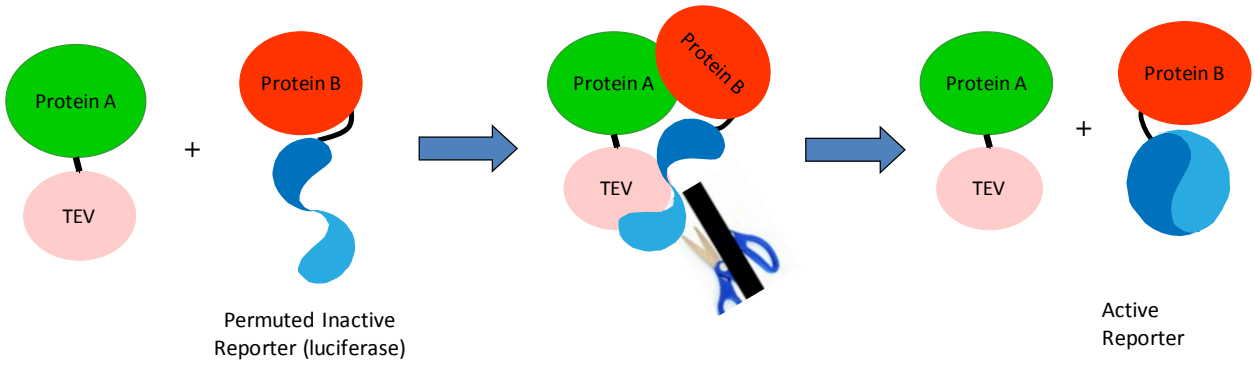
Build Your Own β-arrestin Protein-protein Interaction LinkLight™ Assays

It has become clear that multiple signaling transduction pathways are employed upon GPCR activation. In addition to classic G-protein-dependent signaling pathways, G-protein-independent signaling pathways have attracted significant attention and have been exploited for drug discovery efforts. As new G-protein-independent assay tools have become available (such as β-arrestin based assays), biased ligands that selectively activate one pathway over another, as well as show agonism in one pathway but antagonism in another pathway, have been discovered. Therefore, exploiting biased ligands for therapeutic benefit represents a promising opportunity to develop safer and more efficacious drugs.

β-arrestins as signal adaptor proteins have been implicated to play much broader roles beyond interaction with G-protein coupled receptors (GPCRs) and participate in multiple biological processes. β-arrestins have been indicated to associate with various receptor types such as receptor tyrosine kinases and cytokine receptors. Multiple non-receptor kinases including PI3K, AKT, JNK, p38, and SRC are implicated to interact with β-arrestins.

LinkLight™ technology can detect protein-protein interactions in live cells. The technology consists of two components. A protein A is linked to a Tobacco Etch Virus (TEV) protease and a protein B is linked to a permuted luciferase (pLuc). An inactive pLuc has been created by breaking luciferase into two fragments, rearranging the fragment order in that the N-terminal fragment is moved to C-terminus and C-terminal fragment is moved to the N-terminus and reconnecting them with a TEV protease cleavage sequence. Once a permuted luciferase and a TEV are brought into close proximity during the protein A and B interaction, TEV protease



cleaves a permuted luciferase, and the cleaved permuted luciferase fragment is spontaneously refolded back to form an active luciferase, and a signal can be detected even if the two interaction partners become separated. The luciferase signal intensity is relative to the amount of active luciferase generated. The signal is specific and sensitive for protein A and B interaction. Although such an assay design cannot detect an interaction instantly for mechanism studies, the stable signals enable use in HTS. LinkLight technology overcomes the significant drawback of enzyme or protein fragment spontaneous self-complementation seen in similar assay technologies. The spontaneous fragment self-complementation caused by the high affinity of two fragments increases false interactions and background signals and makes transient protein-protein interactions appear as constitutive interactions, significantly limiting their applications and assay sensitivity. LinkLight assays also have advantages that overcome issues in other assays such as the strict spatial distance and orientation of donor-acceptor complementation requirements in BRET assays, the poor spectral separation between donor and acceptor needed for fluorescence resonance energy transfer (FRET), and off-target signals caused by transcription/translational processes in transcription-based assays.

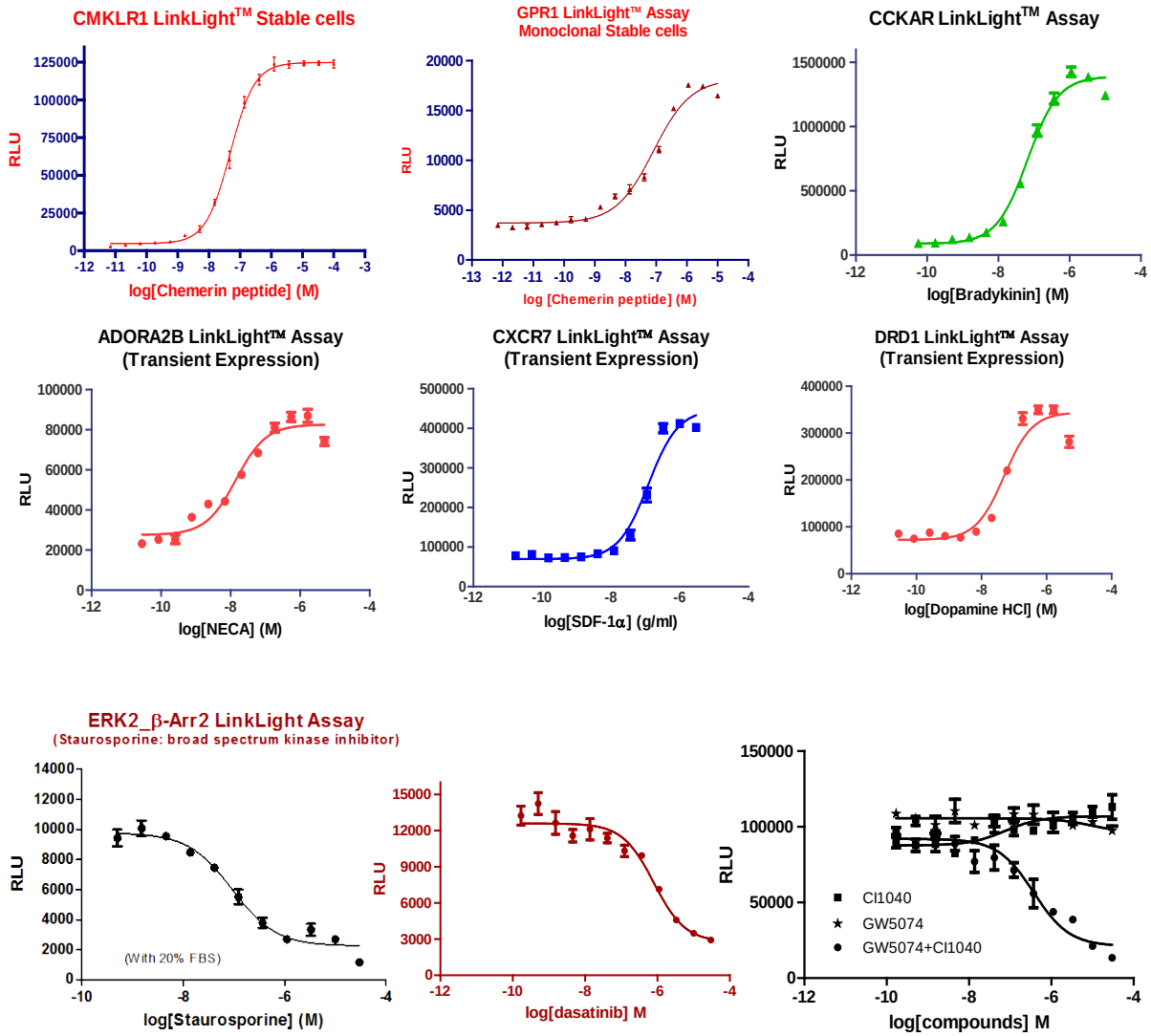
Develop Your Own β-arrestin LinkLight Assays

1). Construct your own target expression plasmids with provided "target-open TEV" vectors.
2). Transfect your target-TEV expression plasmids into our validated β-arrestin-2-pLuc reporter host cells: β-arrestin-2-pLuc/U2OS (cat#: 1000-14); and β-arrestin-pLuc/HEK293 (cat#: 1000-19); and β-arrestin 1&2-pLuc/HEK293 (cat#: 1000-3).
3). Analyze your target/ β-arrestin interaction signals by performing a simple luciferase assay.

Advantages

Broad Application: Applicable to a large number of β-arrestin interaction partners including GPCRs, channels, receptor tyrosine kinases, non-receptor kinases, transcription factors, cytokine receptors, etc..
Cost-effective: Multiple vendors provide firefly luciferase detection reagents.
Simple Operation: Simple luciferase light readout, HTS-ready.
Flexibility: You can do transient expression by plasmids, viruses, or you can generate stable cell lines.

Here are some examples (stable cell lines):



Pan-kinase inhibitor staurosporine blocked the interaction of ERK2/β-arr2, implying the interaction requires kinase activation. Kinase inhibitors such as cancer drug Dasatinib blocked the interaction signals. Raf inhibitor GW5074 and MEK inhibitor CI1040 did not inhibit the ERK2/β-arr2 interaction signals by acting alone but inhibited the interaction when they were combined.

References:

1. Eishingdrelo H, et al. (2011). A cell-based protein-protein interaction method using a per.muted luciferase reporter. Current. Chem. Genomics 5:122-128.

2. Eishingdrelo H. et al. ERK and β-arrestin interaction: a converging-point of signaling pathways for multiple types of cell-surface receptors. J. Biomolec. Screen. 2014; 20: 341-349.