Intracellular Engagement for Clinically Relevant Inhibitors and PROTACs Across the CDK Family Using NanoBRET

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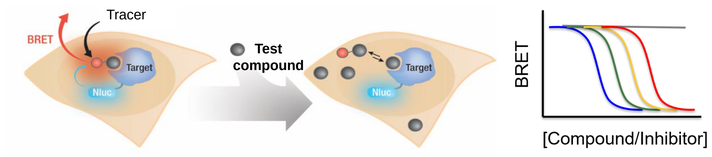
1. Introduction

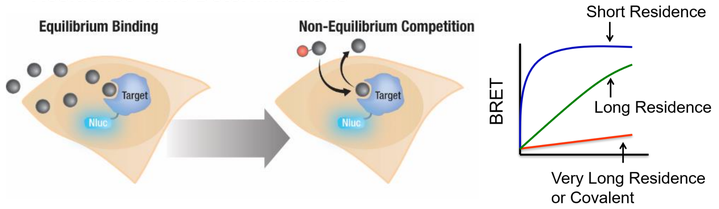
The NanoBRET™ target engagement (TE) technology enables quantitative assessment of target occupancy and residence time for kinase inhibitors and PROTACS in living cells. We have applied this technology to systematic interrogation of kinase target engagement. Cyclin-dependent kinases (CDKs) represent a key opportunity to apply this technology, as CDKs play key roles in diverse cellular functions including cell cycle control, cell proliferation, and transcriptional regulation. The recent clinical successes of CDK inhibitors has helped fuel interest in development of new CDK inhibitors as well as heterobifunctional degraders (PROTACs). The NanoBRET TE technology was used to develop a panel of >30 specific CDK / cyclin or CDKL assays in live cells, allowing a real-time biophysical method to quantitatively assess kinase-inhibitor binding and residence time under physiological conditions. This panel of CDK cellular assays enabled compound potency and selectivity to be determined for type I & II inhibitors. In addition, cyclin dependent effect on inhibitor cellular potency was demonstrated. NanoBRET TE live cell assays were able to quantitate binding of a promiscuous kinase PROTAC to several CDKs. An assessment of kinetic selectivity of compounds with similar cellular equilibrium affinity for CDK9-cyclin T1 revealed the compounds displayed different cellular residence times.

2. Target Engagement (TE) using NanoBRET

Affinity / Potency Determinations

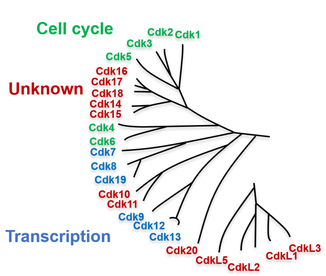


Residence Time Determinations



3. Cyclin-Dependent Kinase Family

CDK Family



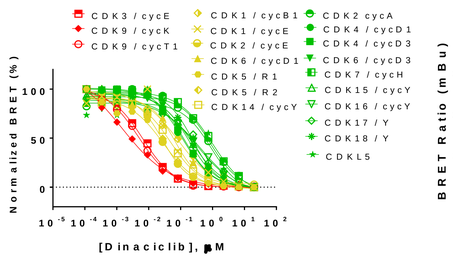
NanoBRET TE Cellular CDK Assays

CDK1 + Cyc B1	CDK5 + CDK5R1	CDK14 + Cyc Y
CDK1 + Cyc B2	CDK5 + CDK5R2	CDK15 + Cyc Y
CDK1 + Cyc E1	CDK6 + Cyc D1	CDK16 + Cyc Y
CDK1 + Cyc K	CDK6 + Cyc D3	CDK17 + Cyc Y
CDK2	CDK7	CDK18 + Cyc Y
CDK2 + Cyc A1	CDK7 + Cyc H	CDK19 + Cyc C
CDK2 + Cyc A2	CDK8 + Cyc C	CDKL1
CDK2 + Cyc E1	CDK9 + Cyc K	CDKL2
CDK3 + Cyc E1	CDK9 + Cyc T1	CDKL3
CDK4 + Cyc D1	CDK10 + Cyc L2	CDKL5
CDK4 + Cyc D3	CDK11A + Cyc K	
CDK5	CDK11A + Cyc L2	

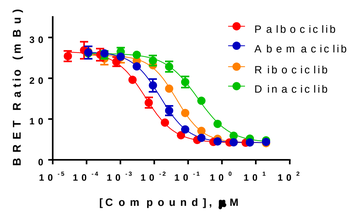
- Over 20 CDKs and CDK-like proteins comprise the human kinome and play key roles in cell cycle control and transcriptional regulation. (left)
- The CDK activity is tightly regulated by interactions with numerous intracellular cyclins, resulting in many unique CDK-cyclin kinase pairs.
- By introducing exogenous cyclin with NanoLuc-CDK fusions, NanoBRET TE cellular assays have been developed for >30 specific CDK-cyclin pairings or CDKLs. (right)

4. Cellular Compound Potency for CDK Family using Numerous Cyclin Pairings

A. Representative CDK NanoBRET TE Assays

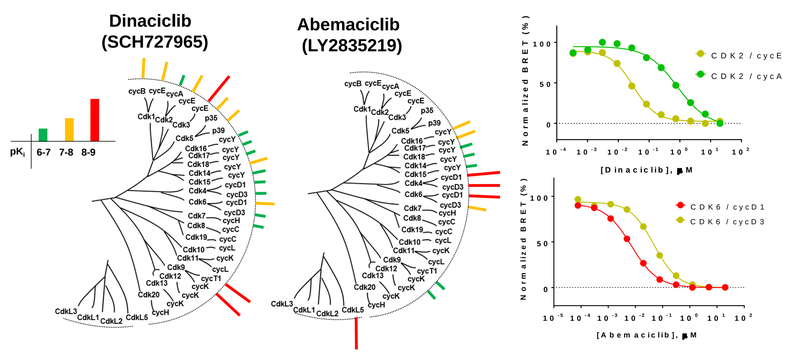


B. CDK6 / Cyclin D1 NanoBRET TE Assays



- Dinaciclib is a CDK inhibitor in clinical trials with FDA orphan drug status. Potency determined for 20 CDK / cyclin pairs or CDKL using NanoBRET TE assays. CDK-NanoLuc fusion expressed with an appropriate cyclin in HEK293 cells. (A)
- Cellular potency of Dinaciclib and clinically relevant CDK inhibitors Abemaciclib (Verzenio®), Palbociclib (Ibrance®), & Ribociclib (Kasqali®), approved for breast cancer, for CDK6 / cyclin D1. (B)

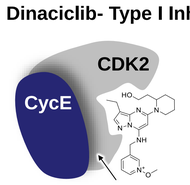
5. Intracellular CDK Selectivity and Cyclin Bias for Clinical Drugs



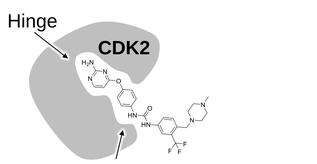
- Cellular selectivity profile of Dinaciclib identifies primary targets of CDKs 1 - 6 & 9, as well as binding to several cyclin Y CDKs (CDKs 14-18). (left)
- Cellular selectivity profile of the selective CDK inhibitor Abemaciclib identified the primary targets of CDKs 4 & 6. It also binds to CDKs 14-18. (center)
- Dinaciclib & Abemaciclib intracellular potencies can depend on cyclin pairing. (right)

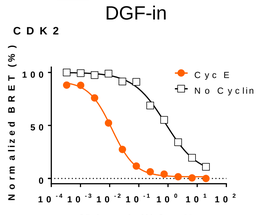
6. Intracellular Potency of CDK Type I & II Inhibitors

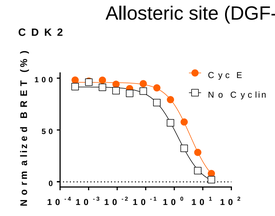
Dinaciclib- Type I Inhibitor



K03861 – Type II Inhibitor

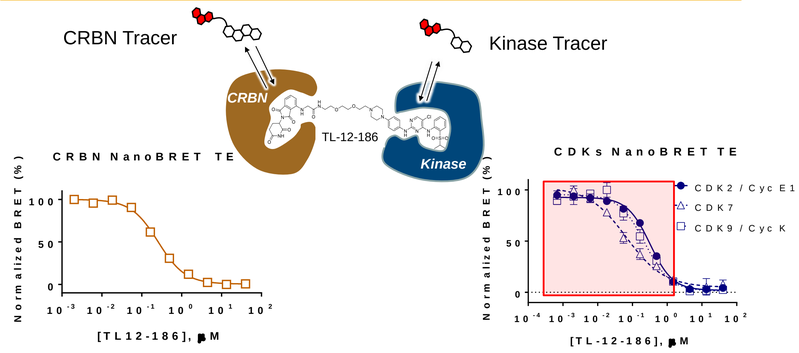






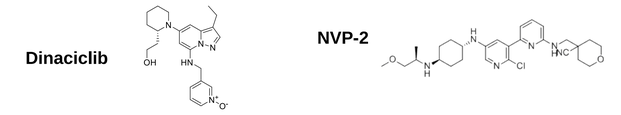
- NanoBRET TE cellular assays enable the study of specific CDK-cyclin pairings by co-expression of CDK and cyclin pairs
- Higher intracellular potency for Dinaciclib with CDK2 / Cyclin E compared to CDK2 (lacking exogenous cyclin expression) was observed. (left)
- No cyclin bias observed with the type II inhibitor K03861 that binds CDK2 in DFG-out conformation associated with catalytically inactive kinase. (right)

7. Kinase PROTAC Target Engagement at CRBN and CDKs Can Be Quantified via NanoBRET

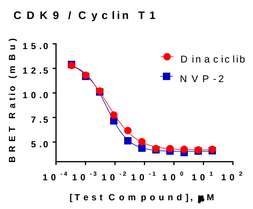


- TL-12-186 is a promiscuous kinase inhibitor conjugated to a cereblon (CRBN) E3 ubiquitin ligase ligand (pomalidomide).
- Intracellular binding of TL-12-186 to CRBN was quantified via CRBN NanoBRET TE assay. (left, IC_{50} = 0.3uM)
- Intracellular binding of TL-12-186 to CDK2, CDK7, & CDK9 was quantified via NanoBRET TE. (right, IC_{50} 0.07 – 0.3uM)

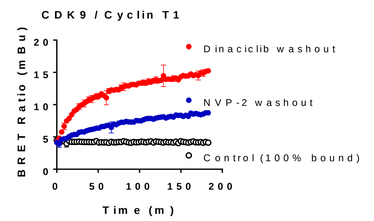
8. Utilizing Cellular Affinity & Residence Time Measurements to Characterize Compounds



A. Equilibrium Binding



B. Residence Time



- NVP-2 is CDK9 selective, while Dinaciclib inhibits several CDKs. NanoBRET TE showed both compounds had similar cellular affinity for CDK9 / cyclin T1 (A).
- Despite the similar intracellular affinity, NVP-2 has a longer intracellular residence time compared to dinaciclib, demonstrating that affinity and residence time don't always correlate (B).

9. Conclusions

NanoBRET TE assays broadly enable the quantitative determination of compound affinity/potency for specific targets inside cells

- Cell permeable NanoBRET Tracers have been developed that allow TE assays for >200 full length kinases
- For the CDK family, a suite of >30 NanoBRET TE kinase enable interrogation of inhibitor potency against different CDK / cyclin pairings

Cellular potency of various kinase inhibitors types is measured using NanoBRET TE assays

- For CDKs, type I & II inhibitor cellular potencies have been determined.
- For other kinases, NanoBRET TE has been used to quantitate type I, II and allosteric compounds cellular potency.

Residence time for specific kinases in live cells is measured with NanoBRET TE

- Using both equilibrium & residence time methods, kinetic selectivity may be revealed- offering unique inhibitor development opportunities.

PROTACs cellular permeability and potency for E3 ubiquitin ligases CRBN and VHL can be quantified via NanoBRET TE assays

- NanoBRET TE assays can be run in live and lytic mode to assess cellular permeability of PROTACs.

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