similar in activity assays but have distinct kinetics

In this poster we present Carna's biotinylated kinases and Carterra's HT-SPR LSA^{XT} and ULTRA instruments. Combined, these products enable exceptionally easy assay development, which are a result of the high quality biotinylated kinases, and exquisite sensitivity and multiplexing abilities of the instruments. Screens of low Mw fragments (over 125,000 data points in 3 days) and small molecule libraries (80,000 data points in 3 days), were performed, and that data and analysis is presented here.

We also show the value of SPR data, in addition to biochemical activity data and cellular data, early on in a drug discovery program. SPR from Carterra's HT-SPR instruments provide quality kinetic data, which may help guide programs in the direction of increased success at later stages, and ultimately in the clinic.

2. CarnaBio's Biotinylated Kinases

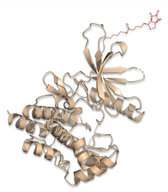


Figure 1A. A cartoon of a BTN-Kinase

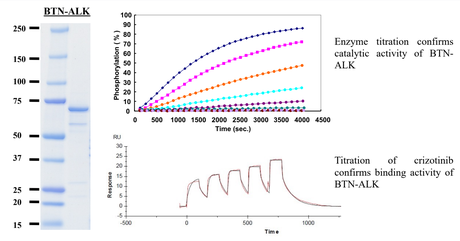


Figure 1B. Quality control: confirmation of BTN-kinase purity, and catalytic and binding activity

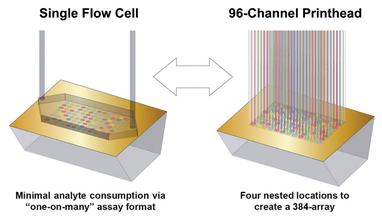
CarnaBio produces the BTN-kinases in Sf21 cells, with a single biotin moiety attached directly to a single lysine-containing linker and then a DYKDDDDK sequence connecting to the N- or C-terminal residue of the kinase. The kinases have their catalytic and binding activity confirmed, and their biotinylation ratio and purity established.

The advantages of using singly biotinylated kinases, rather than other methods, on SPR include:

- The BTN moiety is small, which does not interfere with kinase function, nor add significant mass to the kinase therefore aiding in analyte detection.
- The position of the BTN is specific to one specific residue on the kinase and minimizes any steric issues.
- Binding to a streptavidin chip, creates a homogenous protein surface making kinetic analysis simple
- The BTN/Streptavidin association is extremely tight: kinase does not leach off the surface during long runs.

Over 200 BTN-kinases are in the Carna catalog, with more being added frequently, including clinically relevant mutants.

3. Carterra's HT-SPR Systems, LSA^{XT} & ULTRA



Carterra's HT-SPR family members, the LSA^{XT} and ULTRA are designed (using proprietary single flow cell technology) to provide enhanced sensitivity and fast data collection rates allowing automated workflows in a single run. These runs can analyze interactions between proteins, PROTACs, small molecule inhibitors and fragments. Up to 384 ligands can be immobilized via a 96-channel printhead, and then analytes are flowed across all ligands via a single-flow cell, simultaneously capturing all the binding interaction.

Carterra LSA^{XT} HT-SPR
- Immobilize up to 384 ligands on surface
- Capture kinetics of up to 384 interactions in parallel, and in real time
- Uses 270 ul of analyte per 384-interactions
- Analyte low threshold 500 Da

Carterra ULTRA HT-SPR
- Immobilize up to 191 ligands on surface
- Capture kinetics of up to 191 interactions in parallel, and in real time
- Uses 180 ul of analyte per 191 interactions
- Analyte low threshold approx. 100 Da

4. Carterra LSA^{XT}: TOCRIScreen Kinase Inhibitor Library 3.0 Screen

Using the Carterra LSA^{XT} HT-SPR, 87 of CarnaBio's BTN-kinases and off-target proteins were captured to multiple densities at 384 locations on an SAD200M sensor chip (in HBS, 0.005% Tween-20, 5% glycerol, 0.5 mg/mL BSA, pH 7.4 @ 15° C). The Biotechne TOCRIScreen^{TM} Kinase Inhibitor 3.0 library (Cat. No. 7844) was screened at 1 uM (in HBS, 0.005% Tween-20, 5 mM $MgCl_2$, 5% glycerol, 1 mM DTT, 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.

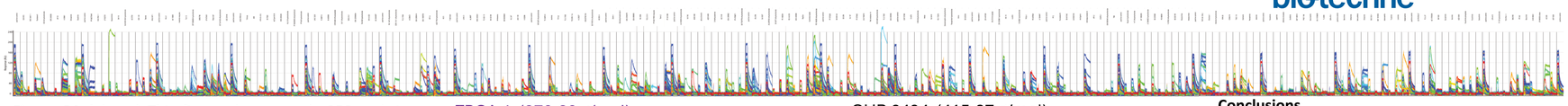


Figure 2A (above) This figure shows nearly 250 real-time, label-free binding cycle sensorgrams. Each column consists of an injection of one compound across the array of 87 biotinylated kinases and controls, with individually colored sensorgrams representing a unique kinase.

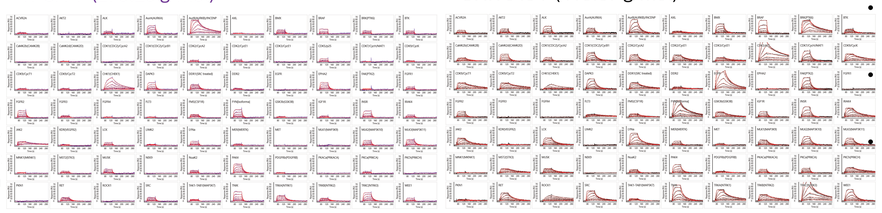


Figure 3 (right). Detailed Kinetic Fingerprints of CHR 6494 and TPCA-1 Against Select Kinases. CHR 6494 binds across many kinases while TPCA-1 is much more selective in the kinases it recognizes. Beyond presence/absence of binding, the data describe the discrete kinetics of each interaction.

Conclusions
- CarnaBio's BTN-kinases enabled easy assay development and show stability across the entire screen on the LSA^{XT}
- Screening of 210 compounds v 104 kinases generated over 80,000 unique interactions, in 3 days, providing a full profile of each compound.
- During the screen, LSA^{XT} measured quantitative kinetics of small molecules of 167 Da while requiring only 300 uL of each compound and ≈ 2 ug of each BTN-Kinase for the entire screen

5. Carterra ULTRA: 1000 Fragment Maybridge Ro3 Diversity Library Screen

The new Carterra ULTRA was used to capture over 100 of CarnaBio's BTN-kinases to the SAD200M chip at an approx. density of 10,000 RU per kinase. The Maybridge Ro3 core 1000 diversity fragment library was screened at 200 uM (in HBS, 0.005% Tween-20, 5 mM $MgCl_2$, 5% glycerol, 1 mM DTT, pH 7.4) in duplicate at 10 ° C, in one continuous experiment over 3 days. Over 125,000 interactions were recorded. Binding levels were normalized using positive control (AMPPNP) responses, and data was exported from Carterra Kinetics software to Datawarrior (https://openmolecules.org) for initial SAR analysis.

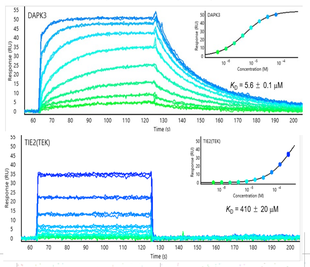


Figure 5 Triplicate AMPPNP dose responses reveal kinetics with a wide range of affinities.

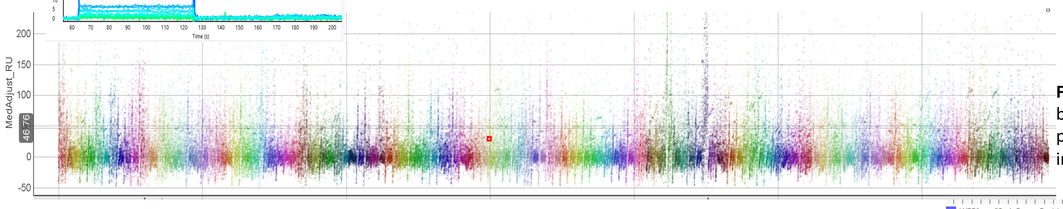


Figure 8 (right). The normalized responses, of approximately 50 fragments are shown in Carterra's analysis software for the kinase BTN-WEE1. Using in-house parameters, the hit threshold was assigned, and as shown, 4 fragments are highlighted as declared hits. The blue points at approx 40 RU at every 25 injections, represent binding of an AMPPNP control, and shows the stability and reliability of the BTN-WEE1 across the screen. In house automated software tools are undergoing development at Carterra, to accelerate correction, normalization and analysis of fragment binding data.

Figure 6 (right). Dose response of ADP as a control during screening shows reproducibility across time of screen for different kinases.

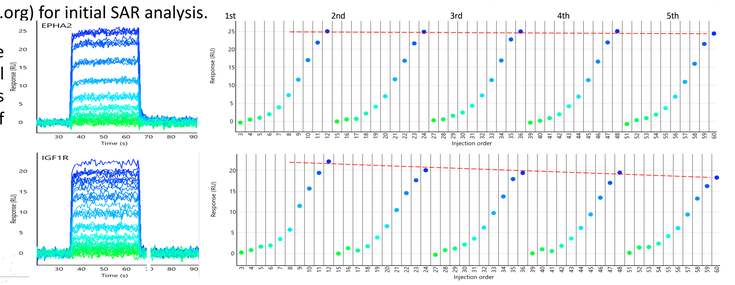
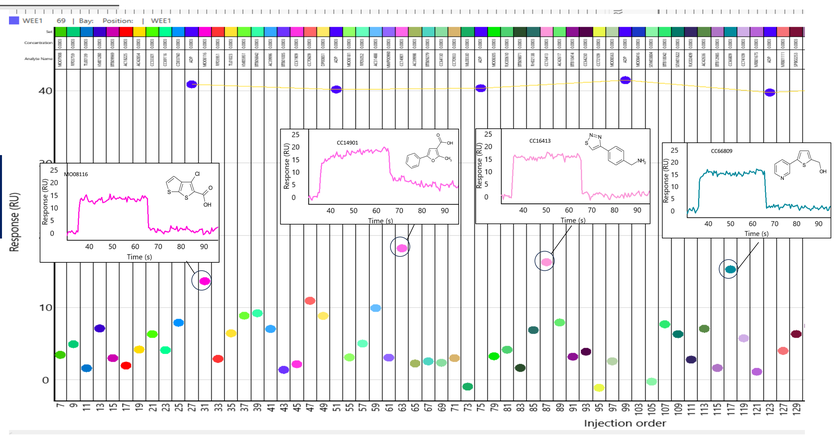


Figure 7 (left). A Datawarrior dashboard showing the corrected and normalized average binding response of each fragment to each kinase. Each kinase is represented as a single point, arranged with each fragment along the x-axis. The magnitude of the response of interaction is indicated on the y-axis. The coloration is for visualization purposes.



6. SPR Kinetic Parameters can Guide Cellular Activity

Successful library or fragment screens produce hits from different chemical scaffold series. These series may have different physical or ADME properties or different off-target profiles. Compounds of these series may show similar affinities (K_D) or activities (IC_{50}) appearing equivalent, but SPR is able to measure intrinsic rate constants (k_{on}, k_{off}) of interactions. This differentiates similar compounds at an early stage – before cellular assays or high [ATP] biochemical assays, where effects of these kinetic parameters may be revealed. This kinetic understanding helps to guide chemistry from an early stage, to maintain focus on what drives activity in a cellular, and potentially clinical context[1].

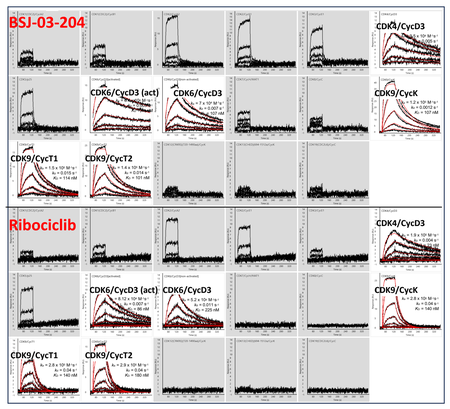


Figure 9. The binding data above show examples of dosed interactions of BSJ-03-204 (PROTAC) and Ribociclib (competitive inhibitor). Both interact with CDK4 & 6, and also with CDK9. Interactions with CDK4 and CDK6 contain noticeably slower k_{off} than with CDK9, even though the all share similar K_Ds.

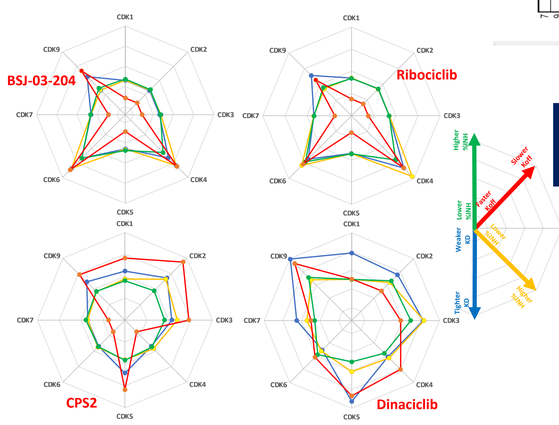


Figure 10. The activities of 2 CDK inhibitors and 2 CDK PROTACs against 8 BTN-CDKs and their cyclins were investigated using the Carterra Ultra (kinetics & K_D), 1 mM ATP activity assays, and NanoBRET^{TM} TE. The normalized data are shown in the logarithmic radar plots above and show a general trend. This is best exemplified by Ribociclib and BSJ-03-204: compounds may show a similar **K_D**, yet their efficacy in competitive environments (1mM ATP & NanoBRET^{TM}) correlates with slow off rates.

7. Reaching New Levels in Kinase Discovery

The combination of Carterra's HT-SPR instrumentation with CarnaBio's biotinylated kinases, allows kinase drug discovery to produce data at levels of quality and quantity as never seen before.

Carterra's ULTRA HT-SPR
- Detects fragments down to 100 Da
- Small sample sizes for analytes and ligands
- Can run unattended for multiple days
- Sensitivity enables screening campaigns to produce actionable affinities *and* kinetic data
- Multiplexing produces quantities of data on a scale never seen before
- Various surface chemistries available

CarnaBio Sciences's BTN-Kinases
- Single biotinylation at N- or C-terminus.
- Produce a homogenous surface
- Shown to be stable and reliable for over 3 days of continuous analysis
- Quality control guarantees activity
- Easy and quick assay development
- Over 200 available, including mutants and different activation states
- New kinases added continuously

To view this, or other Carterra/CarnaBio posters, please scan the QR codes above. For more information, email info@carnabio.com or questions@carterrra-bio.com

Reference: [1]'The drug-target residence time model: a 10-year retrospective', Robert A. Copeland, *Nature Reviews Drug Discovery*, 2016.