A Comprehensive LinkLight™ platform for GPCR Mechanistic Profiling to Support Cancer Drug Discovery

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Abstract

Background: G protein–coupled receptors (GPCRs) are increasingly recognized as important targets in cancer therapy due to their roles in tumor growth, angiogenesis, and metastasis. LinkLight technology is a protein–protein interaction (PPI) detection platform that monitors β-arrestin recruitment to activated G protein–coupled receptors (GPCRs), complementing second messenger–based assays for ligand activity characterization. Here, we use LinkLight stable cell lines co-expressing TEV-protease–tagged GPCRs and permuted luciferase (pLuc)–tagged β-arrestin to study β-arrestin recruitment, cAMP signal, and calcium-influx within the same cellular context.

Methods: Functional assays were conducted in agonist and antagonist modes across three GPCR LinkLight stable cell lines representing distinct G-protein couplings (Gs, Gi, and Gq). β-Arrestin-1/2 recruitment was quantified using the LinkLight PPI luminescence readout, while G-protein-mediated signaling was measured via cAMP or calcium-influx fluorescence, depending on receptor class.

Results: Ligands produced consistent, mechanism-appropriate responses across β-arrestin and second-messenger pathways. For Gq-coupled ADRA1A, the agonist Cirazoline induced robust β-arrestin-1/2 recruitment (EC_{50} = 2.51×10^{-8} M) and calcium influx (EC_{50} = 8.2×10^{-9} M), while the antagonist Prazosin inhibited both (β-arrestin EC_{50} = 2.51×10^{-8} M; calcium EC_{50} = 2.06×10^{-7} M). For Gi-coupled ADRA2A, the agonist Brimonidine stimulated β-arrestin-1/2 recruitment (EC_{50} = 2.64×10^{-8} M) and suppressed cAMP (EC_{50} = 8.88×10^{-10} M), whereas the antagonist Yohimbine blocked β-arrestin recruitment (EC_{50} = 1.78×10^{-8} M) and reversed cAMP inhibition (EC_{50} = 2.69×10^{-7} M). For Gs-coupled ADRB2, the agonist Fenoterol induced β-arrestin-2 recruitment (EC_{50} = 8.83×10^{-10} M) and cAMP signaling (EC_{50} = 1.52×10^{-9} M), while the antagonist Yohimbine (S)-Propranolol inhibited both (β-arrestin EC_{50} = 3.98×10^{-8} M; cAMP EC_{50} = 3.6×10^{-8} M). Across all receptors, β-arrestin recruitment aligned with expected G-protein–specific signaling in response to the same ligand, demonstrating the robustness of the Comprehensive GPCR Portfolio.

Summary: By enabling simultaneous assessment of β-arrestin recruitment and G-protein signaling in a unified cellular context, LinkLight technology offers a comprehensive, mechanistically informative platform for GPCR pharmacology. Integrated with G-protein activation data, LinkLight technology also supports high throughput profiling of ligand efficacy, bias, and receptor regulation for GPCR drug discovery against cancer.

Data Table: EC50

EC50 data table for all targets of all 3 cell-based functional assays

GPCR	LinkLight partner	G-protein partner	GPCR/β-arrestin Recruitment Assay	
			Agonist Mode	Antagonist Mode
			EC50	EC50
ADRA1A	β-arrestin-1&2	Gq	Cirazoline: 2.51E-08	WB4101: 1.8E-08 Prazosin: 2.51E-08
ADRA2A	β-arrestin-1&2	Gi	Adrenaline: 4.04E-08 Brimonidine: 2.64E-08	Yohimbine: 1.72E-08
ADRB2	β-arrestin-2	Gs	Fenoterol: 8.83E-10; Salmeterol: 1.21E-10	(S)-Propranolol: 3.98E-08 ICI118551: 8.18E-08

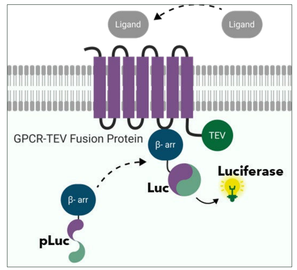
GPCR	LinkLight partner	G-protein partner	GPCR/cAMP Production Assay	
			Agonist Mode	Antagonist Mode
			EC50	EC50
ADRA2A	β-arrestin-1&2	Gi	Brimonidine: 8.88E-10	Yohimbine: 2.69E-07
ADRB2	β-arrestin-2	Gs	Fenoterol: 1.52E-09	(S)-Propranolol: 3.6E-08

GPCR	LinkLight partner	G-protein partner	GPCR/Calcium Flux Assay	
			Agonist Mode	Antagonist Mode
			EC50	EC50
ADRA1A	β-arrestin-1&2	Gq	Adrenaline: 4.56E-09; Cirazoline: 8.2E-09	Prazosin: 2.06E-07

Assay Introduction

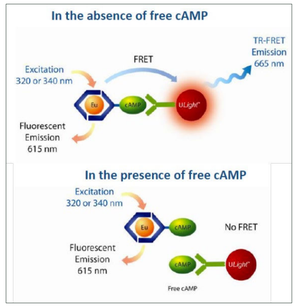
GPCR/β-arrestin Recruitment Assay

The LinkLight™ technology is a protein–protein interaction platform performed in stable cell lines co-expressing protein A fused to a TEV protease and protein B fused to a permuted luciferase (pLuc). Cells are engineered to co-express GPCR–TEV protease and β-arrestin–permuted luciferase (pLuc). Upon ligand-induced GPCR activation, β-arrestin is recruited to the GPCR receptor, bringing TEV protease into close proximity with pLuc. TEV-mediated cleavage triggers refolding of pLuc into its active form, which—upon addition of luciferin—produces a robust, irreversible luminescent signal. This signal quantitatively reports GPCR activation via β-arrestin recruitment.



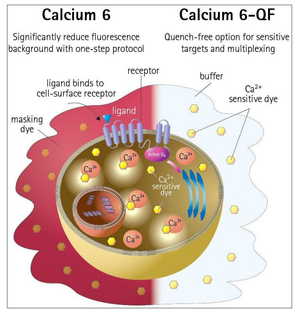
GPCR/cAMP Production Assay

GPCR/cAMP production assay measures changes in intracellular cAMP levels following activation of Gs- or Gi-coupled GPCRs. The LANCE Ultra cAMP assay is a homogeneous time-resolved fluorescence resonance energy transfer (TR-FRET) immunoassay designed to measure cAMP produced upon modulation of adenylyl cyclase activity by G-protein coupled receptors (GPCRs). The assay is based on the competition between the europium (Eu) chelate-labeled cAMP tracer and sample cAMP for binding sites on cAMP-specific monoclonal antibodies labeled with the ULight™ dye.



GPCR/Calcium Flux Assay

GPCR/calcium flux assay detects rapid changes in intracellular calcium triggered by activation of Gq-coupled GPCRs. The FLIPR Calcium 6 Assay Kit contain calcium sensitive florescence dye. Kit components are mixed with buffer and incubated for approximately two hours with cells. During incubation, the indicator passes through the cell membrane, and esterases in the cytoplasm cleave the acetoxymethyl (AM) portion of the molecule. After incubation with the dye, the cells are ready to be assayed. When the target is activated, direct measurement of intracellular fluorescence change due to increased calcium concentration is recorded by FLIPR.



Example Data

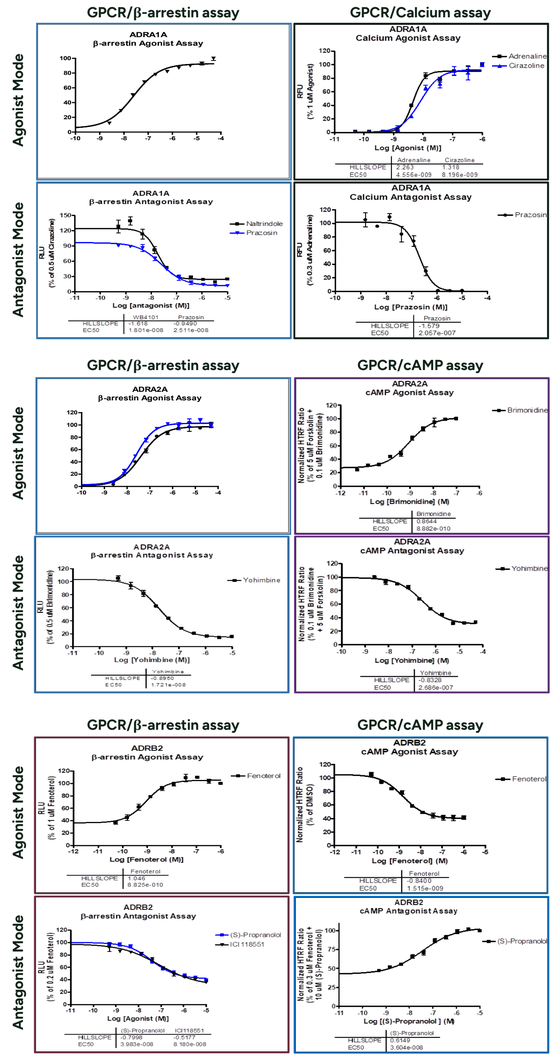


Figure 1. ADRA1A

- ADRA1A (alpha-1A adrenergic receptor) is involved in cell functions like smooth muscle contraction, blood pressure regulation, heart cell survival, and learning/memory.
- Agonist Cirazoline induce the recruitment of β-arrestin-1&2 through the activation of the ADRA1A, while antagonist Prazosin and Naltrindole inhibit the recruitment of β-arrestin-1&2 through deactivation of the ADRA1A.
- Agonist Cirazoline and Adrenaline induce the calcium signal through the activation of the ADRA1A, while antagonist Prazosin inhibit the calcium signal through deactivation of the ADRA1A.

Figure 2. ADRA2A

- ADRA2A (alpha-2A adrenergic receptor) is involved in regulating neurotransmitter release, catecholamine signaling, and responses to stress within the nervous systems.
- Agonist Brimonidine and Adrenaline induce the recruitment of β-arrestin-1&2 through the activation of the ADRA2A, while antagonist Yohimbine inhibit the recruitment of β-arrestin-1&2 through deactivation of the ADRA2A.
- Agonist Brimonidine inhibit cAMP signal through the activation of the ADRA2A, while antagonist Yohimbine induce cAMP signal through deactivation of the ADRA2A.

Figure 3. ADRB2

- ADRB2 (beta-2 adrenergic receptor) is involved in cell functions like regulating immune responses and cardiopulmonary function.
- Agonist Fenoterol induce the recruitment of β-arrestin-2 through the activation of the ADRB2, while antagonist (S)-Propranolol and ICI118551inhibit the recruitment of β-arrestin-2 through deactivation of the ADRB2.
- Agonist Fenoterol induce cAMP signal through the activation of the ADRB2, while antagonist (S)-Propranolol inhibit the cAMP signal through deactivation of the ADRB2.

Summary

1. LinkLight technology serve as a powerful tool for studying **comprehensive GPCR portfolio** for GPCR drug discovery.
2. **Agonist Cirazoline** induce the recruitment of β-arrestin-1&2 and calcium signal through the activation of the **ADRA1A**, while antagonist Prazosin inhibit the recruitment of β-arrestin-1&2 and calcium signal through deactivation of the **ADRA1A**.
3. **Agonist Brimonidine** induce the recruitment of β-arrestin-1&2 and inhibit cAMP signal through the activation of the **ADRA2A**, while antagonist **Yohimbine** inhibit the recruitment of β-arrestin-1&2 and induce cAMP signal through deactivation of the **ADRA2A**.
4. **Agonist Fenoterol** induce the recruitment of β-arrestin-2 and cAMP signal through the activation of the **ADRB2**, while antagonist **(S)-Propranolol** inhibit the recruitment of β-arrestin-2 and cAMP signal through deactivation of the **ADRB2**.

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