NanoBRET Target Engagement and Acoustic Dispensing Technologies for Drug Screening

Gediminas Vidugiris[1], Matt Robers[1], Tracy Worzella[1], Chad Zimprich[1], Cristopher Cowan[1] and Michael Forbush[2]

[1]Promega Corporation, 2800 Woods Hollow Rd., Madison, WI 53711, USA; [2]EDC Biosystems 49090 Milmont Dr. Fremont, CA 94538

Abstract # 5000

1. Introduction

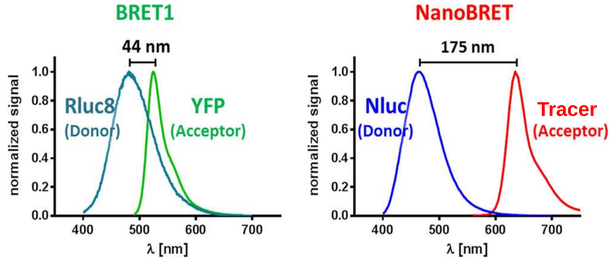
Chemical probes are critical tools for revealing the biological functions of proteins and can lead to new medicines for treating disease. The pharmacological validation of protein function requires verification that chemical probes engage their intended targets in vivo. However, no methods currently exist to quantitatively assess chemical probe affinity or binding kinetics to select target proteins inside intact cells.

The new NanoBRET Target Engagement assay provides a powerful platform to determine compound engagement with an intracellular protein target in a native cellular environment.

We describe the use of acoustic dispensing technology to improve the work flows for the NanoBRET Target Engagement. Acoustic dispensing facilitates cell transfection and NanoBRET reagents delivery, enabling a simple approach to profiling target engagement for protein kinases within intact cells in high density plates.

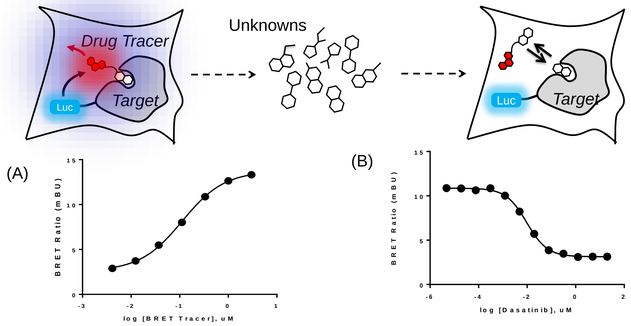
2. NanoBRET Principles and Advantages

(Nano)**B**ioluminescent **R**esonance **E**nergy **T**ransfer



- Energy transfer from bioluminescent donor to fluorescent acceptor resulting in emission of light.
- Allows measurement of target engagement with binding partners in cells.
- Other BRET assays are substantially limited by low light output and resolution.

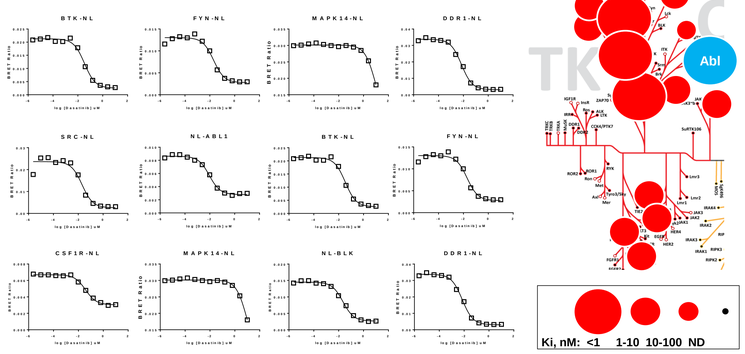
3. NanoBRET Cell-Based Target Engagement Assay



Titration of a cell-permeable NanoBRET kinase tracer (A) or dasatinib (B) onto cells expressing NanoLuc-Abl kinase results in BRET dose-dependent increase (A). Incubation of live cells with a different concentrations of dasatinib results in competitive displacement of the tracer and in a dose-dependent decrease in BRET.

4. Profiling Dasatinib Against Cytosolic Kinases and RTKs in Cells

Binding of dasatinib to Abl1 target is potent but not selective.



5. Nanoliter Acoustic Dispenser ATS from EDC Biosystems

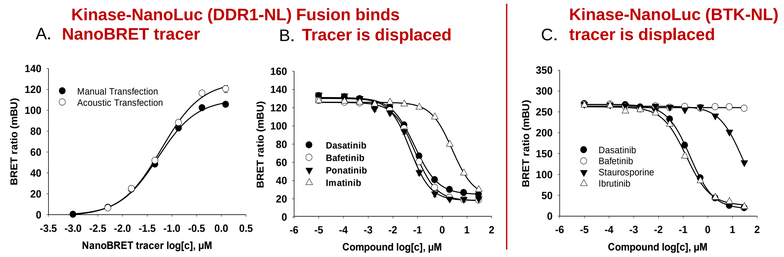
Acoustic dispensing uses sound waves focused on the surface of a liquid to generate precise and accurate droplets in the range of 1nl to 25nl volumes. Multiple ejection of these droplets constitutes a total dispense. These droplets are directed into an inverted well plate where they adhere to the bottom of an empty well or fuse with liquid already in the well.

Acoustic Dispensing Advantages

- Accurate and precise nanoliter transfers
- Low dead volumes
- Dispense range from 1nl to 1μL
- Variable droplet size allows for optimization of speed or fine volume resolution.
- Easy to automate
- Lasting precision and performance
- Open platform for application flexibility
- Wide variety of plates

6. NanoBRET Target Engagement Titrations: Compound Binding or Tracer Displacements

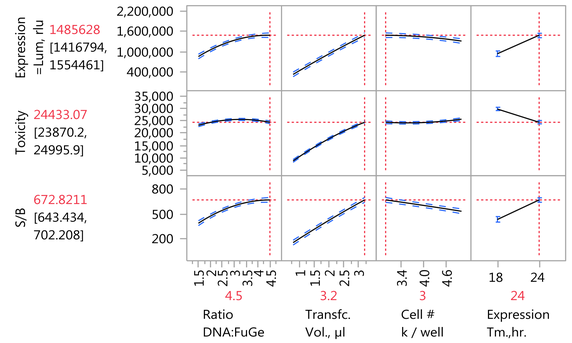
The ATS can be used for acoustic transfer of tracers, compounds, and furimazine substrate



A. NanoBRET kinase tracer was acoustically titrated into cells expressing DDR1 kinase. B and C. Cells expressing DDR1 or BTK targets were dosed with unlabeled compounds of interest in the presence of NanoBRET kinase tracer and furimazine substrate. Results show expected rank order potency for the respective targets. BRET was measured with a GloMax Discover.

8. DOE Accelerates Transfection for Proteins Expression Optimization. Prediction Profiler

Design of Experiment approach and ATS acoustic dispenser streamlines optimization of cell transfections for protein expression in live cells

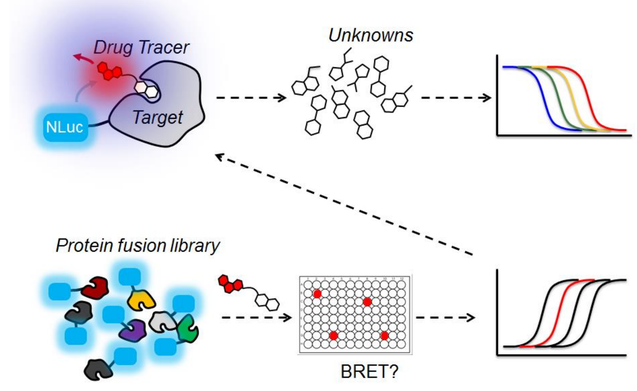


Protein Expression increase with:

- Transfection mix volume increase
- Increase of DNA:FuGENE ratio
- Transfection time

Toxicity evaluated by CellTox-Green Cytotoxicity Assay

8. Acoustic Dispensing Could Enable Profiling of Many Kinases in a Scalable Format



9. Conclusions

NanoBRET is the only method enabling quantitative measurements of compound affinity at selected targets inside live cells.

New NanoBRET technology demonstrates increased detection sensitivity and dynamic range.

Acoustic dispensing benefits:

- Improves workflow of selective target proteins expression in HTS formats for drug screening
- Facilitates dispensing of reagents and titration of compounds from stock and pre-diluted solutions.

Corresponding author: gediminas.vidugiris@promega.com