A Novel GPCR beta-arrestin LinkLight Assay Technology

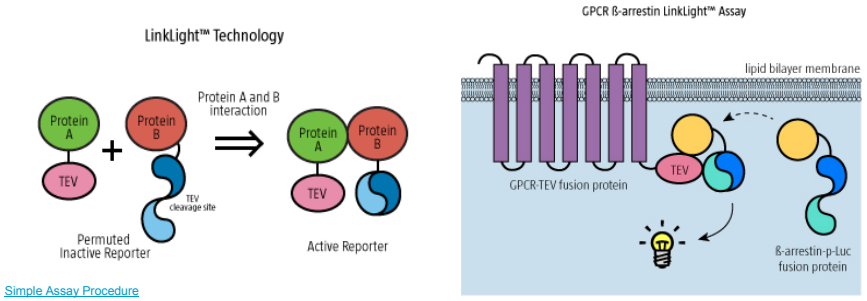
Haifeng Eishingdrelo[1], Jidong Cai[2], Paul Weissensee[2], Praveen Sharma[2], Michael J. Tocci[2] and Paul S. Wright[2]

1: BioInvenu; 2: sanofi

Abstract

We have developed a novel cell-based protein-protein interaction 'LinkLight' assay technology for HTS applications. The basic assay design of protein-protein interaction consists of two components: an inactive permuted luciferase containing a Tobacco Etch Virus (TEV) protease cleavage sequence fused to protein B, and protein A fused to the protease TEV. Upon interaction between protein A and B, inactive permuted luciferase is cleaved, the cleaved luciferase fragments are spontaneous refold, and active luciferase is reconstituted. The luciferase signals are specific and sensitive for specified protein A and B interaction. We named this cell-based protein-protein interaction method as "LinkLight" assay technology. The technology is not another simple enzyme fragment complementation. The LinkLight assay design avoids the significant problem of fragments spontaneous self-complementation, therefore, reducing background interaction noise and auto-luminescent signals. The assay does not involve transcription and translation, and therefore, has reduced off-target signals. We demonstrated assay applicability for ligand-induced protein-protein interactions including G-protein coupled receptors, receptor tyrosine kinases and nuclear hormone receptors.

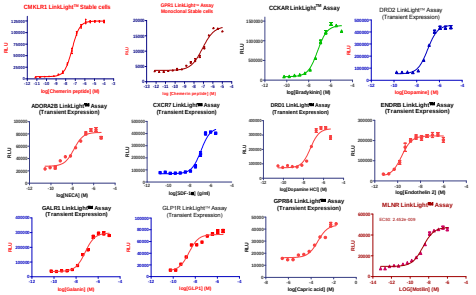
LinkLight™ Technology & GPCR LinkLight™ Assay



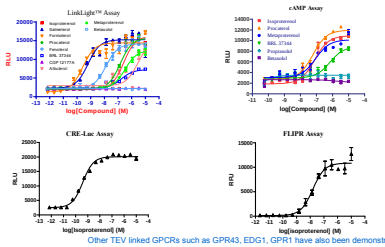
Simple Assay Procedure

1. Seed cells in 384-wel plate, culture for 24 hours.
2. Change to serum-free medium if necessary, add ligands, incubate for one hour.
3. Add luciferase detection reagent, count signals on a luminescent reader.

Examples of GPCR LinkLight™ Assays



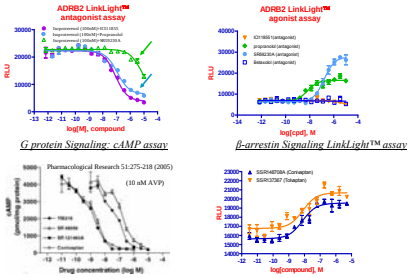
TEV linked GPCR ADRB2 does not alter G-protein signaling pathway



Other TEV linked GPCRs such as GPR43, EDG1, GPR1 have also been demonstrated no interference to G-protein signaling

Identify biased ligands

Some of well-known antagonists showed partial agonist activity in GPCR LinkLight™ assays

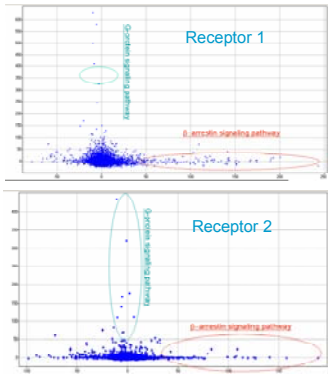


Validate GPCR LinkLight™ Assay in Small Screenings

Receptor	Actives Criteria (positive on triplet: ?3+SD)	Active description
ADRB2 (β 2)	39	All known ADRB2 ligands including ligands of related biogenic amine receptors were picked up
EDG1 (S1PR1)	37	Hits are very diverse including previously unknown EDG1 actives
V2R	24	Diverse hits

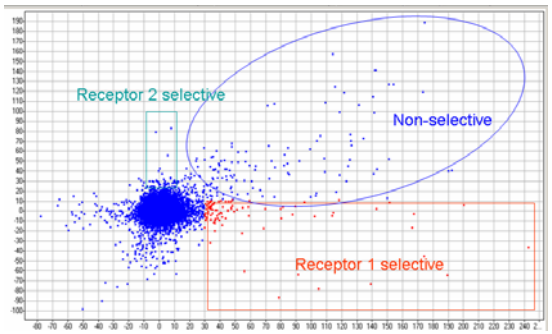
❖A small validation library contains ~5,000 compounds including known GPCR actives and natural ligands.
❖Non-specific hit rate is very low compared to other G-protein signaling based assays. There are a few common non-specific actives that are known cytotoxic agents with anti-tumor properties.

Hit Distribution of LinkLight™ (β-arrestin) vs. FLIPR (G-protein Signaling) Screens



❖Two GPCR receptors were screened using a small validation library containing ~5,000 compounds.
❖The screens were performed in single dose (10 μM) and single point to look for agonists.
❖Hit distributions largely depend on signaling pathway assay methods.
❖The discrete hit distribution pattern suggests "comprehensive" or "saturating" screening approach for GPCR lead discovery.

Identification of Selective Hits by Comparison of Receptor 1 & 2 LinkLight™ Screens



Receptor 1 selective hits are chosen based on E% ≥ 30 for receptor 1 & ≤ 10 for receptor 2 (92 selective positives) receptor 2 selective hits are chosen based on E% ≥ 30 for receptor 2 & ≤ 10 for receptor 1 (18 selective positives)

Potential Applications of LinkLight™ Assay Technology

- Applicable to a broad range of protein interactions, such as receptor and its adaptor interactions, cytoplasmic protein interactions, nuclear protein interactions, membrane protein interactions, cell-cell interactions through membrane proteins.
- Applicable to stimuli-induced transient protein-protein interactions, finding molecules modulating protein-protein interactions.
  - address specific signal transduction pathways via protein-protein interactions as the signal readout
  - build assays for currently intractable targets or targets only tractable by non-specific promoter-reporter methods
- Applicable to constant protein-protein interactions, finding molecules directly blocking protein-protein interactions.
  - Build assays for protein-protein interactions as drug targets
  - Build assays to map protein interaction partners and networks.
- Luciferase can be replaced with other reporter proteins such as fluorescent proteins, or other enzyme reporters.