GPCR panel establishment and application in drug discovery

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Abstract #4712

Introduction

G protein-coupled receptors (GPCRS) are the largest family of membrane proteins of cell surface receptors. It can be activated by various stimuli and play an important role in various physiological and pathological processes. Abnormal regulation of GPCR is associated with various human diseases, such as oncology, metabolic diseases, cardiovascular diseases, and eye diseases.

GPCR panel has high availability. Here, we constructed over 170 GPCR overexpression stable cell lines, covering wide families like 5-Hydroxytryptamine, acetylcholine, dopamine, glucagon, and opioid receptors. We also constructed different function assays, including cAMP assay, calcium flux assay, IP1 assay, reporter assay, and β-arrestin NanoBit assay to detect the different second messengers produced by G protein submits and GPCR-mediated multiple signaling pathways. The SPR binding assay, tag lite binding assay, and FACS binding assay are constructed to validate the affinity between GPCR and compounds. These GPCR panel and function assays can be used for target identification, hit-to-lead and lead optimization, also suitable for high-throughput screening.

GPCR panel application in function assay

1. GPCR HTRF cAMP Assay

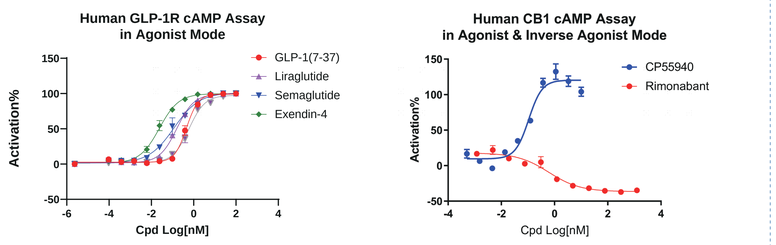


Figure 1. HTRF cAMP assay was constructed for compound screening. Its principal is based on HTRF technology (homogeneous time-resolved fluorescence). The method is an immunoassay designed to measure cAMP produced upon modulation of adenylyl cyclase activity by G-protein coupled receptors (GPCRs). The 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 A. Different compounds (liraglutide, semaglutide, exendin-4, PF-06882961) were tested using GLP-1R-CHO cell. D. Agonist and inverse agonist test of CB1 in CB1-CHO stable cell line.

2. GPCR β-arrestin NanoBit Assay

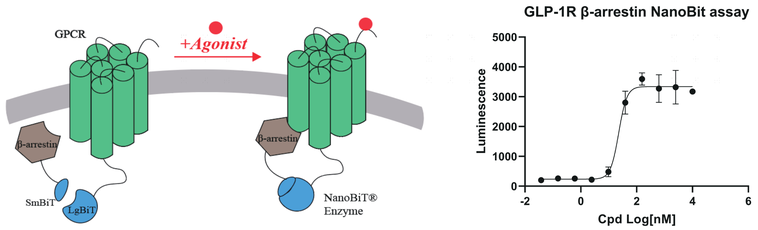


Figure 2. Monitoring recruitment of the GPCR and β-arrestin in cells. A. The principle of NanoLuc® Binary Technology (NanoBiT®) assay. NanoBiT® assay is one of the Top 10 Innovations of 2015 by The Scientist magazine and has been successfully applied in drug screening. The technology is to cleave NanoLuc fluorophore enzyme into two subunits–a large subunit (LgBiT=158 aa) and a small subunit (SmBiT=11 aa), the LgBiT large and SmBiT small subunits were fused to GPCRs and β-arrestin1/2.B. The NanoBiT assay was used to characterize the GLP-1R and β-arrestin recruitment in GLP-1R/β-arrestin co-expression stable cell line.

3. GPCR Internalization IFA assay

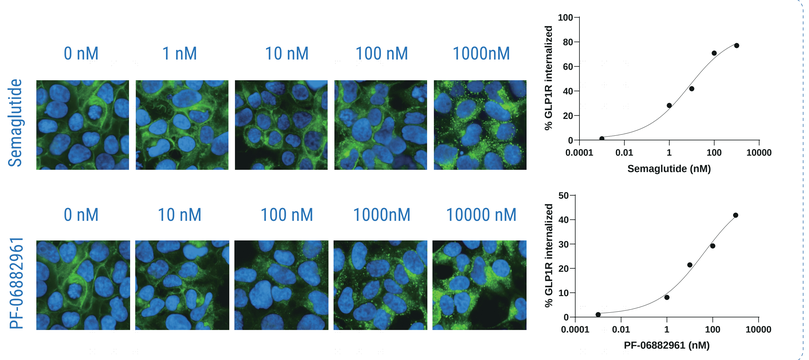
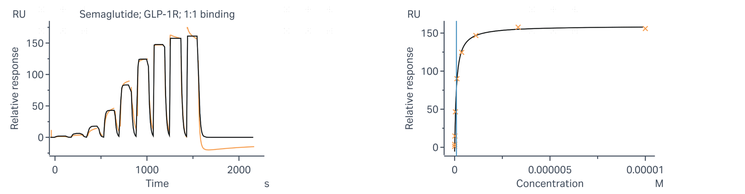


Figure 3. GLP-1R IFA internalization assay. Once activated, GPCRs induce signals at the cell surface. This is often followed by internalization, a process that results in the transfer of receptors from the plasma membrane to membranes of the endosomal compartment. The cells expressing GLP-1R, which fused with GFP protein, employ HCS to measure the GFP intensity. Semaglutide and small molecular PF-06882961 were used to activate GLP-1R.

4. GLP-1R SPR binding assay



Immobilized ligand	Injection variables Analyte 1 Solution	Kinetics model	1:1 binding ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Quality Kinetics Chi² (RU²)	U-value	Affinity model	Steady state affinity KD (M)	Rmax (RU)
GLP-1(7-37)	Semaglutide	1:1 binding	6.51e+05	7.45e-02	1.14e-07	162.9	1.20e+02	7	Steady state affinity	9.35e-08	162.8

Figure 4. GLP-1R SPR binding assay by Biacore. The signal changes during protein association and dissociation were recorded, and the affinity and kinetic parameters could be fitted.

ICE_Obesity Panel Establishment

Assay Targe	Assay Format
CALCR	Agonist
CALCR-RAMP	Agonist
CCKAR	Agonist
CB1	Inverse agonist
GCGR	Agonist
GIPR	Agonist/Antagonist
GLP1R	Agonist
MC4R	Agonist
NPY1R	Agonist
GPR75	Antagonist
HCRTR1	Antagonist
HCRTR2	Antagonist

Specie	GLP-1R(EC50/nM)	GIPR(EC50/nM)	GCGR(EC50/nM)
human	0.015	0.014	0.0003
mouse	0.007	0.200	0.003
rat	0.017	0.037	0.002
chicken	0.028	0.029	/
dog	0.022	0.018	/
rabbit	0.021	0.007	/

Figure 5. Obesity-Relevant Target Profiling: A. GPCR receptors related to obesity and metabolic disease were chosen to construct the ICE_Obesity Panel. B. Different species of glucagon receptors（GLP-1R, GIGR, GCGR）stable cell lines were established for species selectivity screening.

5. CNS-related targets validation by FLIPR assay and HTRF IP1 assay

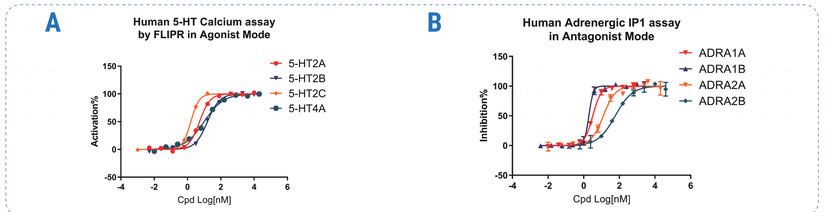
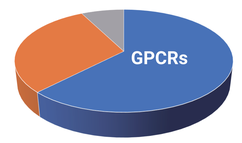
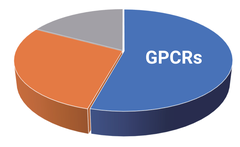


Figure 6. CNS-related targets validation by FLIPR assay, HTRF IP1 assay, and HTRF cAMP assay. A. The Calcium 6 kit from Molecular Devices provides a homogeneous assay designed to work for the majority of GPCRs, including multiple CNS-related targets. The cells stably express the serotonin family receptors that were used to conduct calcium flux assay by FLIPR. B. HTRF IP1 assay was constructed to identify adrenergic receptors

ICE_GPCR CNS Safety Panel Establishment (Based on FDA and EMA Guidance Files)



Panel Name	Target	Assay
ICESTP DrugAbuse SafetyPanel	53	99
ICESTP CNS Core SafetyPanel	70	123



ICESTP DrugAbuse SafetyPanel　　ICESTP CNS Core SafetyPanel

Figure 7. GPCRs involved in CNS panel establishment. Based on as an initial assessment of both the abuse potential of drugs and CNS off-target screening. The guideline issued by FDA and EMA, the ICE has developed the drug dependent panel which could serve. A. DrugAbuse SafetyPanel includes 33 GPCRs, which are related in dopamine, serotonin,gamma-aminobutyric acid (GABA), opioid, cannabinoid, etc. B. The ICESTP CNS Core SafetyPanel includes 38 GPCRs. All the assays included in this panel are functional in vitro assays.

Family	Receptor	Family	Receptor	Family	Receptor	Family	Receptor
Adrenoceptors	Alpha 1A	Acetylcholine	M1	Dopamine	D1	Cannabinoid	CB1
	Alpha 1B		M2		D2L		CB2
	Alpha 1D		M3		D2S	Orexin	OX1
	Alpha 2A		M4		D3		OX2
	Alpha 2B		rat M4		D4	Chemokine	CCR1
	Alpha 2C		mouse M4		D5		CCR2
	Beta 1		monkey M4	Opioid	delta		CCR5
	Beta 2		dog M4		kappa		CCR6
5-Hydroxytryptamine	5-HT1A		rabbit M4		mu		CCR7
	5-HT1B		M5		rat mu		CCR8
	5-HT1F	Tachykinin	NK1		mouse mu		CCR9A
	5-HT2A		rat NK1		NOP		CCR9B
	5-HT2B		mouse NK1		rat NOP		CXCR1
	5-HT2C		NK2		mouse NOP		CXCR2
	5-HT4A		mouse NK2	Somatostatin	SSTR1		CXCR4
	5-HT4B		rat NK2		SSTR2	Class A Orphan	GPR6
	5-HT5A		NK3		SSTR3		GPR84
	5-HT7A		rat NK3		SSTR4		GPR40
	5-HT7B		mouse NK3		SSTR5		MRGPX2

Summary

We successfully constructed multiple stable cell lines expressing GPCRs and lots of function assays, which could be applied in compound screening until get the clinical candidate. In addition, the GPCR panel was suitable for the validation of agonists, inverse agonists, antagonists, or even biased agonists and PAM. The establishment of the GPCR panel and multiple function assays provide powerful tools for drug discovery and screening.

References

[1] World Obesity Federation, World Obesity Atlas 2023. https://data.worldobesity.org/publications/?cat= 1

[2] Norskov-Lauritsen, L., A.R. Thomsen, and H. Brauner-Osborne, G protein-coupled receptor signaling analysis using homogenous time-resolved Forster resonance energy transfer (HTRF(R)) technology. Int J Mol Sci, 2014. 15: .2554-72.

[2]R., Zhang, X., Xie, Tools for GPCR drug discovery. Acta Pharmacologica Sinica, 2012. 33: 372-384.