Assay and array technologies for G-protein coupled receptors

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Abstract

The overall aim of this thesis is to investigate strategies to aid in the measurement of G-protein coupled receptor (GPCR) activity for high-throughput screening and sensing applications. GPCRs are cell surface receptors which have seven membrane spanning domains. They are the largest family of membrane proteins in the human genome and are involved in a number of physiological and pathophysiological pathways. They are the most widely targeted protein family for therapeutics being the target for over 30% of the currently available prescription drugs (Jacoby *et al.* 2006). For this reason commercial interest and investment into compound screening using these receptors as targets is of high importance in lead drug discovery. Additionally, the extensive ligand range of the GPCR superfamily, which includes light, odorants/ volatiles, neurotransmitters and hormones, make them an attractive biological recognition element in biosensor applications.

This thesis demonstrates the functional expression of the H_1-histamine, M_2-muscarinic and α_{2a}-adrenergic receptors of the G-protein coupled receptor family, along with their associated G-proteins (Gα, Gβ and Gγ). Expression was achieved using the *Sf9*/baculovirus expression system. The G-proteins were successfully incorporated into an assay system using time-resolved fluorescence resonance energy transfer (TR-FRET). TR-FRET was used in order to create a homogeneous assay format capable of monitoring GPCR activation through the movement of the G-protein subunits. Fluorescence changes in the TR-FRET assay indicated a change in distance between the Gα subunit and G$\beta\gamma$ dimer. The separation of the Gα subunit and the G$\beta\gamma$ dimer after activation resulted in a significant decrease in TR-FRET measurement.

The homogeneous set-up of the TR-FRET assay could potentially be adaptable to an array based format. This thesis describes the capture of vesicles containing functional GPCRs onto a solid substrate via the specific interaction between complementary oligonucleotides. GPCR presence and function within the immobilized vesicles, was demonstrated using fluorescent ligands. Further to this, alternative lipid hosts (to the vesicles), known as cubosomes, were introduced. When tagged with an oligonucleotide, these cubosome particles were also shown to immobilize site specifically onto a complementary oligonucleotide surface.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

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Abbreviations

α_{2a}-AR	α_{2a}-adrenergic receptor
[^{35}S]GTPγS	Radioactive isotope S^{35} conjugated, non-hydrolysable guanosine triphosphate
AMP-PNP	Adenylyl- 5`-imidodiphosphate tetralithium salt
AFM	Atomic force microscopy
a.f.u	Arbitrary fluorescence units
a.u	Arbitrary units
AlF_4^-	Tetrafluoroaluminate
βγ	G-protein beta gamma dimer
BA1	Biotinylated oligonucleotide (A1 sequence)
BB1	Biotinylated oligonucleotide (B1 sequence)
BCIP	5-bromo-4-chloro-3`-indolyphosphate p-toluidine salt
Bmax	The maximum value for specific binding (number of functional receptors)
BODIPY	4,4-difluoro-5,7-dimethyl-4-bora-3a, 4a-diaza-s-indacene-3-alkyl
bp	Base pairs
BRET	Bioluminescence resonance energy transfer
BSA	Bovine serum albumin
C-terminus	Carboxyl terminus of polypeptide
CA1`	Cholesterol tagged oligonucleotide (A1` sequence [complementary to A1])
CB1`	Cholesterol tagged oligonuceotide (B1` sequence [complementary to B1])
CCR5	Chemokine receptor
CHAPS	(3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate)
CHO	Chinese hamster ovary
CLSM	Confocal laser scanning microscopy
CNS	Central nervous system
CS124	7-amino-4-methyl-2(1H)-quinoline
CSIRO, MHT	Commonwealth scientific & industrial research organisation, molecular and health technologies
Cys	Cysteine residue
dCA1`	Double cholesterol tagged oligonucleotide (A1` sequence [complementary to A1])
dCB1`	Double cholesterol tagged oligonucleotide (B1` sequence [complementary to B1])
DHB	2,5-Dihydroxybenzoic acid
DMSO	Dimethyl sulfoxide
DNA	Deoxyribose nucleic acid
DTPA	Diethylenetriaminepentaacetate
E.coli	*Escherichia coli*
EC_{50}	Effective concentration at 50%
FITC	Fluorescein isothiocyanate
FRET	Fluorescence resonance energy transfer
G-protein	Guanine nucleotide binding protein
Gα	G-protein alpha subunit
GDP	Guanosine-5`-diphosphate
GF/C	Glass fibre filter (class c)
GFP	Green fluorescent protein

GPCR	G-protein coupled receptor
GTP	Guanosine-5`-triphosphate
GTPγS	Non-hydrolysable guanosine triphosphate
H_1R	H_1-histamine receptor
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
hexahistidine	Six sequential histidine residues
His6	Hexahistidine
HTS	High throughput screening
IgG	Immunoglobulin G
K_d	Dissociation constant
Ki	Dissociation constant (obtained in competition binding studies)
M_2R	M_2-muscarinic receptor
MALDI MS	Matrix-assisted laser desorption/ionisation mass spectrometry
MANT	N-methylanthraniloyl (fluorescent conjugate)
MOI	Multiplicity of infection
mRNA	Messenger ribonucleic acid
NA	Numerical aperture
N-terminus	Amino terminus of the polypeptide
NBT	Nitro-blue tetrazolium chloride
Net TR-FRET	FRET fluorescence @ 572nm/donor fluorescence @ 545nm
Ni-NTA	Nickel-nitrilotriacetic acid
OG	Oregon green
PBS	Phosphate buffered saline
PEG	Polyethylene glycol
PEG-biotin	Polyethylene glycol with 50% of the side chains consisting of a biotinylated polyethylene glycol
PFU	Plaque forming units
PLL	Poly-L-lysine
PLL-*g*-PEG	Graft copolymer: Poly(L-lysine)-grafted-poly(ethylene glycol)
PDMS	Polydimethylsiloxane
PMSF	Phenyl methyl sulfonyl fluoride
QCM-D	Quartz crystal microbalance with dissipation
R_o	Distance between two spectrally matched fluors at which half the energy is transferred during resonance energy transfer
RET	Resonance energy transfer
RGS	Regulator of G-protein signalling
RNA	Ribonucleic acid
RT	Room temperature
SEM	Standard error of the mean
S/B	Signal to background
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Sf9	*Spodoptera frugiperda 9*
SPR	Surface plasmon resonance
TAC	Telenzepine amine congener

Tb	Terbium
TEG	Tri-ethylene glycol
TEM	Transmission electron microscopy
TIRFM	Total internal reflection fluorescence microscopy
TR	Time-resolved
Tris	Tris hydroxymethylaminoethane
TR-FRET	Time-resolved fluorescence resonance energy transfer
UK	Synthetic adrenaline analogue (5-bromo-N-[4,5-dihydro-1H-imidazol-2-yl]-6-quinoxalinamine)
UV	ultraviolet