NanoBRET™ Assays for Monitoring Protein Interactions in Living Cells

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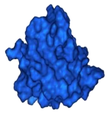
1. Introduction

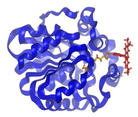
Identifying small molecule modulators, inhibitors or activators, of protein-protein interactions (PPI) remains challenging, largely due to the difficulty of developing robust, high-throughput screening tools that can used to interrogate these interactions within a biologically relevant context. Bioluminescent resonance energy transfer (BRET) has been used to monitor real-time protein:protein interactions in live cells, but current approaches suffer from limited sensitivity and narrow dynamic range. Here we present a new BRET method, termed NanoBRET, based on a small and extremely bright NanoLuc luciferase coupled to a HaloTag - long-wavelength fluorophore. This highly effective energy donor-acceptor combination boosts BRET performance, and the higher sensitivity facilitates application of the method in high density plates and high throughput screening. Here we present several examples of protein interaction biology that can be interrogated with NanoBRET including epigenetic interactions, adaptor recruitment to membrane receptors, and proteasomal recruitment of proteins targeted for degradation.

2. NanoBRET Assay Overview



NanoLuc Luciferase:

- Thermal stable, monomeric enzyme
- Small size, 19kDa
- Extremely bright
- Narrow, blue-shifted emission spectrum
- Live cell substrate
- Active over a broad pH range

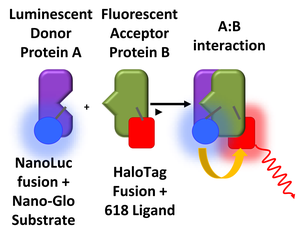


HaloTag Technology:

- Monomeric protein, 34kDa
- No endogenous equivalent
- Irreversibly binds chloroalkane ligands
- Extremely fast association rate
- HaloTag 618 ligand is cell permeable NanoBRET acceptor

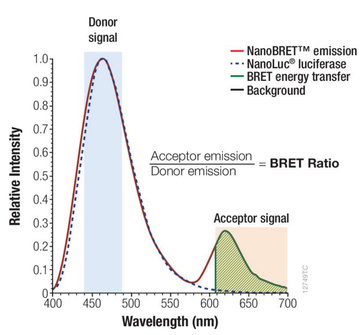
NanoBRET PPI

Bioluminescence Resonance Energy Transfer



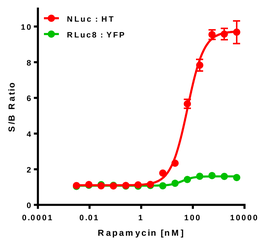
3. NanoBRET Improves over other BRET Assays

Optimized donor and acceptor pairing significantly improves S:B



Improved S:B over BRET2

FKBP:FRB + Rapamycin



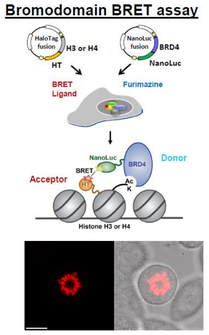
NanoBRET is ratiometric

$$\frac{\text{Acceptor signal}}{\text{Donor signal}}_{\text{with ligand}} - \frac{\text{Acceptor signal}}{\text{Donor signal}}_{\text{no ligand}} = \text{Corrected BRET Ratio}$$

4. NanoBRET Applied to Epigenetic Interactions

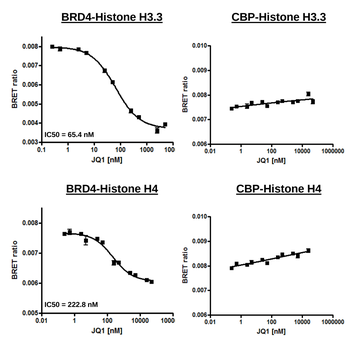
Study interaction in native chromatin in live cells

Bromodomain BRET assay



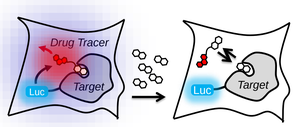
Staining with TMR ligand demonstrates chromatin incorporation of Histone H3.3-HaloTag

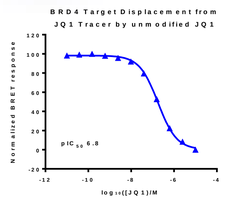
Measuring Effect of BET Inhibitor on Histone-Bromodomain Interactions



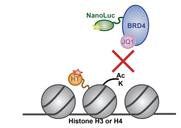
5. NanoBRET PPI Correlates with Compound Binding

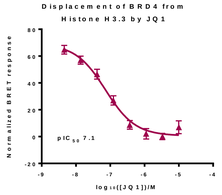
Live Cell Target Engagement of BRD4 with JQ1





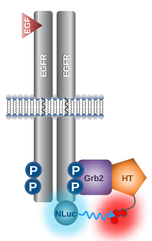
Displacement of BRD4 from Chromatin with JQ1



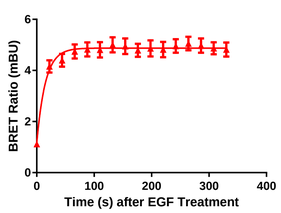


6. NanoBRET EGFR/Grb2 Interaction Assay

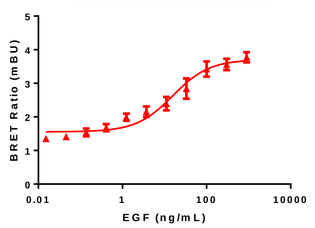
Stable Expression of EGFR-NL/HT-Grb2 in HEK293 Cells, Grb2 recruitment induced by EGF treatment



Real-Time NanoBRET kinetic assay

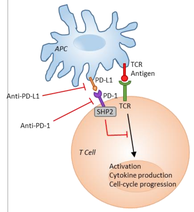


EGF dose response curve

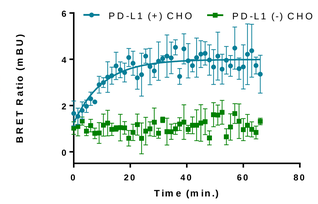


- **Generation of single or dual stable cell line possible with NanoBRET**
- **Real time kinetic measurements of interactions possible with transient or stable expression**
- **See expected induction of interaction and response to EGF treatment**

7. NanoBRET PD-1/SHP2 Interaction Assay

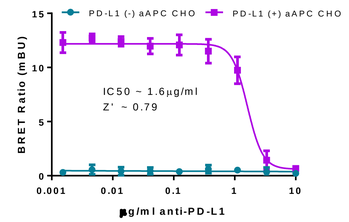


Time course showing the PD-LI mediated recruitment of SHP2 to PD1



NanoBRET PD-1/SHP2 fusions expressed transiently in Jurkat cells from bi-directional promoter

Inhibition of SHP2 recruitment by anti-PD-L1 antibody

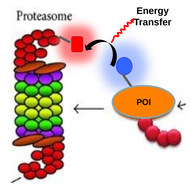


Stable expression of NanoBRET PD-1/SHP2 fusions in Jurkat cells

8. NanoBRET Proteasomal Recruitment Assay

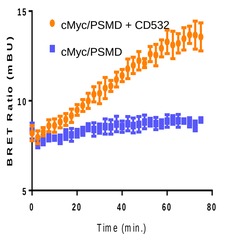
Measure real-time changes in recruitment to proteasome after addition of small molecules to promote degradation of targets

Small molecule induced interactions with proteasome

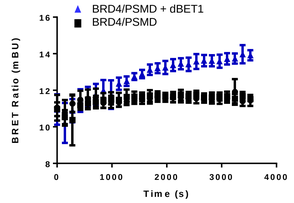


- Proteasomal Subunit = HaloTag fusion energy acceptor
- Protein targeted for degradation = NanoLuc fusion energy donor

cMyc:PSMD +/- CD532



BRD4:PSMD +/- dBET1 (BRD4 degrader)



9. Summary

NanoBRET PPI System is composed of two components

- NanoLuc luciferase energy donor
- HaloTag labeled with HaloTag618 ligand as energy acceptor

NanoBRET offers improved S:B over other BRET assays

- Bright, blue-shifted donor signal and red-shifted acceptor create optimal spectral overlap, increased signal and lower background compared to conventional BRET assays

NanoBRET provides sensitive live-cell method to study protein interactions

- Monitor both protein association and dissociation events in real time
- Use low, native expression levels
- Applicable to diverse intracellular interactions including interactions on native chromatin, membrane receptors, and proteasome recruitment