# Intracellular Target Engagement Enabled by NanoLuc Luciferase Chemistries

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**Abstract #2127**

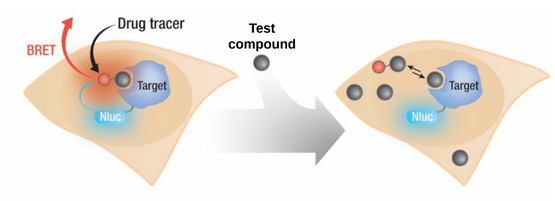
## 1. Introduction

The physical binding of a drug with cellular targets is known as target engagement. Measuring target engagement in a cellular context is an important part of the drug discovery process, as it can address compound cell permeability, equilibrium binding affinity, as well as kinetic binding rates of compound binding and dissociation. However, assessing target engagement in a cellular context has been challenging. We present a biophysical method to directly measure target engagement within intact mammalian cells using bioluminescence energy transfer (BRET).

Four key components are needed:
- Cellular expressed target-NanoLuc fusion
- Cell-permeable fluorescent drug tracer
- Substrate for NanoLuc (Furimazine)
- Extracellular NanoLuc inhibitor

## 2. Target Engagement using BRET Measurements

BRET is achieved by the luminescent energy transfer from luciferase to the proximal fluorescent tracer that is bound to the target protein-luciferase fusion. The BRET assay specificity is governed by tight distance constraints.
Test compounds that are applied to the cells and specifically engage the intracellular target protein-luciferase fusion displaces the tracer and results in a decrease in BRET.




## 3. Assay Components

**Target**
- Protein of interest with NanoLuc fusion
- Known ligand or inhibitor

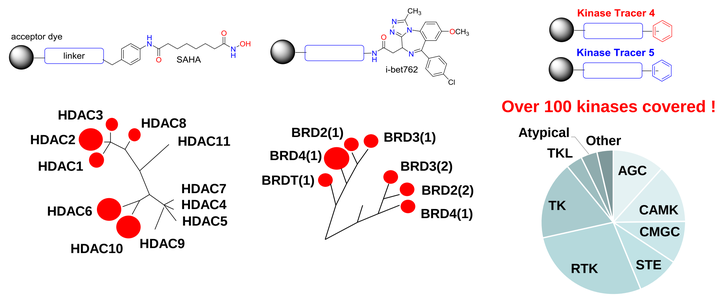
**Tracer**
- Cell-permeable fluorescent tracer
- Available from Promega or can be custom made for novel targets

**Substrate for NanoLuc**
- Furimazine reacts with NanoLuc inside cell
- Emits blue light (450 nm), energy transfer to fluorescent dye (>600 nm)

**Extracellular NanoLuc inhibitor**
- Cellular debris is commonly observed in populations of rapidly-dividing live cells in culture
- Extracellular NanoLuc inhibitor ensures BRET measurements originate only from inside intact cells

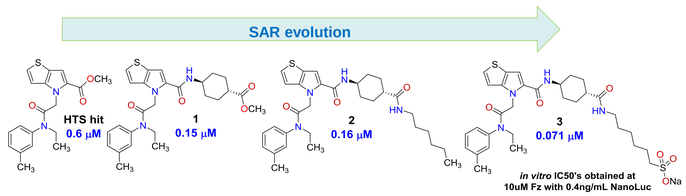
## 4. Tracer Development

- Broad coverage ligand-dye tracers have been developed
- SAHA for HDAC; IBET for Bromodomains; Kinase tracers 4 and 5

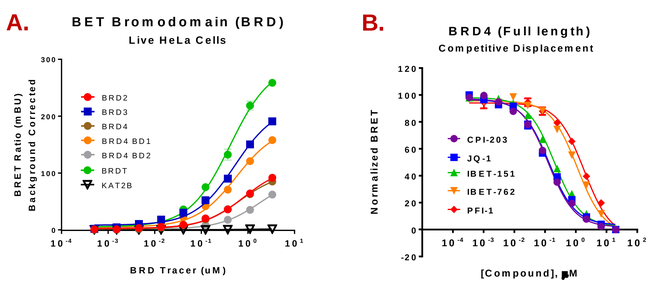



## 5. Extracellular NanoLuc Inhibitor For "Live-Cell Only" NanoLuc Detection

- Elaboration of HTS hit yielded potent, cell-permeable inhibitors
- Selective and competitive inhibition ($IC_{50}$'s of <2nM obtained)
- Appending polar groups at the end of the alkyl chain affects permeability
- Quenches NanoLuc signal from damaged, leaky cells but not healthy cells
- Inhibitor **3** has high water solubility (>1 mM) and no toxicity (CellTiter-Glo Viability Assay)

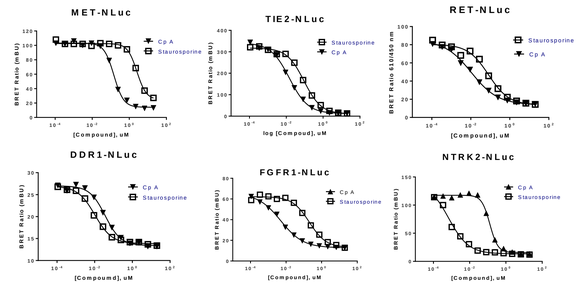



## 6. Profiling Compound Binding to BET Bromodomains in Live Cells via BRET



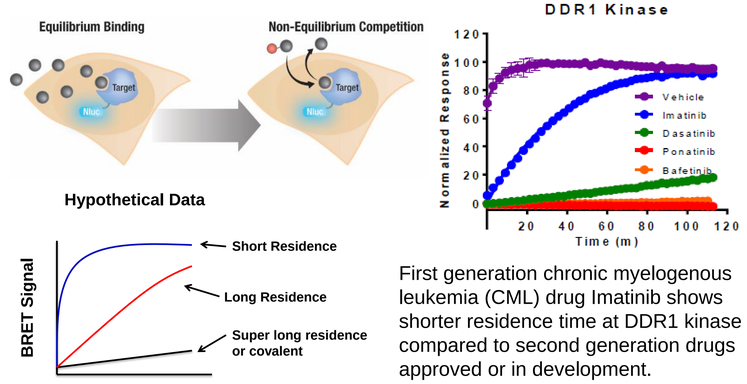

**Panel A.** The BRD tracer engages full-length Bromodomain proteins BRD2, BRD3, BRD4, and BRDT expressed in Hela cells. In addition it directly binds to BRD4 Domain 1 (BD1) and Domains 2 (BD2). **Panel B.** Using a competitive displacement experiment, BRD4 inhibitors were profiled for binding to BRD4 full length protein in HeLa cells. By profiling against other BRDs, compound selectivity of compounds can be determined.

## 7. NanoBRET Tracers Enable Live-Cell Assays for Multiple Classes of Full-Length Kinases Including 37 RTKs




Cell-permeable kinase tracers enable >100 kinase target engagement assays spanning multiple kinase families. Representative in-cell competitive displacement data is shown for several full-length receptor tyrosine kinases. These enable assessment of compound permeability and affinity.

## 8. Monitoring In-Cell Residence Time




First generation chronic myelogenous leukemia (CML) drug Imatinib shows shorter residence time at DDR1 kinase compared to second generation drugs approved or in development.

## 9. Conclusions

**NanoBRET enables direct physical measurement of target engagement in live cells**
- Cell-permeable fluorescent tracers and an extracellular NanoLuc inhibitor ensure BRET measurements arise from inside the cell

**Intracellular affinities of test compounds against proteins from various target classes can be measured in live cells**
- We have developed live-cell BRET target engagement assays for numerous kinases, bromodomains, and HDACs

**Intracellular target residence time can be measured with NanoBRET assays**
- Fast binding tracers can enable real-time analysis of test compound dissociation rates
- Measurement of compound-target binding dynamics is made possible by the long signal half-life of NanoLuc



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