Targeting G Protein-Coupled Receptors in Cancer Pharmacotherapy: A comprehensive screening platform establishment and application in drug discovery

Xiaoyan Wang, Dandan Hu, Tiejun Bing
GPCR Department, ICE Bioscience InC.

Innovative CRO+Explorer
ICE Bioscience

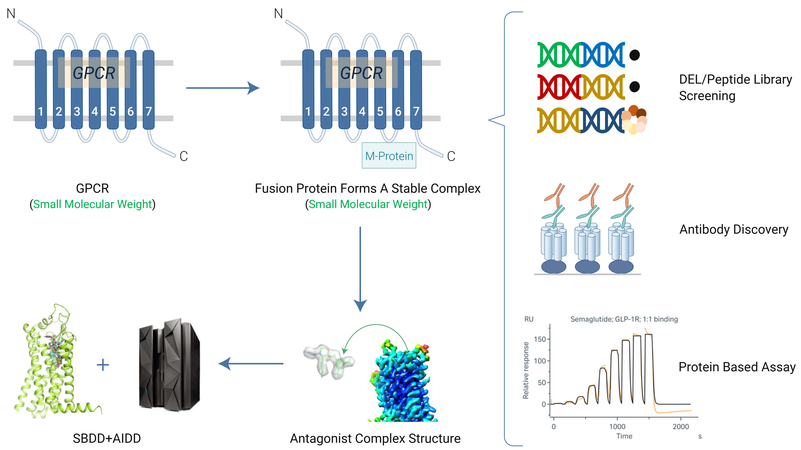
Abstract Number: 5677

Introduction

G protein-coupled receptors (GPCRS) are the largest family of cell surface receptors (~800 members in humans). It can be activated by various stimuli and couple with different G proteins to initiate multiple signaling pathways. Dysregulation of GPCR is related to a variety of human diseases and disorders, such as metabolic diseases, cardiovascular diseases, hypertension, including cancer initiation and development.

This research established the biophysical binding platform. MegaR-engineered GPCR protein was purified for Spectral Shift technology, one of the approaches possible using the Dianthus, and Surface Plasmon Resonance assay, which is the “gold standard” for detecting interaction between ligand and targets. Furthermore, over 170 GPCRs stable cell lines were constructed for multiple assays establishment to promote GPCR drug discovery and screening. The cell-based functional assays like HTRF cAMP assay, ADCC reporter assay and β-arrestin2 recruitment are suitable for agonist, antagonist and inverse agonist validation. Consequently, the GPCR platform offers a comprehensive screening cascade for the assessment of compounds, thereby supporting diverse methodologies that are instrumental in advancing the discovery of novel anti-cancer therapeutics.

GPCR Protein Production



A	ADORA2A	ADRA2B	ADRB1	ADRB2	AGTR1	AGTR2
	APLNR (APJ)	BDKRB2	BRS3	C3AR1	C5AR1	C5AR2
	CB1	CCKAR	CCKBR	CCR2	CCR3	CCR4
	CCR5	CCR6	CCR7	CCR8	CCR9	CMKLR1
	CXCR1	CXCR2	CXCR3	CXCR4	CXCR5	CXCR6
	DRD2	DRD3	DRD4	EDNRA	EDNRB	FFAR1
	FPR1	FPR2	FPR3	GALR1	GHSR	GNRHR
	GPR35	GPR39	GPR52	GPR75	GPR84	GRPR
	HCRTR2	HRH1	HTR1A	HTR1B	HTR2B	HTR2C
	LPAR1	LPAR2	LPAR3	MC3R	MC4R	MCHR1
	CHRM1	CHRM2	CHRM3	CHRM4	CHRM5	MLNR
	MTNR1B	NPBWR2	NPFFR1	NPFFR2	NPY1R	NPY4R
	NTSR1	OPRD1	OPRK1	OPRL1	P2RY1	PRLHR
	PTGDR	PTGDR2	PTGER1	PTGER2	PTGER3	PTGER4
	RXFP3	S1PR1	SSTR2	SSTR3	SSTR4	SSTR5
B1	CALCR	CALCRL	GCGR	GHRHR	GIPR	GLP1R
	CRHR1	CRHR2	PTH1R	PTH2R	VIPR1	VIPR2
C&F	GPRC5C	GPR158	GPRC5D	SMO	FZD7	

Figure 1. MegaR technology enables GPCRs to achieve enhanced stability. To date, we have successfully produced over 120 GPCRs. This technique maintains the ligand-free, native conformation of MegaR-engineered GPCRs at their transmembrane and extracellular regions, making them particularly suitable for small-molecule and peptide screening and antibody discovery.

MegaR-engineered CCR8 Protein

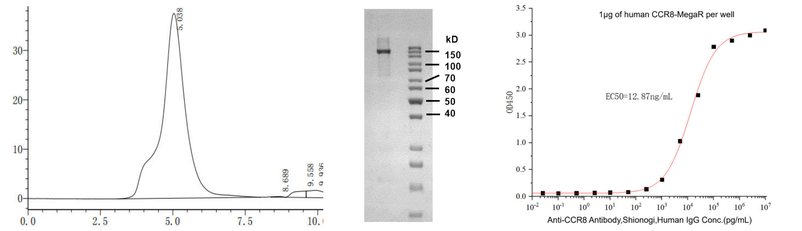


Figure 2. A. High-performance liquid chromatography (HPLC) analysis results of a compound. The x-axis represents retention time (minutes), and the y-axis represents signal intensity (mV). B. Analysis of the purified protein by SDS-PAGE. The final sample is highly pure as observed by western blotting with an anti-CCR8 antibody. C. Dose-response relationship of the anti-CCR8 antibody (Shionogi, human IgG) and CCR8 protein by Elisa. The x-axis indicates antibody concentration (pg/mL), and the y-axis shows absorbance at 450 nm (OD450). The EC50 value of 12.87 ng/mL, indicating the half-maximal binding concentration.

CCR8 Cellular Assay Establishment

Family	Target/cell	Ready assay
Chemokine	CCR1-CHO	HTRF cAMP
	CCR2-HEK	HTRF cAMP
	CCR3-CHO	HTRF cAMP
	CCR4	CCR4 Chemotaxis assay on CD4+(Treg)
	CCR5-CHO	HTRF cAMP
	CCR6-CHO	HTRF cAMP
	CCR7-CHO	HTRF cAMP
	CCR8-HEK	HTRF cAMP
		ADCC reporter assay
	CCR9A-CHO	HTRF cAMP
	CXCR1-CHO	HTRF cAMP
	CXCR2-HEK	HTRF cAMP
	CXCR3-CHO	HTRF cAMP
	CXCR4-CHO	HTRF cAMP

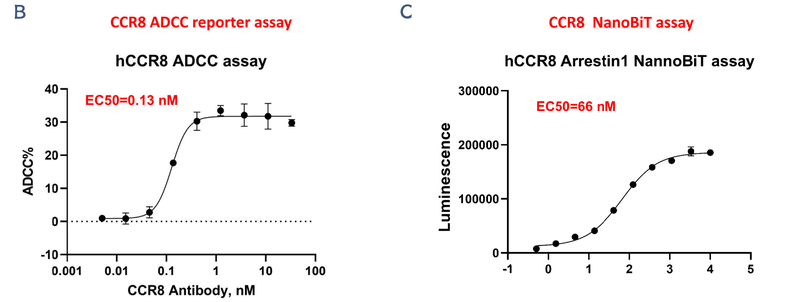
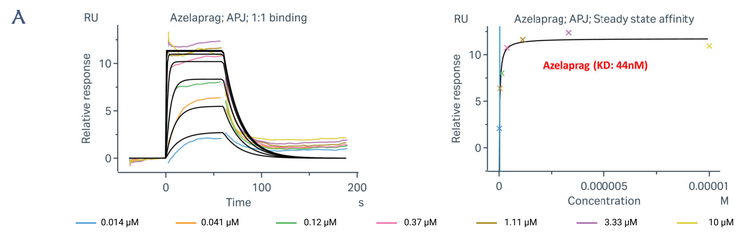


Figure 3. CCR8 function assay construction for compound validation. Chemokines are a class of small signaling proteins that play a role in inflammation and cell migration during immune responses, and are associated with the occurrence and progression of cancer. Agents for several chemokine receptors such as CCR6, CCR8 and CXCR2 are currently in clinical trials, the CC and CXC receptor overexpression cell panel were construction for compound and off-target screening. A. AZ084 is an oral active CCR8 allosteric antagonist, which restrains the formation of the immunologically tolerant pre-metastatic niche (PMN) and tumor cells metastasis in lung by downregulating Treg differentiation, can be used in studies of cancer. HTRF cAMP Assay was constructed to test the AZ084 potency with CCR8-CHO stable cell. B. CCR8 antibody is being studied for solid tumours therapeutic, Antibody-dependent cell-mediated cytotoxicity, ADCC reporter assay was used to detect the activity of anti-CCR8. C. ZK756326 is a nonpeptide CCR8 agonist, the NanoBiT assay was constructed to characterize the CCR8 and β-arrestin1 recruitment stimulated by 756326 in CCR8/β-arrestin1 co-expression cell.

Screening Assay Establishment for APJ

Apelin/APJ is involved in the development and poor prognosis of a variety of cancers, such as lung cancer, liver cancer, cholangiocarcinoma, breast cancer, glioblastoma, prostate cancer, ovarian cancer, and so on. Accumulating evidence has also shown that the apelin/APJ system acts as a biomarker and predictor of postoperative effects in multiple cancers, which can also affect the tumor microenvironment and the efficacy of cancer immunotherapy. Considering that the apelin/APJ system may be a potential target for cancer treatment, it is of great significance for the study of new cancer treatment targets.



C

Item	Description	Assay	Apelin-13 (EC50 nM)
Cell based assay	hAPJ function assay	HTRF cAMP	1.7
		β-arrestin2 recruitment assay	3.4
	Selectivity	CHO-Mouse APJ	3.35
		CHO-Rat APJ	2.69
		CHO-Monkey APJ	1.77
		CHO-Dog APJ	16.85
	Off-target screening	PDE selectivity panel (10 and 26 targets)	Ready
		GPCR panel (13 targets)	Ready
		Safety panel (44 and 90 targets)	Ready

Figure 4. APJ screening assay construction for compound validation. A. Surface Plasmon Resonance (SPR) is an optical method that enables the real-time measurement of molecular interactions and determination of the equilibrium dissociation constant (KD), quantifying the strength of molecular binding affinity. Azelaprag, functioning as a balanced agonist for the APJ receptor, was utilized as a reference compound in an SPR assay alongside the MegaR-engineered APJ protein, yielding a dissociation constant (KD) of 44 nM. B. Spectral Shift (SPS) technology, one of the approaches possible using the Dianthus, was employed to determine the binding affinity between the APJ receptor and Azelaprag, the KD is 51 nM, which aligns with the findings from SPR assay. These two biophysical binding assays are helpful for identifying binders during the early stages of drug discovery. C. Multiple cell-based assays were developed for compound validation, meeting target MOA study, species selectivity, and safety panel screening.

Summary

- Access to high-quality GPCR protein was achieved using MegaR technology to improve protein solubilization and stabilization from cells.
- The purified protein's stability and functionality were confirmed through SPS and SPR analyses, indicating that it retained its native state and activity throughout the screening process.
- More than 170 GPCR overexpression cells were ready to develop different cell-based assays, eg. HTRF cAMP assay, β-arrestin2 recruitment assay or internalization assay.

References

[1] Lorente JS, Sokolov AV, Ferguson G, Schiöth HB, Hauser AS, Gloriam DE. GPCR drug discovery: new agents, targets and indications. Nat Rev Drug Discov. 2025 Mar 3. doi: 10.1038/s41573-025-01139-y. Epub ahead of print. PMID: 40033110.

[2] Korbecki J, Grochans S, Gutowska I, Barczak K, Baranowska-Bosiacka I. CC Chemokines in a Tumor: A Review of Pro-Cancer and Anti-Cancer Properties of Receptors CCR5, CCR6, CCR7, CCR8, CCR9, and CCR10 Ligands. Int J Mol Sci. 2020 Oct 15;21(20):7619. doi: 10.3390/ijms21207619. PMID: 33076281; PMCID: PMC7590012.

[3] Chen J, Li Z, Zhao Q, Chen L. Roles of apelin/APJ system in cancer: Biomarker, predictor, and emerging therapeutic target. J Cell Physiol. 2022 Oct;237(10):3734-3751. doi: 10.1002/jcp.30845. Epub 2022 Aug 7. PMID: 35933701.