Generation of Biased Ligand Screening Prediction Platform

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Introduction:

G protein-coupled receptors (GPCRs) are essential drug targets, constituting 30% of clinically used drugs. Biased agonism as a drug discovery paradigm, wherein GPCRs signal through specific pathways, is of significant interest. However, the current understanding of effector driven GPCR conformational changes is limited due to standard binding assay constraints, which routinely measure only low-affinity receptor forms. Nanobodies provide an alternative measure but are expensive, receptor-specific, and time-consuming to produce.

Our research aims to bridge this knowledge gap by developing a universal system for studying drug affinity for specific receptor-effector conformations. We propose a fusion protein model system that combines GPCRs and modified G protein to recapitulate native receptor-effector conformations, eliminating the need for nanobodies.

Aims of the project:

(1) Identify candidate ligands capable of inducing biased agonism, either towards G_s protein or arrestin signalling pathways, specific to the β_2 adrenergic receptor (β_2AR).
(2) Investigate the kinetics of drugs binding to different receptor-effector combinations.
(3) Explore the potential impact of temperature and salt levels on experimental outcomes.

Methods:

Saturation binding assays: Fluorescent propranolol affinity was determined through saturation binding assays employing terbium-labelled fusion protein within an assay buffer composed of Hank's Balanced Salt Solution (HBSS), 5 mM HEPES, 0.1% BSA, and 0.02% F-127 detergent. Total binding was assessed with a DMSO solution, while nonspecific binding was assessed with 1 µM alprenolol.

The dissociation kinetics of F-propranolol were assessed by introducing a high saturation concentration of 1 µM alprenolol to F-propranolol (40nM) which was prebound to the receptors.

Competition binding assays: This assay contained a fixed concentration of green propranolol (10nM), a serial dilution of competitor, and terbium-labelled fusion protein in assay buffer. Assays were conducted at room temperature. Subsequently, data were obtained using a PHERAstar FSX plate reader, processed with MARS software, and subjected to further analysis within GraphPad PRISM 10.

Result:

β_2AR	Saturation Binding		Association	Dissociation	
	K_d (nM)	B_{max}	K_{on} ($M^{-1}min^{-1}$)	K (min^{-1})	Half-life (0.693/k)
WT	4.40 ± 0.67	2243.5 ± 552.86	11620540 ± 2893259	0.08 ± 0.005	8.48 ± 0.53
mG_s	4.40 ± 0.95	1942 ± 127.04	10563274 ± 6824183	0.03 ± 0.001	21.39 ± 0.81
Arrestin	3.69 ± 0.44	1791.6 ± 120.22	12943659 ± 2165306	0.06 ± 0.005	11.00 ± 0.90

Table 1: *Dissociation constants (K_d) and maximum receptor densities (B_{max}) in β_2AR-based binding assays for both the wild-type (WT) and miniG-coupled (mG) forms, alongside with ligand binding association and dissociation rates.*

Result 1: F-propranolol has similar receptor affinity for WT, mG_s and arrestin fusions.

Result 2: The differences observed in fluorescent propranolol's binding kinetics. The association rates across the different membrane preparations were similar but dissociation rates of F-propranolol differed for different receptor-effector combinations, suggestive of different receptor conformations.

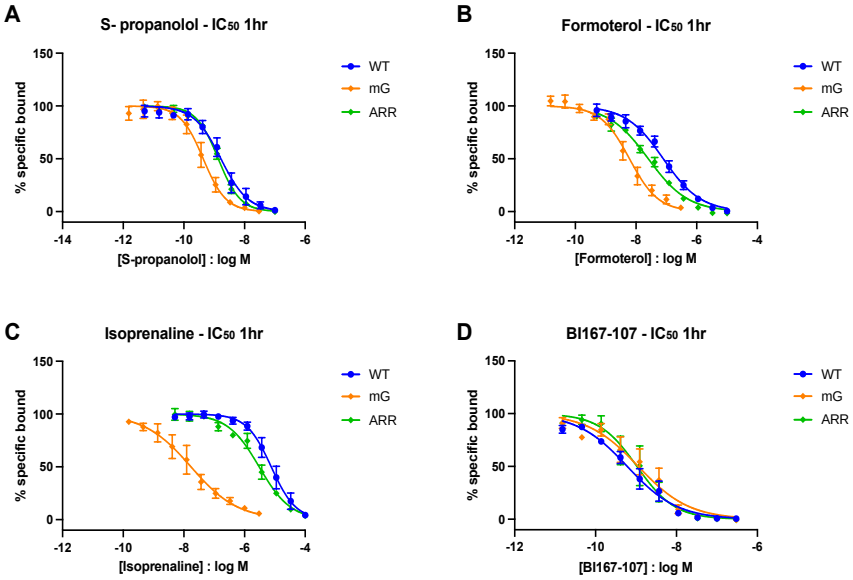

Figure 1: *Concentration-response curves showing half maximal inhibitory concentration (IC_{50}) values of (A) S- propanolol; (B) Formoterol; (C) Isoprenaline; (D) BI167-107, competing with F-propranolol for different receptor conformations (native WT, mG and arrestin bound). Measurements were taken at the 1-hour time point. Data are mean ± S.E.M.*

Compounds	*WT*	*mG*	*Arrestin*
S – Propanolol	9.18 ± 0.19	9.62 ± 0.18	9.35 ± 0.08
Formoterol	7.51 ± 0.11	8.61 ± 0.17	8.09 ± 0.12
Isoprenaline	5.58 ± 0.14	7.94 ± 0.09	6.07 ± 0.11
BI167-107	9.03 ± 0.14	8.76 ± 0.11	9.29 ± 0.11

Table 2: *Experimental pK_i values for the competitors shown in Figure 1, which bind to the β_2AR-based WT, mG-coupled and arrestin bound forms, are presented. Values are presented as the mean ± S.E.M. with data from at least three independent experiments.*

Result 3: A clear and distinct shift is observed in favor of mG compared to arrestin for certain competitors.

S-propanolol, formoterol, and isoprenaline display bias behaviour, characterised by their higher potency and stronger affinity for mG compared to arrestin and WT receptors.

Competitor BI167-107 presents a neutral response for binding to either β_2AR-based WT or mG forms. These findings are supported by the IC_{50} curves and the pKi values presented in Figure 1 and Table 2 respectively.

Outcomes:

In this study, using the β_2AR as a model system, we demonstrate that our fusion proteins effectively measure high affinity binding compared to the wild-type receptor, offering a promising avenue for studying bias agonism at GPCRs. This innovative approach eliminates the need for nanobodies, ensuring native receptor-effector conformations. Our methodology provides an efficient and cost-effective means of investigating GPCRs, and drug induced conformational changes, with the potential to discover effective biased agonists. This research has the potential to lead to more effective treatments for a variety of diseases.

Future directions:

This methodology can be extended to explore the interactions with other β_2AR-coupled proteins, such as G_i, thereby expanding our knowledge of GPCR signalling pathways. Furthermore, its versatility can be leveraged for studying a wide range of GPCR receptors, enabling a comprehensive understanding of their functional characteristics and bias agonisms. These insights have the potential to drive drug discovery efforts, potentially leading to the development of novel therapies for a variety of diseases, ultimately improving healthcare options and patient outcomes.

Values of studentship to student and the lab:

During my time in Veprintsev lab, I developed proficiency in conducting binding assays and utilising Prism software for data analysis. I have also enhanced my critical thinking and scientific communication skills. This period also ignited my passion for GPCR research and reinforced my commitment to pursuing a biochemistry-related Ph.D.

Looking back, I deeply appreciate the unwaving support from my supervisors and lab colleagues, and the generous funding provided by the Biochemical Society, which were instrumental in my growth and achievements.

The studentship also contributed to the ongoing Veprintsev lab research and will be featured in upcoming publications.

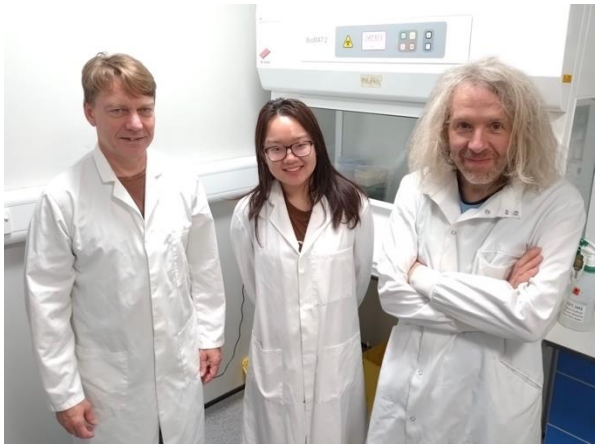

Figure 3: *Image of Prof. Dmitry Veprintsev, Ngan Phan and Dr David Sykes. From left to right.*