Anti-Obesity Related Target Assay Development and Application

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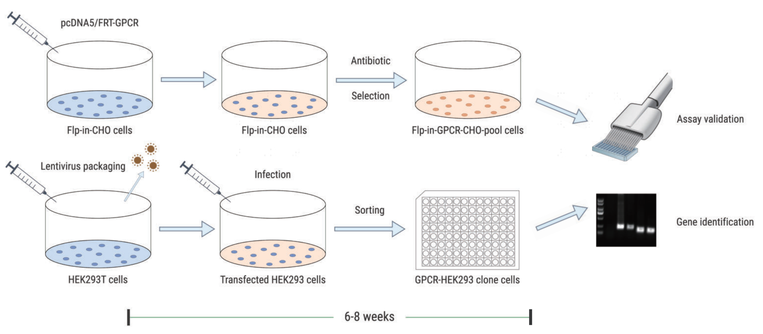
Introduction

In the past 50 years, obesity has become an international public health issue that affects the quality of life, increases the risk of illness, and raises health-care costs in countries in all parts of the world. Anti-obesity drugs will be the most impactful trend of 2024, followed by personalized and precision medicine, immuno-oncology drug development, real-world evidence and cell and gene therapies. Research all over the world has been carried about around multiple anti-obesity targets on different mechanism like genetic, epigenetic, physiological, behavioural, sociocultural, and environmental factors.

Here we briefly introduced our Biology Building Blocks platform which including the target, assay and technology for Cannabinoid Receptor 1 (CB1), the Apelin receptor (APJ) and G Protein-Coupled Receptor 75 (GPR75), which are all related to anti-obesity pathway with different mechanisms. This study described the recombinant protein purification, cell line generation, functional assay development and application. Meanwhile, several CDX modes are constructed for promisingly conducting in-vivo experiments and biomarker detection. Combine with small molecules library, AI/ML and MedChem collaboration, our Bioscience platform may accelerate drug discovery process.

GPCR Overexpression Cell Line Construction Strategy



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

* Indicates that the protein is available for use in SPR

Figure 1. A. GPCR Cell Line Generation. Flip-In-CHO cell system and Lentiviral system were used to generate stable cell pool or monoclonal for assay validation and screening. Currently we have 175 GPCR overexpression cell line ready-to-use.**B. Obesity-Relevant Target Profiling:** GPCR receptors related to obesity and metabolic disease were chosen to construct the ICE_Obesity Panel.

Results

1. HTRF cAMP Assay

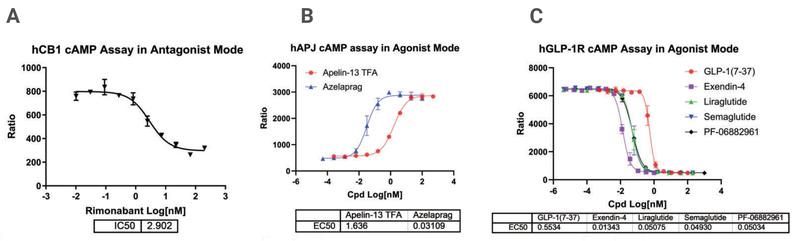
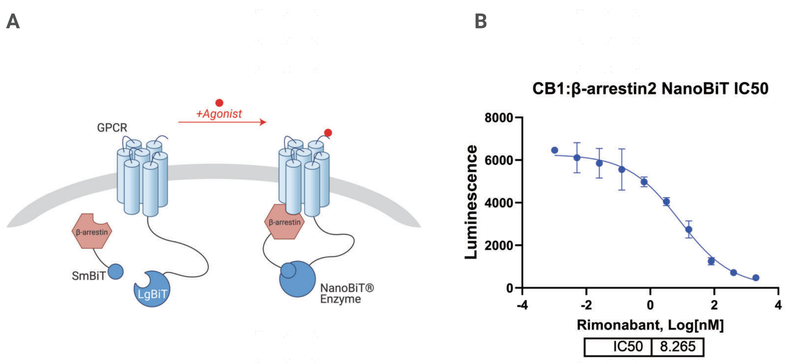


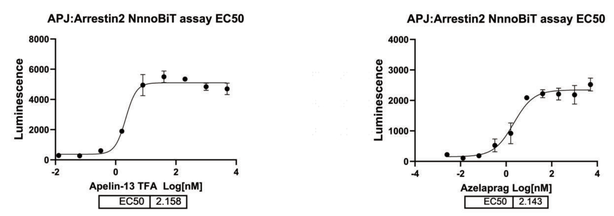
Figure 2. HTRF cAMP assay was constructed for compound screening. The principle of this assay is grounded in HTRF (Homogeneous Time-Resolved Fluorescence) technology. It is formatted as an immunoassay for quantifying cAMP levels, which are generated in response to the modulation of adenylyl cyclase activity by GPCRs. A&B&C . Human CB1, APJ and GLP1R stable cell lines were constructed for agonist, inverse agonist and antagonist validation.

2. β-Arrestin NanoBiT Assay



C

APJ NanoBiT assay_Compound: Apelin-13 TFA & Azelaprag



D

GLP1R NanoBiT assay _Compound: GLP1(7-37)

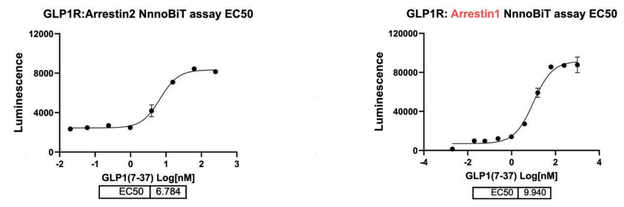
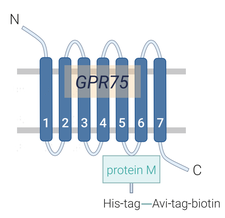


Figure 3. Monitoring Recruitment of the GPCR and β-Arrestin in Cells. A. The NanoBiT® assay operates on the principle that when two protein tags are in proximity to one another, the energy from a bioluminescent luciferase donor can be transferred non-radiatively to an acceptor tag, resulting in the emission of light from the acceptor. Transfer of energy is dependent on the distance and orientation of the tags to each other. Consequently, the assay can be effectively utilized to detect and measure the interactions occurring between proteins. **B.** The NanoBiT assay was used to characterize the CB1 and β-arrestin recruitment. **C.** Apelin-13 TFA & Azelaprag are uesde in the APJ beta-arrestin2 NanoBiT assay. **D.** GLP1R and beta-arrestin1/2 NanoBiT assay was developed for dru discovery and compounds screening.

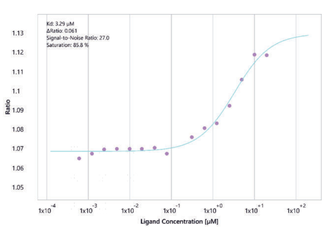
3. Biophysical Assay for GPCR Recombinant Protein

A



Spectral shift

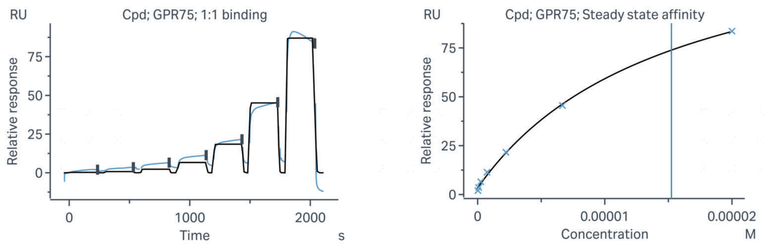
Dianthus



Ligand	Evaluator
Compound	Spectral Shift
KD	Signal-to-Noise Ratio
3.29 μM	27.0

B

SPR Analysis –GPR75 vs. Compound

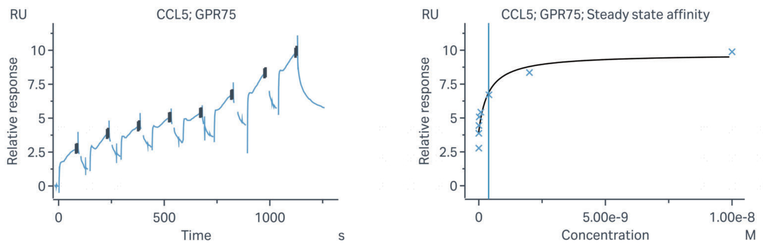


0.02743 0.0823 0.24691 0.74074 2.22222 6.66667 20 μM

Immobilized ligand	Injection variables Single cycle kinetics 1 Solution	Cpd MW	Steady state affinity KD (M)	Rmax (RU)
GPR75	Compound	359.46 Da	1.52e-05	141.0

C

SPR Analysis - GPR75 vs. CCL5

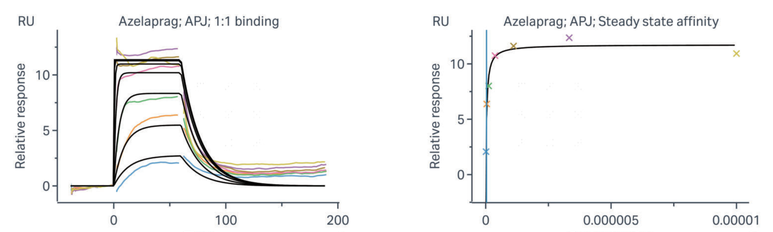


0.000128 0.00064 0.0032 0.016 0.08 0.4 2 10 nM

Immobilized ligand	Injection variables Single cycle kinetics 1 Solution	Affinity model	Steady state affinity KD (M)	Rmax (RU)
GPR75	CCL5	Steady state affinity	3.88e-10	5.7

D

SPR Analysis - APJ vs. Azelaprag



0.014 μM 0.041 μM 0.12 μM 0.37 μM 1.11 μM 3.33 μM 10 μM

Immobilized ligand	Injection variables Analyte 1 Solution	Kinetics model	1:1 binding ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
APJ	Azelaprag	1:1 binding	1.28e+06	5.69e-02	4.44e-08	11.4

Figure 4. GPCR Binding Assay by Dianthus and Biacore 8K. A. MST-TRIC (Microscale Thermophorescence with Transient Intermolecular Complex) is a biophysical technique that combines MST with the detection of transient intermolecular complexes. This technology was used to study interactions between molecules and GPR75. **B&C&D.** SPR (Surface Plasmon Resonance) binding assay is a biophysical method that uses surface plasmon resonance technology to monitor molecular interactions in real time. This technique was used to study interactions between molecules or CCL5 and GPR75(B,C), also the binding between APJ protein and Azelaprag (D).

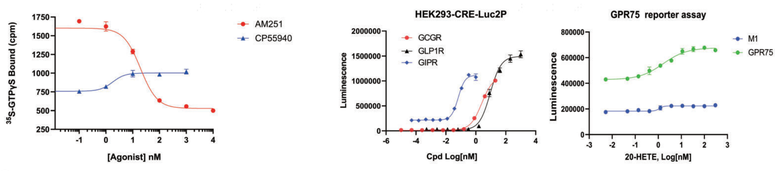
4. Other GPCR Function Assay Establishment

A

35S-GTPγS binding assay

B

Reporter assay



C

GLP1R internalization assay- Compound: Sumaglutide

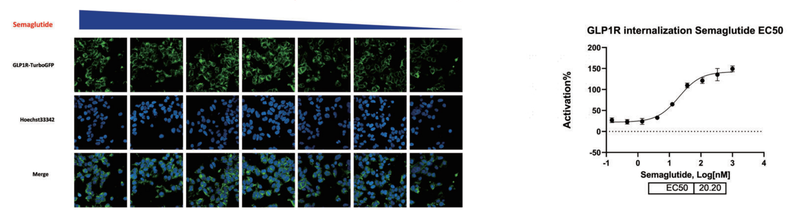


Figure 5. A. 35S-GTPγS binding assay was used to study the efficacy of CB1R compounds. **B.** Different receptors stable cell lines were established for application in multiple screening assays. GLP1R,GIPR and GCGR stable cell lines were used in GPCR CRE-Luc reporter assay, and GPR75-SRE-Luc reporter assay can measure the activation or inhibition of GPR75 in response to different compounds. **C.** The cell expressing GLP1R, which fused with GFP protein, employ HCS to measure the GFP intensity. Semaglutide was used to activate GLP1R.

5. Efficacy Testing of Semaglutide on DIO Mouse Model

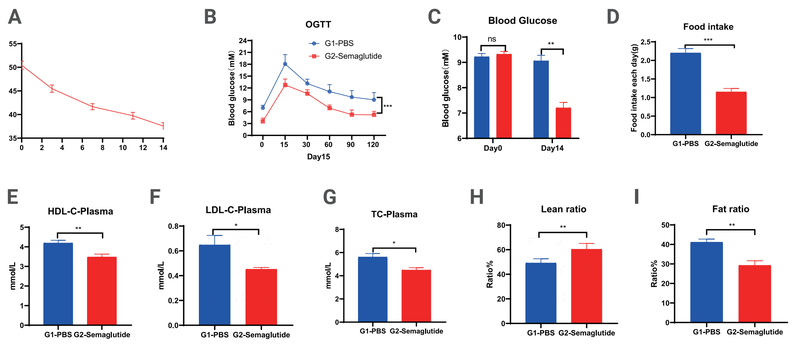


Figure 6. Efficacy of Semaglutide on High fat diet induced obese (DIO) mouse model. A. Body weight changes after 2 weeks of treatment with semaglutide. **B.** Glucose tolerance test at Day15. **C.** Fasting blood glucose levels on Day 0 and Day 14 after 6-hour fasting. **D.** Average daily food intake during the experimental process. **E&F.** Plasma cholesterol of high density of lipoprotein (HDL-C) and cholesterol of low density of lipoprotein(LDL-C) after 2 weeks of treatment with semaglutide . **G.** Plasma total cholesterol (TC) after 2 weeks of treatment with semaglutide . **H&I.** Lean ratio and fat ratio tested by MRI after 4-weeks treatment of semaglutide. N=5. t-test, *p＜0.05, **p＜0.01, ***p<0.001.

Summary

We have successfully developed a range of stable cell lines that express GPCRs, which can be used in various functional assays, suitable for application in the compound screening process to identify clinical candidates. Moreover, our GPCR SPR platform is well-suited for validating the binding between different types of compounds eg. peptide and small molecular and proteins from the perspective of biophysics. The creation of this GPCR cell lines, coupled with the diverse functional assays in vitro and in vivo, offers robust tools for the discovery and screening of new drugs.

References

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