Monitoring intracellular protein interactions using NanoLuc® Binary Technology (NanoBiT™)

Brock Binkowski[1], Andrew Dixon[2], Marie Schwinn[1], Mary Hall[1], Kris Zimmerman[1], Paul Otto[1], Thomas Lubben[1], Braeden L. Butler[1], Thomas Machleidt[1], Monika G. Wood[1], Christopher Eggers[1], Lance P. Encell[1], Frank Fan[1] & Keith Wood[1]

[1]Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711-5399;

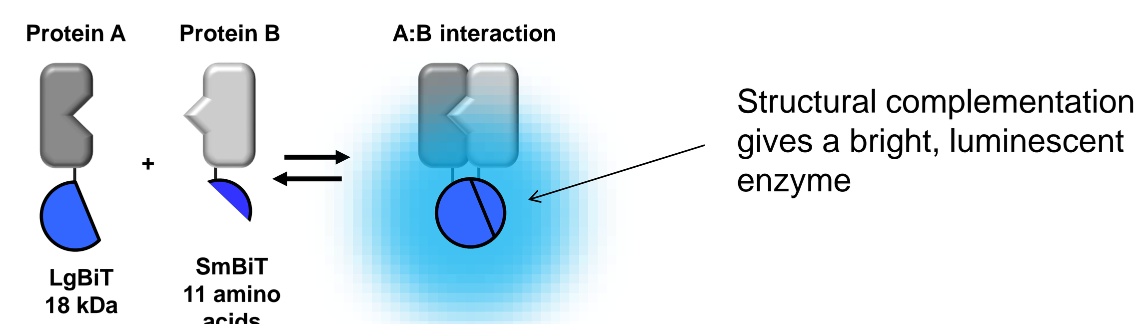
[2]Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT 84112

Abstract #4830

1. Introduction

Protein:protein interactions (PPIs) are essential to the cellular signal transduction pathways that contribute to cancer. Although numerous approaches exist to monitor PPIs *in vitro*, methods for intracellular detection have been more limited. We developed NanoLuc® Binary Technology (NanoBiT), a two-subunit system based on NanoLuc® luciferase that can be applied to the intracellular detection of PPIs. Large BiT (LgBiT; 17.6 kDa) and Small BiT (SmBiT; 11 amino acid peptide) subunits are expressed as fusions to proteins of interest, where PPI facilitates subunit complementation to give a bright, luminescent enzyme. Unlike related approaches where an enzyme or protein is simply split, LgBiT was independently optimized for structural stability and SmBiT was selected from a peptide library specifically for the PPI application. The result is a subunit pair that weakly associates (K_D = 190 µM) yet shows only 3 fold lower activity at saturation vs. NanoLuc *in vitro*. In contrast to many split systems, the LgBiT:SmBiT interaction is reversible, allowing the detection of rapidly dissociating proteins. PPI dynamics can be followed in real-time in living cells using the Nano-Glo® Live Cell Reagent, a non-lytic detection reagent containing the cell-permeable furimazine substrate. Advantages over split systems include better sensitivity, reversibility, fusion to a peptide or a small, structurally stable protein domain, real-time measurements using a non-lytic assay format, and subunits with reduced affinity for self-association. We have applied this system to several PPIs associated with cellular transformation.

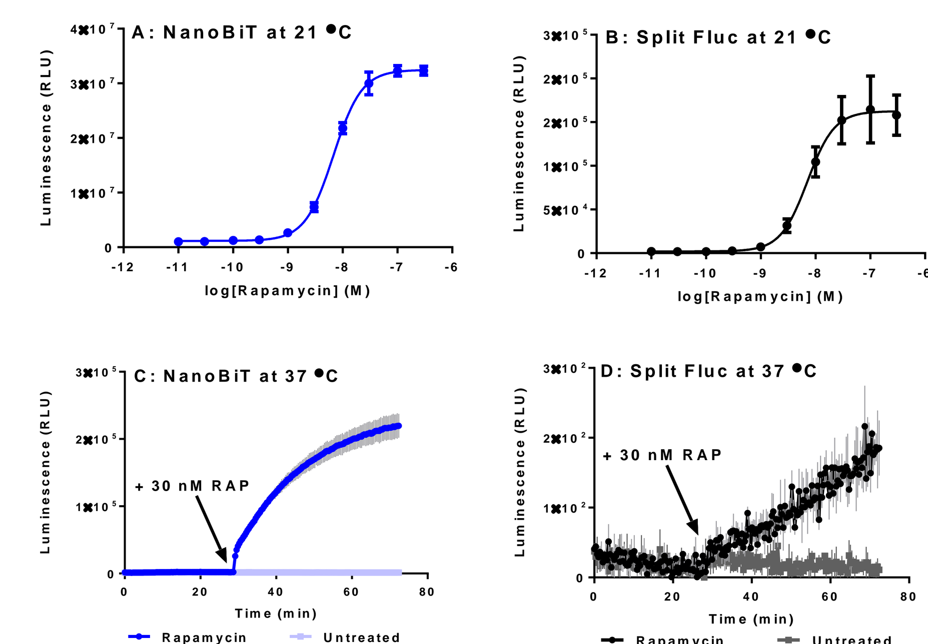
2. NanoBiT overview



Structural complementation gives a bright, luminescent enzyme

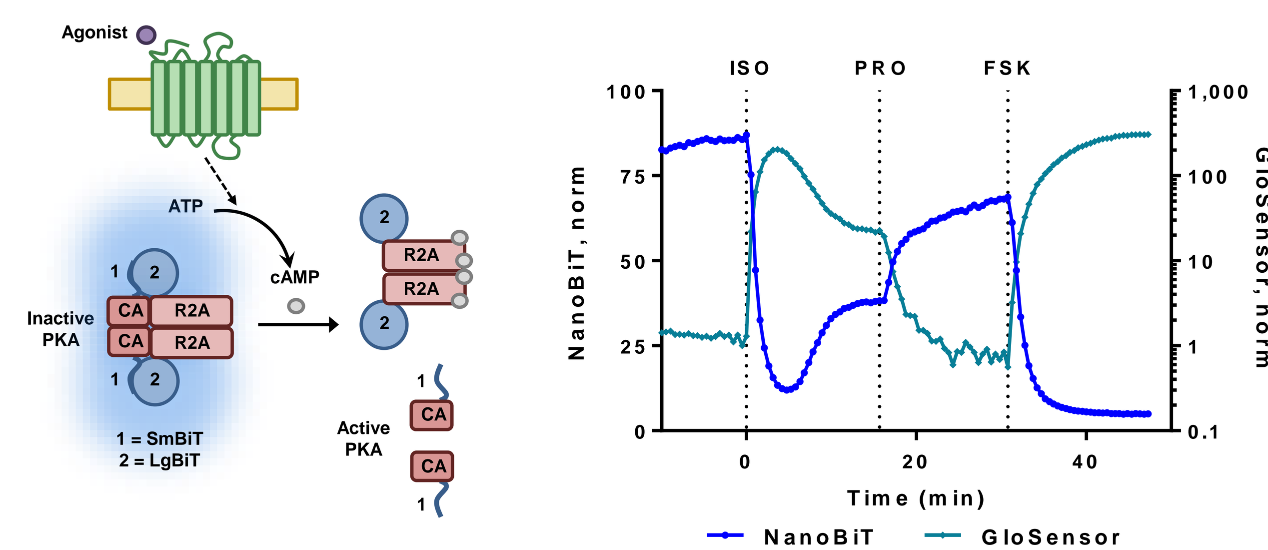
- LgBiT and SmBiT are fused to proteins A & B
- A:B interaction facilitates LgBiT:SmBiT interaction, generating a bright luminescent enzyme
- LgBiT:SmBiT with low affinity (K_D = 190 µM), limiting non-specific association and reducing assay background
- LgBiT:SmBiT interaction is reversible (kon = 500 $M^{-1}sec^{-1}$; koff = 0.2 sec^{-1})
- LgBiT evolved for increased structural stability making it a better fusion partner
- Non-lytic assay format allows real-time measurements of protein interaction dynamics for 1-2 hrs

3. NanoBiT is logs brighter than split firefly luciferase



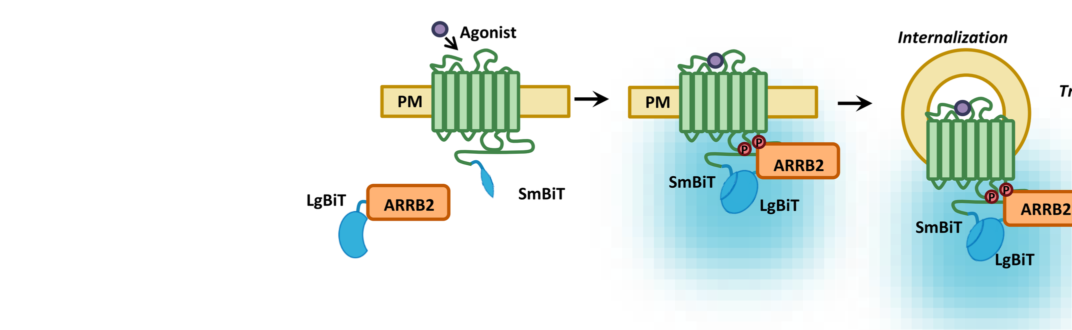
- Optimal orientation identified for both NanoBiT & split Fluc (4-398 + 394-544)
- NanoBiT >300 fold brighter at room temperature (panels A & B)
- NanoBiT>1,000 fold brighter at 37 °C (panels C & D)

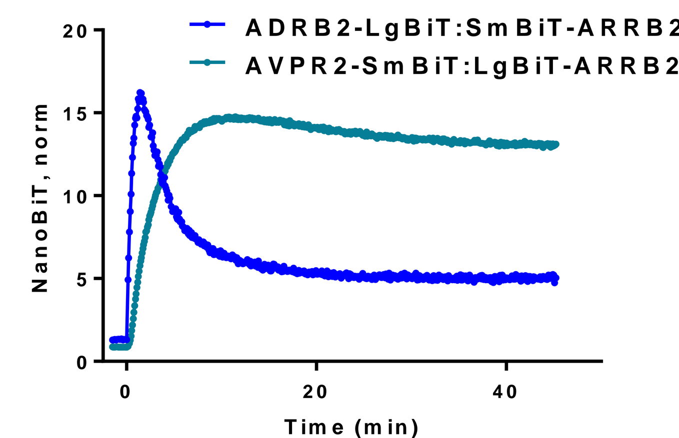
4. NanoBiT is reversible



- NanoBiT compared to a firefly luciferase-based biosensor for cAMP (GloSensor cAMP 22F) following transient expression in HEK293 cells (endogenous ADRB2)
- Modulators of intracellular cAMP added sequentially at indicated time points

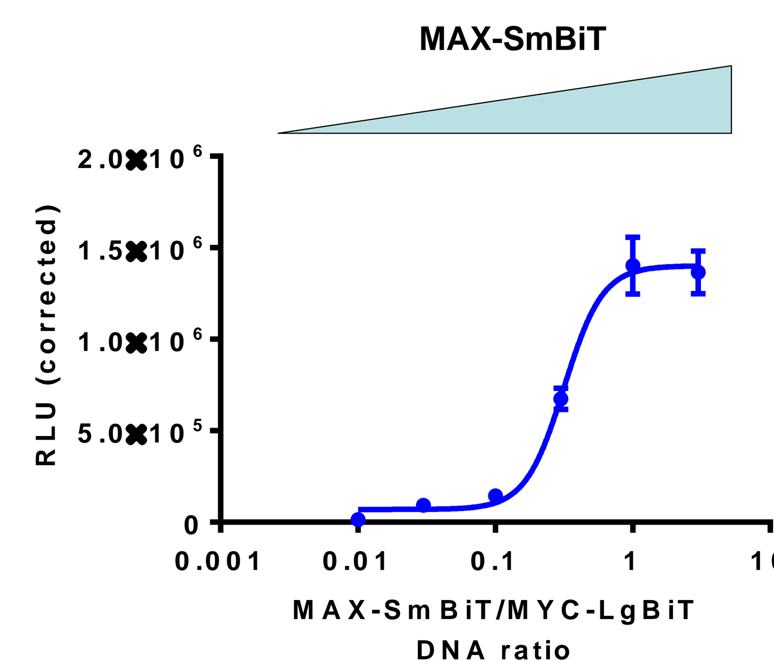
5. NanoBiT applied to GPCR:ARRB2





- Class A receptors (e.g. ADRB2) recycle quickly and show a transient association with ARRB2
- Class B receptors (e.g. AVPR2) recycle more slowly and show a stable association with ARRB2
- GPCR:ARRB2 pairs transiently expressed in HEK293 and treated with saturating agonist at time zero
- AVPR2:ARRB2 validated in 1536-well format with Z' = 0.52

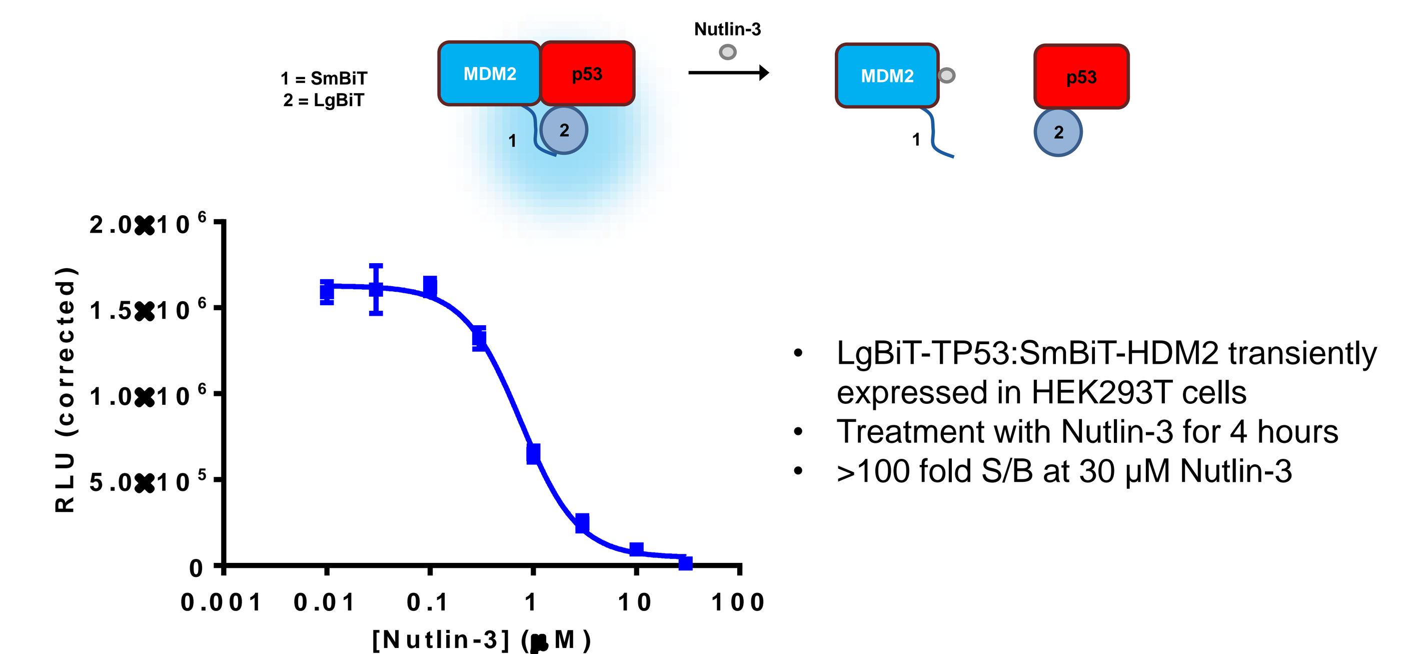
6. NanoBiT applied to MYC:MAX



Increasing MAX-SmBiT expression with fixed MYC-LgBiT

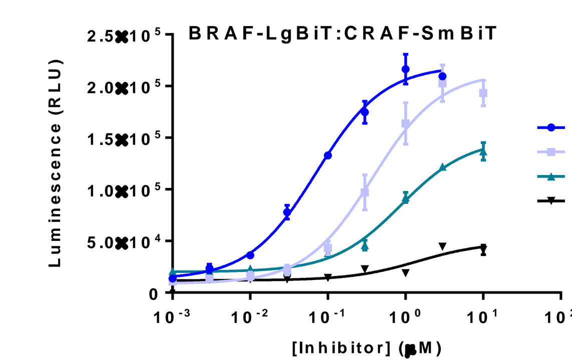
- Varying ratios of plasmid DNA transiently transfected in HEK293T
- MYC-LgBiT:MAX-SmBiT >1,000 fold brighter than MYC-LgBiT expressed alone

7. NanoBiT applied to p53:MDM2

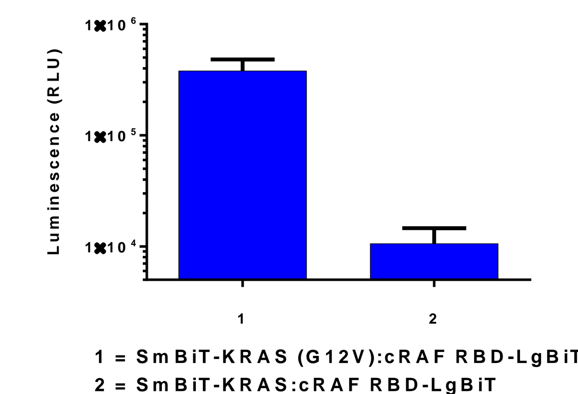


- LgBiT-TP53:SmBiT-HDM2 transiently expressed in HEK293T cells
- Treatment with Nutlin-3 for 4 hours
- >100 fold S/B at 30 µM Nutlin-3

8. NanoBiT applied to cRAF:bRAF & KRAS:cRAF



- bRAF-LgBiT:cRAF-SmBiT transiently expressed in HEK293
- Treatment with bRAF inhibitors for 2 hrs at 37 °C
- Expected rank order potency observed
- Using full-length bRAF & cRAF



- Wild-type KRAS or KRAS G12V pairs transiently expressed in HEK293
- Serum starve for 4 hrs
- >10 fold S/B for constitutively activated KRAS G12V vs. wild-type KRAS
- Using cRAF Ras binding domain (RBD)

9. Conclusions

NanoBiT is extremely bright
- >1,000 fold brighter than split firefly luciferase at 37 °C
- Fusion partners can be expressed at very low levels, minimizing potential artifacts

NanoBiT components are small & stable
- LgBiT, 17.6 kDa; SmBiT, 11 amino acids
- LgBiT evolved for increased structural stability, providing a stable fusion partner

NanoBiT is reversible
- Monitor both protein association and dissociation events

NanoBiT offers experimental flexibility
- Monitor protein interaction dynamics at a single time point or continuously for 1-2 hours
- Room temperature or 37 °C measurements
- Validated in 96-, 384- & 1536-well formats

Corresponding author: brock.binkowski@promega.com