

Assay and array technologies for G-protein coupled receptors

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Contents

1	Introduction.....	1
1.1	How and why: investigating G-protein coupled receptors as recognition molecules in biosensors and biochips?	1
1.2	G-protein coupled receptors.....	3
1.2.1	Structure and function.....	3
1.3	G-proteins.....	8
1.3.1	Structure and function.....	8
1.4	GPCRs and G-proteins: potential for therapeutics.....	11
1.5	Current assay technologies for monitoring G-protein coupled receptors	13
1.5.1	Cell-based assays	13
1.5.2	Cell-free assays	14
1.5.3	Conformational changes in the receptor and/or G-proteins	17
1.6	Creating protein arrays.....	18
1.6.1	Array reading	18
1.7	Membrane protein immobilisation strategies.....	19
1.7.1	Supported and tethered lipid bilayers.....	19
1.7.2	Supported lipid bilayers from native membrane sources.....	20
1.7.3	Immobilised vesicles.....	21
1.8	Optical and biophysical techniques to monitor GPCR function.....	21
1.9	Chapter summary	22
1.10	Scope of thesis	23
2	GPCR and G-protein expression, preparation and purification	25
2.1	Introduction.....	25
2.1.1	Choosing an appropriate expression system	25
2.1.2	Baculovirus amplification and infection	28
2.1.3	Model receptors.....	29
2.1.4	Membrane preparations of GPCRs	35
2.2	Materials and methods	35
2.2.1	Materials.....	35
2.2.2	Methods.....	37
2.3	Results and discussion	43
2.3.1	Receptor expression	43
2.3.2	Radioligand competition assay	45
2.3.3	G-protein purification	49
2.3.4	Functional [³⁵ S]-GTPγS reconstitution assays	52
2.4	Chapter summary	53
3	Monitoring Specific G-protein Interactions by Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET)	54
3.1	Introduction.....	54
3.1.1	Fluorescence techniques in high-throughput screening	54
3.1.2	Protein labelling	55
3.1.3	Fluorescence resonance energy transfer	57
3.1.4	Spectrally matched fluorophores for FRET applications.....	58
3.1.5	G-protein TR-FRET assay	62
3.1.6	Assay development and assessment.....	67

3.2	Materials and methods	67
3.2.1	Materials.....	67
3.2.2	Methods.....	68
3.2.3	Data analysis	72
3.3	Results and discussion	72
3.3.1	Fluorescently labelled G-protein activity.....	72
3.3.2	Characterisation of G-protein TR-FRET Assay.....	74
3.3.3	Z'-factor.....	75
3.3.4	GTP γ S binding to G α subunit in the absence of receptor.....	76
3.3.5	Validation of G-protein interaction assays.....	79
3.3.6	Increasing assay throughput.....	83
3.3.7	TR- FRET reconstitution assays	84
3.4	Chapter Summary.....	88
4	Surface immobilisation of GPCR molecular assembly.....	89
4.1	Introduction	89
4.1.1	GPCR array technologies.....	89
4.1.2	Functional vesicle arrays.....	90
4.1.3	Oligonucleotide directed vesicle immobilisation.....	93
4.1.4	Alternative protein scaffolds.....	94
4.2	Materials and methods	97
4.2.1	Materials.....	97
4.2.2	Methods.....	98
4.3	Results	105
4.3.1	Membrane extracts	105
4.3.2	Electron microscopy.....	106
4.3.3	Lipid extraction	108
4.3.4	Tagging vesicles: cholesterol-lipid molar ratios	109
4.3.5	Capture of oligonucleotide tagged native vesicles.....	110
4.3.6	Extruding membrane extracts	112
4.3.7	Site-directed immobilisation of extruded GPCR vesicles.....	115
4.3.8	Spotted arrays.....	118
4.3.9	Specific G-protein immobilisation with GPCR vesicles.....	122
4.3.10	An alternative membrane protein array platform.....	125
4.4	Chapter summary	131
5	Final discussion - applications and future directions	133
5.1	Summary of thesis.....	133
5.2	Applications	135
5.3	Future directions.....	135
5.4	Conclusion	137
	Bibliography.....	138
6	Appendices.....	166
6.1	No changes in TR-FRET indicate little non-specific interactions	166
6.2	Extrusion changes turbidity of membrane extracts.....	166
6.3	Phase diagrams of monoolein and phytantriol.....	167
6.4	Multiplexing arrays	168
6.5	Calculating total lipid concentration in membrane extracts.....	168

6.6	Bradford protein assay of extruded membrane extracts.....	170
6.7	TAC-Alexa binding to M ₂ -muscarinic receptor vesicles	170
6.8	Patterned oligo arrays.....	172
6.9	Quantitative analysis of arrays	172
6.10	Manuscript	174

List of Figures

Figure 1.2.1 Schematic representing membrane spanning GPCR and coupled G-protein heterotrimer.	4
Figure 1.2.2 Pharmacological model for G-protein coupled receptor activation.....	6
Figure 1.3.1 Schematic representing the activation cycle of the G-protein heterotrimer	10
Figure 2.3.1 ³ H-ligand binding to <i>Sf9</i> membrane extracts containing the H ₁ -histamine receptor.....	43
Figure 2.3.2 Specific ³ H-ligand binding in <i>Sf9</i> membranes.....	44
Figure 2.3.3 M ₂ R radioligand competition assay with pirenzepine.....	46
Figure 2.3.4 M ₂ R radioligand competition assay with atropine and TAC.....	47
Figure 2.3.5 Ligand affinity for the H ₁ -histamine receptor with or without fluorescent label.....	48
Figure 2.3.6 Gα _{i1} His purification PAGE.....	49
Figure 2.3.7 Gβ ₄ γ ₂ purification PAGE.....	50
Figure 2.3.8 Gα _q purification PAGE.....	50
Figure 2.3.9 Gα _{i1} and Gα _q PAGE and Western Blot:.....	51
Figure 2.3.10 [³⁵ S]-GTPγS Functional Reconstitution Assay.....	52
Figure 3.1.1 Inter and intra-molecular FRET.....	58
Figure 3.1.2 Fluorophore structures.....	59
Figure 3.1.3 Spectral overlap.....	60
Figure 3.1.4 Emission lifetime.....	61
Figure 3.1.5 [³⁵ S]-GTPγS reconstitution assay with labelled proteins.....	63
Figure 3.1.6 Time Resolved Resonance Energy Transfer between Gα and βγ.....	64
Figure 3.1.7 Unlabelled binding partners compete for binding to Gα _{i1} or Gβ ₄ γ ₂ causing a decrease in TR-FRET.....	65
Figure 3.1.8 AlF ₄ ⁻ decreases G-protein affinity.....	66
Figure 3.3.7 Increasing assay throughput from 96 to 384 well plates.....	84
Figure 4.1.1 Schematic of vesicle immobilisation using complementary oligonucleotides.....	94
Figure 4.1.2 Cubic phase surface curvatures.....	95
Figure 4.2.1 Schematic representation of the immobilisation protocol.....	101
Figure 4.2.2 Experimental summary for production of a GPCR array platform.....	105
Figure 4.3.1 Transmission electron microscopy images of non-extruded M ₂ R membrane preparations.....	107
Figure 4.3.2 Transmission electron microscopy images of non-infected cell membrane vesicles.....	108
Figure 4.3.3 DNA specific <i>Sf9</i> cell membrane vesicle immobilisation.....	111
Figure 4.3.4 QCM data showing similar final immobilisation masses for all non-extruded receptors.....	112
Figure 4.3.5 Cryo TEM of extruded M ₂ R vesicles.....	113
Figure 4.3.6 ³ H-ligand binding in <i>Sf9</i> membranes.....	114
Figure 4.3.7 [³⁵ S]-GTPγS functional reconstitution assay.....	115
Figure 4.3.8 <i>Sf9</i> cell membrane vesicle immobilisation.....	116
Figure 4.3.9 QCM data showing changes in frequency for various sizes of vesicles...	117
Figure 4.3.10 Confocal Laser Scanning Microscopy of H ₁ R containing vesicle attachment to complementary oligonucleotide modified surfaces.....	119
Figure 4.3.11 Replicate dots show little non-specific binding on neutravidin.....	119

Figure 4.3.12 Fluorescent ligand binding to vesicles containing the M ₂ -muscarinic receptor.....	120
Figure 4.3.13 Gα _{i1} His Alexa capture with immobilised M ₂ -muscarinic receptor in oligo-tagged vesicles	122
Figure 4.3.14 Cubosome preparations	127
Figure 4.3.15 Site specific immobilization of cubosomes	129
Figure 6.1.1 TR-FRET of heterotrimer formation and βγ subunit specific competition	166
Figure 6.2.1 Extruding membrane extract.	167
Figure 6.3.1 Phase diagrams of (a) monoolein (Qiu & Caffrey 2000) and (b) phytantriol (Barauskas & Landh 2003).	167
Figure 6.4.1 Example of multiplexed array strategy.....	168
Figure 6.6.1 Bradford protein assay results prior to and post extrusion of membrane extracts	170
Figure 6.7.1 Specific binding of TAC-Alexa to vesicles containing the M ₂ -muscarinic receptor.....	171
Figure 6.7.2 Relative fluorescence intensity of specific immobilisation of liposomes containing the M ₂ -muscarinic receptor as measured by TAC-Alexa binding.	171
Figure 6.8.1 Site-specific rhodamine cubosome binding on array pattern	172
Figure 6.9.1 Relative fluorescence intensity of site specific cubosome binding	173
Figure 6.9.2 Relative fluorescence intensity of site specific vesicle binding	173

List of Tables

Table 2.1.1-1 Comparison of the main advantages and disadvantages of various commonly used expression systems	28
Table 2.1.3-2 Binding affinity (K_i)* (mean values) of agonists, inverse agonists and antagonists at human cloned M_2R in HM2-B10 cells.....	33
Table 2.1.3-3 Binding affinity (K_i)* (mean values) of agonists, inverse agonists and antoagonists at human cloned H_1R in Cos-7 cells	34
Table 2.2.1-1 Buffer Compositions.....	37
Table 4.3-1 A summary of the overall changes in frequency and dissipation for both extruded and non-extruded membrane extracts	118
Table 4.3-2 Cubosome size determination.....	127

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 H₁-histamine, M₂-muscarinic and α_{2a} -adrenergic receptors of the G-protein coupled receptor family, along with their associated G-proteins (G α , G β and G γ). 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 G α subunit and G $\beta\gamma$ dimer. The separation of the G α 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]GTP γ S	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
$\beta\gamma$	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 oligonucleotide (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
<i>E.coli</i>	<i>Escherichia coli</i>
EC ₅₀	Effective concentration at 50%
FITC	Fluorescein isothiocyanate
FRET	Fluorescence resonance energy transfer
G-protein	Guanine nucleotide binding protein
G α	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
GTP γ S	Non-hydrolysable guanosine triphosphate
H ₁ R	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
K _i	Dissociation constant (obtained in competition binding studies)
M ₂ R	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
<i>Sf9</i>	<i>Spodoptera frugiperda</i> 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