Profiling Biotinylated CDK/Cyclin Complexes Using HT-SPR Compared to Biochemical (MSA) and Cell-Based NanoBRET™ Assays

John Rosenfeld[1], Adam Shutes[1], Takeomi Inoue[2], Tomohito Fujimoto[2], Rebecca Rich[3], Tony Gianetti[3] , Chris Silva[3], Yusuke Kawase[2]

[1] CarnaBio USA, Inc., 209 West Central Street, Suite 307, Natick, MA 01760, USA
[2] Carna Biosciences, Inc., BMA, 1-5-5, Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan
[3] Carterra, 825 N. 300 W. Ste. C309, Salt Lake City, UT 84103

Abstract

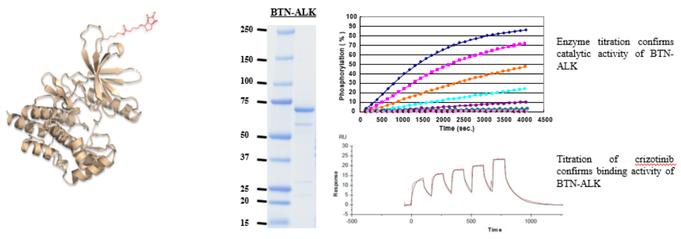
Measurement of inhibitor binding kinetics can play an important role in guiding medicinal chemistry efforts in the early stages of kinase drug discovery programs. In this study, we present a detailed analysis of four compounds, (dinaciclib, ribociclib inhibitors, and CPS2 & BSJ-03-204 PROTAC) binding activity using HT-Surface Plasmon Resonance (SPR) capillary Mobility Shift and cell based NanoBRET assays. By integrating both biochemical and cell-based binding kinetics data, we provide a comprehensive understanding of these compound interactions with their target kinases.

Cyclin-dependent kinases (CDK) are enzymes critical for cell cycle regulation. Their inhibitors, such as dinaciclib, show potential as therapeutics in oncology (with several already approved). In simple biochemical assays, Dinaciclib inhibits select CDK family members, including CDK2, CDK5, CDK1, and CDK9. It has also been shown to cause the inhibition of cell cycle progression and proliferation in various tumor cell lines. Dinaciclib is being evaluated in clinical trials for various cancer indications, including chronic lymphocytic leukemia (CLL), multiple myeloma, and non-small cell lung cancer, although has yet to receive FDA approval as a single agent therapeutic.

HT-SPR assays were employed to determine the real-time binding kinetics and affinity of dinaciclib to purified CDK:cyclin complexes. These biophysical assays revealed differences in KD for the different CDKs, with CDK5 & CDK9, showing increased affinity over CDK2 and CDK1, driven mostly by their slower off-rate. Combining HT-SPR kinetics with NanoBRET residence time analysis suggests various modes of distinct behavior of the on/off rates depending upon the target evaluated. For ribociclib, high affinity binding to CDK9 was observed in SPR that was not replicated when performing biochemical assays at 1 mM ATP. The combined data of all 4 compound challenges on these assay platforms are presented.

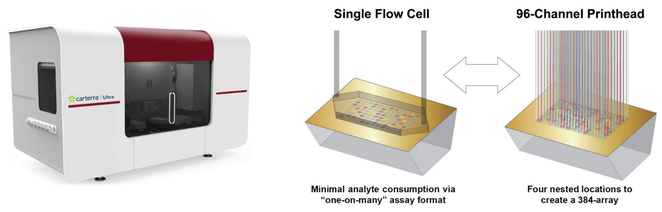
Materials and Methods

1) Biotinylated kinases



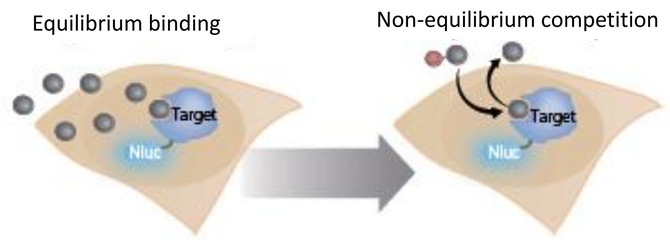
Single-site biotinylated kinases were produced by Carna Biosciences. Briefly, baculoviruses co-expressing relevant kinase and cyclin partners with in-cell biotinylation of an epitope tag was performed in insect Sf21 cells. Following purification, enzyme activity is assessed using a mobility shift assay (MSA). Multiple CDK family members were profiled by HT-SPR, MSA biochemical assay, as well as NanoBRET™ IC_{50} and residence time measurement.

2) Carterra Ultra™ HT-SPR



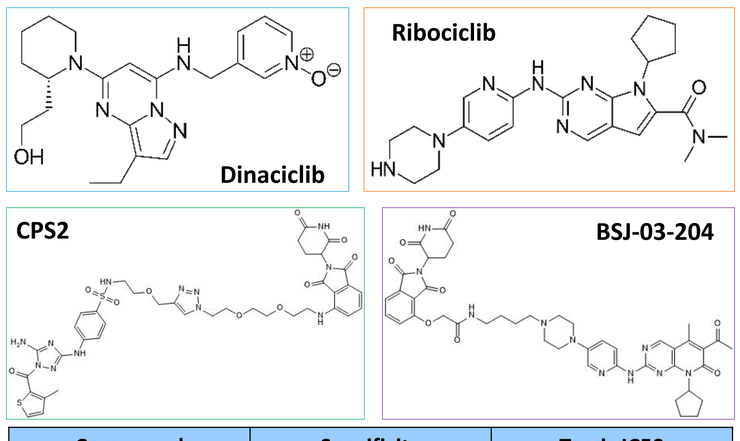
- Immobilize up to 191 ligands on surface
- Capture kinetics of up to 191 interactions in parallel, and in real time
- Uses 180 ul of analyte per 191 interactions
- Analyte low threshold approx. 100 Da
- Applied 2-5 ug kinase per feature

3) NanoBRET™ Target Engagement Intracellular Kinase Assay



The NanoBRET™ TE Intracellular Kinase Assay utilizes BRET in living cells by molecular proximity of the NanoBRET™ Tracer to the NanoLuc® luciferase-fused kinase. After equilibrating with a near-saturating concentration of compound, the cells are washed to remove unbound compound and treated with a near-saturating concentration of a cell-permeable tracer. BRET is measured repeatedly in a luminometer to observe tracer association as a proxy for compound dissociation.

4) Tocris Compounds



Compound	Specificity	Tocris IC50
Dinaciclib	CDK1, 2, 5, 9	3, 1, 1, 4 nM
Ribociclib	CDK4/6	306, 416 nM
CPS2	CDK2 PROTAC	24 nM
BSJ-03-204	CDK4/6 PROTAC	NA

Results

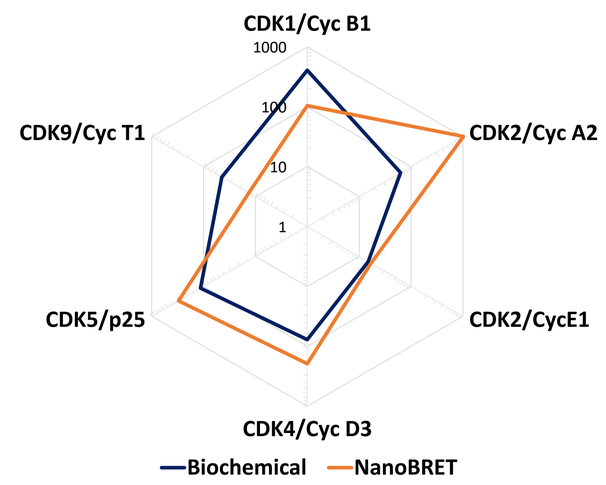


Figure 1. Intracellular potency of dinaciclib against CDK2/CycA2 reduces dramatically in comparison to the result for biochemical assay. Half-maximal inhibitory concentrations (IC_{50}s, nM) were plotted for both biochemical and cell-based assay (NanoBRET™). Electrophoretic mobility shift assay was used to obtain the IC_{50}s of dinaciclib against 6 CDK/cyclin complexes in the presence of 1mM ATP as a biochemical assay, while the NanoBRET™ assay was performed to evaluate intracellular target engagement of the compound.

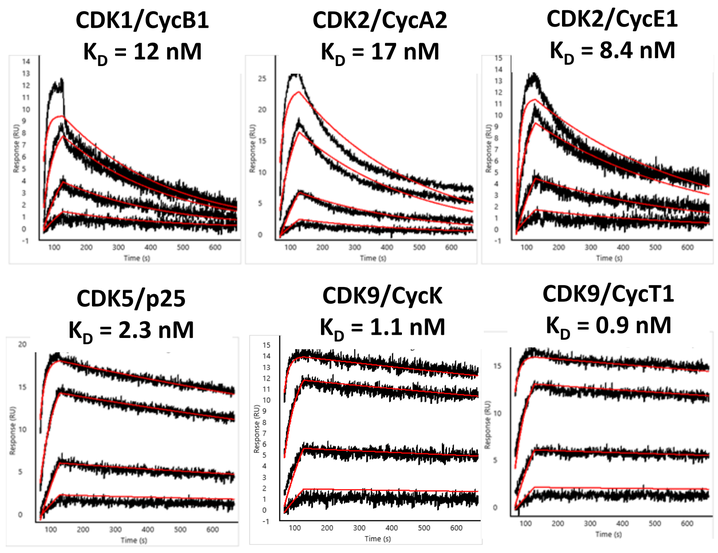


Figure 2. Representative SPR sensorgrams for different CDK/cyclin complexes reveal marked difference in binding kinetics of dinaciclib. The binding experiments were performed in single-cycle kinetic format using the Carterra Ultra.

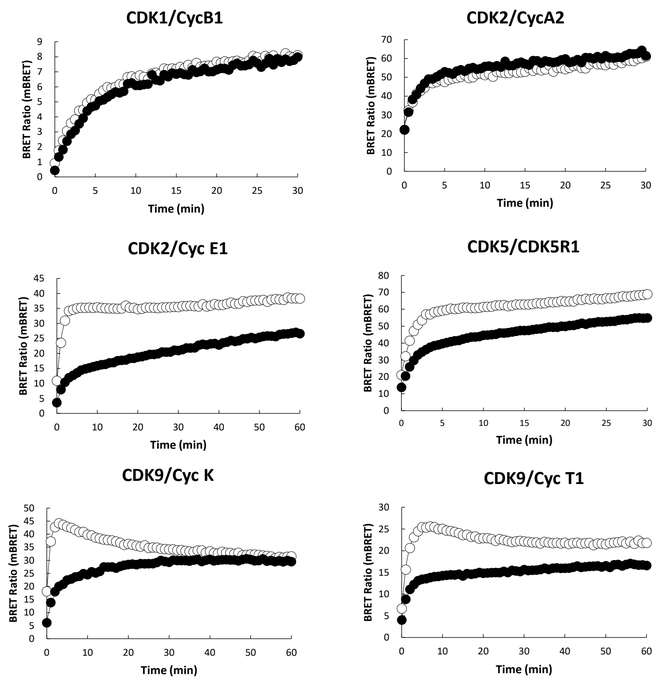


Figure 3. Dinaciclib dissociates rapidly from CDK1/CycB1 and CDK2/CycA2 comparing to other CDK/cyclin complexes including CDK2/CycE1 in live cells. HEK293 cells expressing different CDK/cyclin complex were pre-incubated with a near-saturating concentration of dinaciclib for 2 hours followed by a brief wash. The NanoBRET™ tracer was added immediately and the BRET was repeatedly measured using the GloMax Discover (open circle: DMSO control, closed circle: dinaciclib).

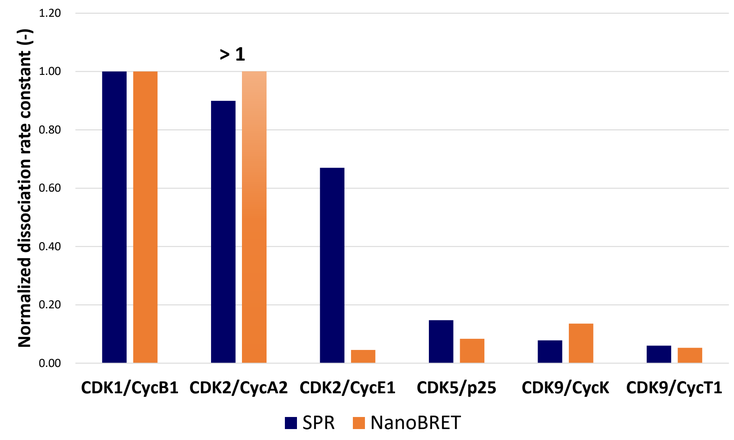


Figure 4. Intracellular kinetic study unveils extremely slow dissociation of dinaciclib from CDK2/CycE1 in live cells. Normalized dissociation rate constants (normalized k_ds) for different CDK/cyclin complexes relative to the k_d for CDK1/CycB1 were plotted. The k_ds for CDK1/CycB1 in the SPR and NanoBRET™ study were $3.09x10^{-3}$ (1/sec) and $6.18x10^{-3}$ (1/sec), respectively. Note that the k_d for CDK2/CycA2 in the NanoBRET™ experiment was expected to be larger than that for CDK1/CycB1, since the dissociation of dinaciclib from CDK2/CycA2 was too fast to calculate its k_d.

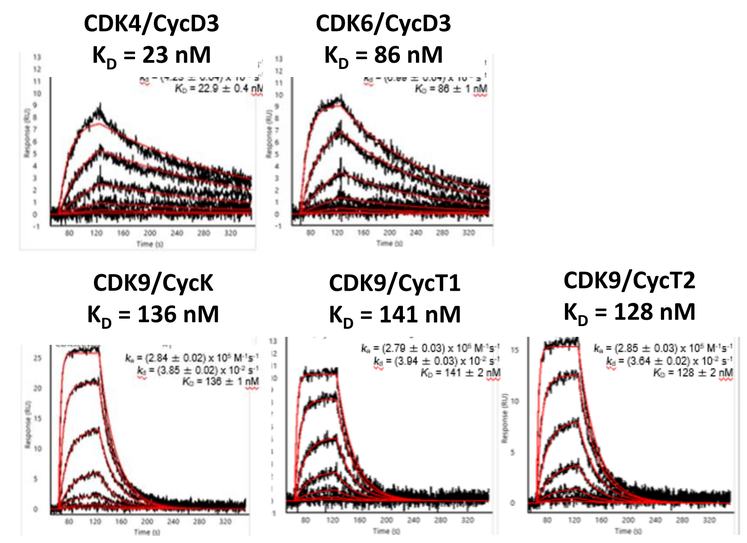


Figure 5. HT-SPR Profiling of ribociclib on Multiple CDK/cyclin complexes. Ribociclib, a CDK4/6 inhibitor demonstrates high affinity binding to CDK9, although it doesn't show any binding to CDK1, CDK2 and CDK5 (data not shown).

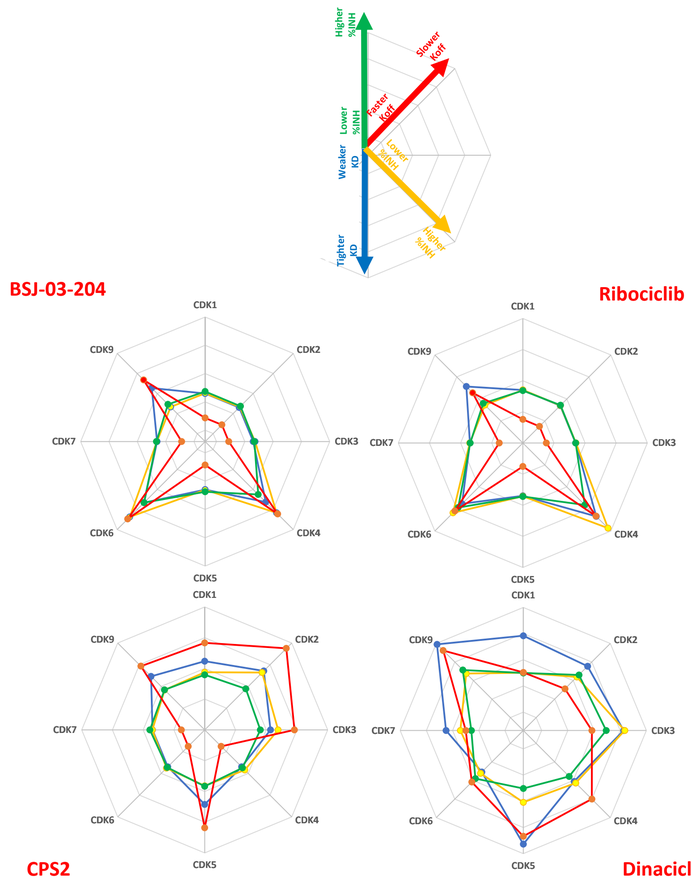


Figure 6. The activities of 2 CDK inhibitors and 2 CDK PROTACs against 8 BTN-CDKs and their cyclins were investigated using the Carterra Ultra (kinetics & K_D), 1 mM ATP activity assays, and NanoBRET™ TE. The normalized data are shown in the logarithmic radar plots above and show a general trend. This 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™) correlates with slow off rates.

Conclusions

1. **Generally, the dissociation rate constant of dinaciclib obtained in SPR experiment and intracellular target engagement assay agrees well and rapid dissociation from cell-cycle CDKs (CDK1 and CDK2) are shown compared to that from non-cell cycle CDKs (CDK5 and CDK9).**
2. **Dinaciclib shows extremely slow dissociation from CDK2/CycE1 in live cells, possibly due to dynamic cyclin interaction within the cells distinct from CDK2/CycA2, a feature only observable with the live cell assay.**
3. **ATP concentration influences the in vitro interaction of ribociclib with CDK9 complexes.**