

Welcome to the

2nd GPCR-UK Network Meeting 2026

#GPCRUK2026

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Second GPCR-UK Network Meeting

3rd - 4th of September 2026
Advanced Research Centre, Glasgow

GPCR-UK.org

University of Glasgow

ABOUT

Organising Committee:

Local Organising Committee

Nicole Perry-Hauser (University of Glasgow)
Brian Hudson (University of Glasgow)
Abbey Begen (University of Glasgow)

ECR Committee

Abbey Begen (University of Glasgow)
Moa Safar (Imperial College London)
Katie Sharrocks (The Francis Crick Institute)

GPCR-UK Executive Committee

Meritxell Canals (University of Nottingham)
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PROGRAMME

Thursday 3rd September 2026

Exhibition Space ARC Engage, 237ABC

14:00 Welcome ECR networking

15:00 Registration

15:30 Keynote – **Brian Bender** (Isomorphic Labs) and **Andrew Brown/Maria Waldhoer** (Ikherma)

16:30 Poster & Sponsor Flash Talks

18:00 **GPCR-UK Network Ceilidh** — GUU, Debates Chamber

Friday 4th September 2026

Exhibition Space ARC Engage, 237ABC

Session 1 – Context-specific GPCR signalling

09:30 **Maria Shchepinova** (University of Bath)

Proximity proteomics approaches to resolve GPCR interactomes

09:50 **Emily Russell** (BioAscent)

Context-dependent pharmacology of 5-HT_2 receptors – implications for Drug Discovery

10:10 **Maria Marti-Solano** (University of Cambridge)

Reconstructing GPCR signalling networks to uncover cell-specific rewiring and new pathway to phenotype relationships

10:30 **Bianca Plouffe** (Queen's University of Belfast)

When Membranes Decide: Lipid Control of GPCR Signalling Specificity

10:50-11:10 Coffee break

Session 2 – Structural insights into GPCRs

11:10 **Isabella Russell** (AstraZeneca)

Cryo-EM structures of constitutively active PTH1R mutants

11:30 **George Hedger** (Imperial College London)

Subcellular lipid modulation of mitochondrial GPCRs

11:50 **Satya Bhamidimarri** (Domainex)

From Native Membranes to Drug Discovery: Stabilizing Native GPCRs in Polymer Nanodiscs

12:10 **Peter McCormick** (University of Liverpool)

12:30-14:30 Lunch break

13:00-14:00 Poster Session Judging (Exhibition Space ARC Engage Lobby)

Session 3 – GPCR function in physiology and disease

14:30 **Caroline Gorvin** (University of Birmingham)

MMRAP2: an accessory for appetite regulation

14:50 **Rob Lane** (University of Nottingham)

Targeted inhibition of mu-opioid receptors in neuronal subpopulations by membrane-tethered Naloxo-DART antagonists

15:10 **Stuart Mundell** (University of Bristol)

Inverse agonism at platelet G protein-coupled receptors in patients with acute coronary syndromes

15:30 **Brian Hudson** (University of Glasgow)

Physiological FFA4 Free Fatty Acid Receptor Signalling in 3D Cultured Adipocytes

16:00 Closing remarks & Prizes



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EVENTS:

ECR networking

Are you an Early Career Researcher (ECR)? If so, we'd love for you to join us for the **ECR Networking Event** on Thursday 3rd of September at 2pm!

We are keen to help build a stronger and more connected ECR community across GPCR-UK. We know conferences can be a great opportunity to meet new people, but it's not always easy to know who to talk to or how to start a conversation, so we're hoping to make it a little easier and more fun!

Our networking event will be a fun, informal speed networking session, giving you the chance to meet other ECRs, hear about the research they're working on, share your own experiences and hopefully make some new friends and connections along the way.

☕ **Coffee, tea and light refreshments will be provided**

📍 **The event will take place at the conference venue (ARC) in Glasgow**

🤝 **Open to ECRs attending the GPCR-UK Network Meeting**

You don't need to be a networking expert, have a big research project to talk about, or know anyone beforehand. The whole point is to give everyone an easy way to meet and chat with fellow ECRs from across the community.

We'd love to see as many of you as possible and hope this can be the start of an even more connected and supportive **GPCR-UK ECR community**.

Your ECR representatives for GPCR-UK, Abbey, Katie and Moa.

Dinner and Ceilidh

The meeting this year includes a social event on Thursday evening, featuring a buffet dinner and a traditional Scottish ceilidh. The event will begin immediately after the flash talks and will take place at the Glasgow University Union, a short 10-minute walk from the meeting venue.

For those who have not attended a Scottish ceilidh before, you can expect an evening of lively group dancing with plenty of hopping, jumping, and spinning. No previous experience is required, as the dances will be called and explained throughout the evening.

Please keep the energetic nature of the dancing in mind and wear suitable footwear. Lightweight clothing, or layers that can be removed, is also recommended.



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PRACTICAL INFORMATION

Venues:

The meeting will take place at the Exhibition Space ARC Engage, 237ABC. The ARC is located on the Gilmorehill Campus of the University of Glasgow:

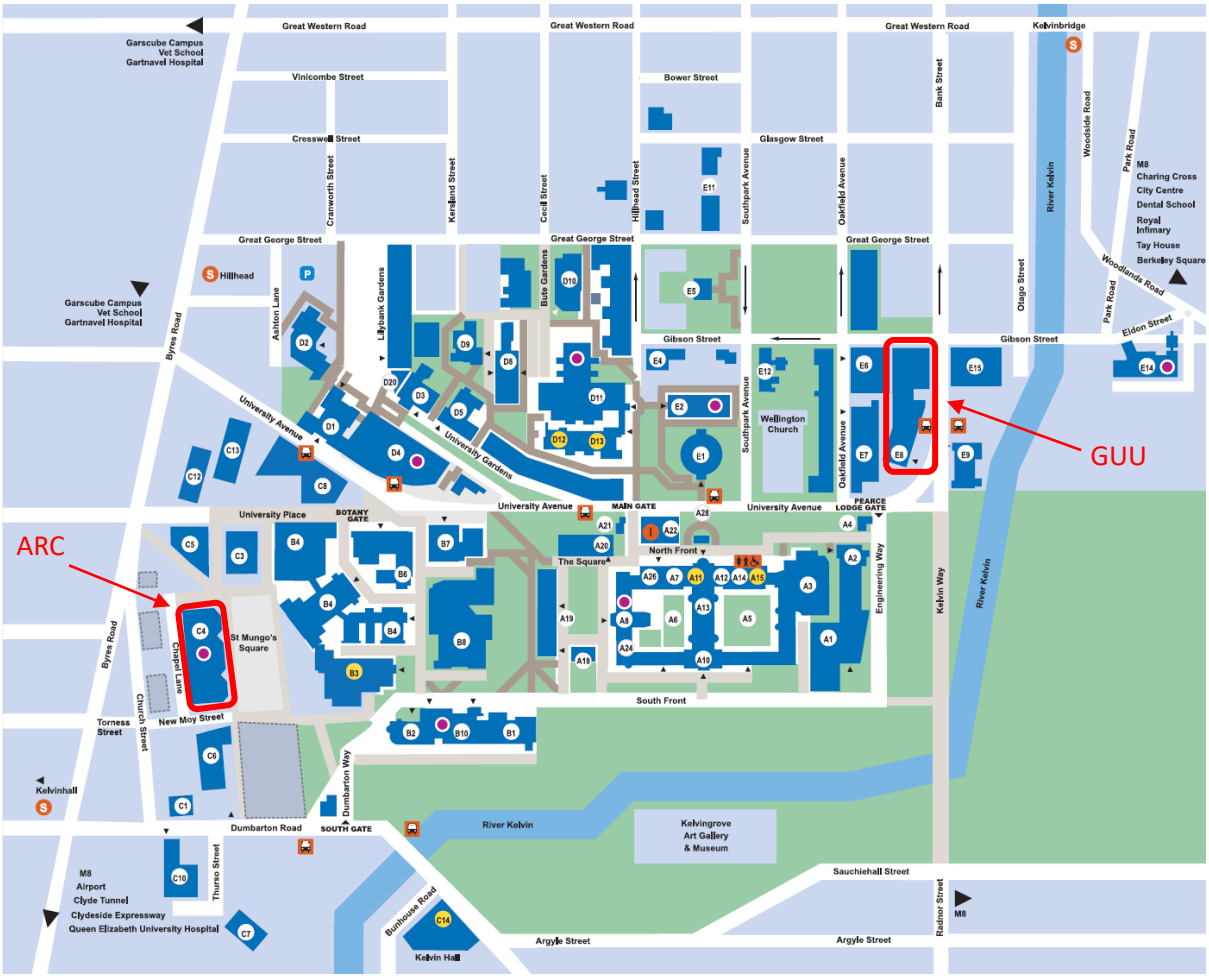
The Mazumdar-Shaw Advanced Research Centre

11 Chapel Lane, University of Glasgow, G11 6EW

More details on how to travel to the ARC are available on the ARC website.

The dinner and ceilidh will take place at the Glasgow University Union (GUU) building:

32 University Ave, Glasgow G12 8LX



Sustainability tip:

To help reduce waste and support our sustainability goals, we kindly encourage all attendees to bring a reusable water bottle. Water refill stations will be available throughout the venue. Thank you for helping us make this event more environmentally friendly.



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POSTER PRESENTATION

Posters will be available to view for the duration of the meeting. Poster should be printed in portrait orientation and A1 size. Poster presentation will be held during the lunch break (13:00-14:00) on Friday the 4th of September. Poster prizes will be awarded at the end of the meeting.

#	Presenter	Affiliation	Title
1	Rudi Prihandoko	Sygnature Discovery	Signalling diversity and subtype selectivity of serotonergic psychedelics
2	Yier Yao	University of Liverpool	Comparative Structure-Based Virtual Screening for CB2-Selective Allosteric Modulators
3	John Pediani	University of Glasgow	FRET real-time tracking of cAMP activity in live cancer cells to identify which PDE target therapeutic drugs have the optimal potential to halt cancer cell tumour proliferation.
4	Sarah Hungerford	University of Glasgow	Real time monitoring of GPCR activity at early endosomes using genetically encoded biosensors
5	Emem Ekong	University of Glasgow	Species selectivity in Succinate Receptor synthetic agonists is largely driven by a single residue in ECL1
6	Katie Brannan	University of Bristol	ATP demonstrates agonism at the platelet P2Y12 receptor
7	Chan Yi Ang	University of Bristol	GRK-independent Recruitment of β-arrestin to the P2Y12 Receptor
8	Eilidh McKay	University of Glasgow	Structural and functional consequences of naturally occurring human GPR84 single nucleotide polymorphisms
9	Ashley Smith/Thomas Gruhl	BMG LABTECH	Dual Channel Kinetic assays for detecting ligand bias at GPCRs
10	Alison Porter	BioAscent	Using label-free cellular impedance to characterise GPR35 regulation in an endogenous cell system.
11	Lily Tosh	University of Strathclyde	Computational Modelling of GnRHR Conformational Activation Dynamics
12	Peter Ashdown	University of Aberdeen	ICL1 in Opioid Receptor Effector Engagement
13	Samantha Collister	Queen's University Belfast	Investigating the spatial properties of EP2 activation by different agonists
14	Rowan Ives	University of Oxford	Identification of Mammalian Fatty Acid Metabolism-Derived Endogenous Agonists Acting at the Orphan Receptor GPR84
15	Adam Wright	Queen's University Belfast	Investigation of the role of Frizzled 6 receptor in diabetic retinopathy
16	Louise Cope	University of Aberdeen	IPR-PDE1C in Pulmonary Hypertension: Uncovering the Signalosome
17	Salsabeel Shaalan	University of Nottingham	Janelia Fluor® Probes for Live-Cell Imaging of Adenosine A2A and A2B Receptors
18	Rana Dawood	University of Nottingham	Genetically encoded intrabody biosensors to detect activation states of the atypical chemokine receptor 3 (ACKR3)
19	Kyara De Oliveira Taborda	University of Cambridge	CaSR Signalling in Sensory Neurons: A GPCR Mechanism for Visceral Pain in IBS
20	Emily Cope	University of Aberdeen	FPR2 regulation by GRKs: implications for pro-resolution pharmacology
21	Aude Aravinthan	University of Nottingham	GRK-dependent trafficking of NK1R and its impact on subcellular signalling dynamics
22	Abbey Begen	University of Glasgow	Mapping Muscarinic Circuits: Unravelling mAChR1 and mAChR4 Expression and Connectivity in the Brain
23	Maryah Kotbi	University of Nottingham	TRPing receptors – modulatory effect of TRP channels on GPCRs
24	Olivia Lucianno	University of Glasgow	Exploring Succinate Receptor Signalling in the Mitochondria
25	Euan Purdie	University of Glasgow	FFA4 inverse agonism and Piezo1 agonism regulate cellular mechanics of 3T3- L1 adipocytes
26	Andreia Moniz e Castro	University of Glasgow	Measuring Gi/o subtype signalling by metabolite sensing GPCRs with novel BRET-based biosensors



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27	Claire Kelly	University of Nottingham	Dissecting the Subcellular Trafficking and Signalling of PAR2
28	Alexander Demby	University of Oxford	GPCRs actively specify the spatiotemporal compartmentalisation of cAMP signalling in cardiac myocytes
29	Sakura Likar	University of Nottingham	Investigating the Interactome of Human Adenosine Receptor A2A at Endogenous Expression Levels
30	Sara Fazzini de Alba	University of Cambridge	FFA3 activation engages visceral sensory pathways associated with dysbiosis-mediated abdominal pain
31	Christina Thomson	University Of Aberdeen	Pharmacological characterisation of novel FPR2 ligands reveals distinct receptor subtype and species selectivity
32	Anna Moelders	University of Aberdeen	GPR75 – Cracking the Function Code
33	Richard Anozia	University of Nottingham	Developing novel bifunctional compounds for targeted proteolytic degradation of CXC Chemokine Receptor 2 (CXCR2)
34	Amberene Qayyum	University of Nottingham	Investigating the Ability of NanoLuciferase and mGOLD-tagged Nanobody AD101 to Target Active-State A2A Receptors in Living Cells
35	Jack Campbell	University of Glasgow	Spatial Biology, a Novel Strategy to Better Understand Opioid Addiction Pathology
36	Xintong Yang	University of Bristol	Evaluating AI-Based Predictions of Ligand Binding to the P2Y12 Receptor
37	Ruijing Yin	University of Glasgow	Novel Ligand to explore the function of GPR84
38	Isabella Maiellaro	University of Nottingham	Sensitisation Drives Dendritic Localisation of TrpA1 mRNA and Enhances Nociceptive Behaviour in Drosophila
39	Paolo Annibale	University of St Andrews	Effects of altered gravity on β-adrenergic-mediated cAMP signalling in intact cells
40	Areej Abuhammad	University of Jordan/University of Bristol	Structural Investigation of FFAR1 Activation Using Cryo-Electron Microscopy
41	Rose Wellens	University of Bristol	G protein coupling class dictates GPCR susceptibility to ERAD
42	Abigail Pearce	University of Cambridge	A NanoBRET Conformational Biosensor for the Glucagon-Like Peptide 1 Receptor
43	Mona Reichel	University of Nottingham	What β-arrestin2 conformational change fingerprints can teach us: a cross-receptor comparison
44	Zsombor Koszegi	University of Birmingham	Single-molecule analysis reveals long-lived RAMP1–GPCR complexes in living cells
45	Tianyi Ding	University of Bristol	The Novel Synthetic Opioid Cychlorphine is a highly potent agonist at the μ opioid receptor
46	Tayla Rees	University of Birmingham	Free fatty acid receptor 4 (FFAR4) variants associated with disease have distinct signalling profiles
47	Li-Chiung Lin	University of Glasgow	Mechanisms of GPR35 Signalling in Protection Against Hepatic Steatosis
48	Lucy Adam	University of Nottingham	Differential regulation underlies unappreciated complexity of pH-sensing GPCR signalling
49	Moa Safar	Imperial College London	Impact on Kisspeptin Receptor (KISS1R) Regulation from its Interaction with G Protein-Coupled Estrogen Receptor (GPER)
50	Ashraf Alruwaili	University of Glasgow	Ortholog Selective Agonism at Succinate Receptor 1 Reveals Species-Dependent Differences in G Protein Activation
51	Katie Sharrocks	The Francis Crick Institute	PD-PALM single-molecule microscopy as a tool to study membrane receptor oligomerization
52	Noemi Karsai	University of Nottingham	Complex Formation Between A2A and A2B Adenosine Receptors in Live Cells.
53	Aqfan Jamaluddin	University of Birmingham	Spatiotemporal APEX2 proximity proteomics reveals potential novel protein interactions of the ghrelin receptor (GHSR)
54	Haoyang Ying	King's College London	Phosphokinome analysis of spatially restricted, ligand specific and temporal GPCR signalling.
55	Abdul-Akim Guseinov	University of Glasgow	GPR84: from structure to pharmacology and bias
56	Alexandre Jean	Revvity	pHSense Eu Reagents for Time-Resolved Fluorescence-based monitoring of GPCR internalization
57	Joshua Sopp	Promega	Seeing β-arrestin recruitment in real time

POSTER ABSTRACTS

#1 Signalling diversity and subtype selectivity of serotonergic psychedelics

Rudi Prihandoko, Justin Kewney, Eleanor Parker, Grace Chan, Emma Crawford, Daniel Smith, Rob Clayton and Lesley Gerrard.
Sygnature Discovery - Cellular Pharmacology. Telford Pavilion, Todd Campus, West of Scotland Science Park, Glasgow, G20 0XA.

Serotonergic psychedelics are a class of compounds that target the serotonergic system and are currently under investigation as therapies for psychiatric disorders, including anxiety and major depression. Whilst the primary molecular target for psychedelic drugs is the 5-HT_{2A} receptor, many psychedelics can also act as agonist at multiple 5-HT receptor subtypes. Interactions with receptors including 5-HT_{1A}, 5-HT_{2B} 5-HT_{2C} 5-HT_{6} and 5-HT_{7} may contribute to both therapeutic and adverse effects making these important targets for pharmacological investigation. Furthermore, differences in agonist intrinsic efficacy and signalling profiles at the 5-HT_{2A} receptor have been proposed as mechanisms that may enable development of therapeutically beneficial compounds with reduced hallucinogenic liability. Therefore, we aim to establish a comprehensive suite of cell-based assays to characterise the signalling diversity, efficacy and receptor selectivity of psychedelics in support of drug discovery programs targeting the serotonergic system.

#2 Comparative Structure-Based Virtual Screening for CB2-Selective Allosteric Modulators

Yier Yao[1], Peter McCormick[1]
[1] University of Liverpool, Liverpool, UK

The cannabinoid receptor 2 (CB2) is a promising therapeutic target, but achieving selectivity over the closely related cannabinoid receptor 1 (CB1) remains challenging. In this study, we developed a comparative structure-based virtual screening workflow to identify putative CB2-selective allosteric modulators. Allosteric binding pockets in CB2 and CB1 were prepared using available receptor structures, followed by molecular docking and comparative analysis of predicted binding poses, scores and protein–ligand interactions. Candidate compounds showing favourable predicted binding within the CB2 allosteric pocket, together with weaker or less consistent interactions in the corresponding CB1 pocket, were prioritised. Several shortlisted compounds formed predicted interactions with key CB2 pocket residues, including Gly122, Leu125, Leu126, Ser161, Phe197 and Phe200. The resulting candidates represent preliminary computational hits with potential CB2-selective allosteric profiles. This workflow provides a foundation for further molecular dynamics simulations, selectivity assessment and experimental validation of candidate CB2 allosteric modulators.

#3 FRET real-time tracking of cAMP activity in live cancer cells to identify which PDE target therapeutic drugs have the optimal potential to halt cancer cell tumour proliferation.

John D. Pediani, Alina Zorn and George S. Bailley
School of Molecular Biosciences, University of Glasgow.

Introduction: Breast cancer and colorectal cancer are among the most common and impactful diseases globally. Phosphodiesterase 4B (PDE4B) and the PDE7 family regulate intracellular cyclic AMP (cAMP) levels, making them critical knockout targets to induce apoptosis and halt tumor proliferation in colorectal cancer (CRC) and breast cancer, respectively.
Objectives: This study aimed to spatially map real-time, FRET-linked cAMP activity in living cancer cells using the EPAC-SH187 biosensor and FRET microscopy, while identifying the most effective PDE-targeted drugs to prevent cAMP degradation.
Methods: FRET microscopy tracked conformational sensor changes in human colorectal carcinoma cells (HCT166 cell line) and breast cancer cells (MCF7 cell line), where a decreasing FRET ratio directly quantified rising cAMP accumulation following PDE inhibition.
Results: In the colorectal cell line, the PROTAC KTX207 degrader drug suppressed PDE4B activity and accumulated cAMP significantly more effectively than the small-molecule catalytic inhibitor BI1015550 (BI). In the breast cancer cell line (MCF7), all assessed PDE7 inhibitors successfully reduced PDE7B activity.
Conclusion: In live HCT166 cells event-driven PROTAC KTX207 targeted degradation offers a more robust, transformative therapeutic oncology strategy than occupancy-driven PDE inhibition. By driving robust cAMP accumulation and overcoming the limits of conventional inhibitors, PROTACs represent a vital therapeutic oncology treatment pathway. Furthermore, assessing diverse small-molecule modulators across distinct isoforms (such as PDE7B in MCF7 cells) highlights the enormous viability of manipulating cyclic nucleotide pathways in oncology cell model cell lines to identify which drugs have the optimal potential to effectively disrupt and halt cancer cell tumour proliferation. Furthermore, FRET imaging is a highly effective technique for screening therapeutic drug modulators to disrupt cancer cell proliferation.

#4 Real time monitoring of GPCR activity at early endosomes using genetically encoded biosensors

Sarah Hungerford, Brian D. Hudson
Centre for Translational Pharmacology, University of Glasgow

G protein-coupled receptors (GPCRs) regulate a wide range of physiological processes and are an important therapeutic target. Recent studies have demonstrated GPCRs signalling from intracellular compartments and there is particularly strong evidence for GPCR signalling from early endosomes (EE), in addition to the plasma membrane (PM). This has led to a need for biosensors to detect EE GPCR activity across Gα subfamilies (Gαs, Gαi, Gαq and Gα13). Here we develop a novel bioluminescence resonance energy transfer (BRET)-based biosensor to measure GPCR activation, through binding with the G protein α5 helix from each Gα subfamilies within the EE. Furthermore, to confirm whether G-protein coupling

corresponds to G-protein activation, assays were performed using the synthetic peptide KB1753 and effector p63RhoGEF, which detect GTP bound Gi and Gq respectively. Findings from these experiments demonstrated that most GPCRs were able to couple to their respective G protein α5 helix at both the PM and EE, although the degree of EE activation varied across receptors. However, studies with KB1753 and p63RhoGEF suggested that despite most receptors being able to couple to their G protein in the EE, only some receptors actually produced effective coupling in the EE, leading to GTP bound G protein. This study establishes new tools to study intracellular GPCR signalling, and uses these tools to demonstrate diversity in the way GPCRs signal at the EE.

#5 Species selectivity in Succinate Receptor synthetic agonists is largely driven by a single residue in ECL1

Emem Ekong, Ashraf Alruwaili, Brian D. Hudson
Centre for Translational Pharmacology, University of Glasgow

Succinate receptor (SUCNR1) is a metabolite-sensing GPCR that primarily signals through Gi proteins, linking extracellular succinate to inflammatory and metabolic responses. Although SUCNR1 has emerged as a promising therapeutic target, marked differences in receptor pharmacology between species present a challenge for translating preclinical findings. Notably, clear differences are observed among synthetic SUCNR1 agonist between mouse (m) and rat (r) SUCNR1: Cmp 131(S) is specific for rSUCNR1, while TUG-1688 and TUG-1689 are specific for mSUCNR1. In this study, we have identified two residues within the predicted SUCNR1 ligand binding pocket that are not conserved between mSUCNR1 and rSUCNR1: position 181.31 (Asn in mouse vs Lys in rat) and 84EL1 (Gly in mouse vs Trp in rat). We have made species swap mutations of these residues either individually or in combination and then assessed the activity of SUCNR1 agonists using a BRET-based Gi dissociation assay. In these studies, mSUCNR1-N18K and rSUCNR1-K18N had little effect on ligand pharmacology. In contrast, mSUCNR1-G84W and rSUCNR1-W84G each largely reversed the mouse vs rat species selective pharmacology. The double mutants also displayed pharmacology similar to mSUCNR1-G84W and rSUCNR1-W84G. These findings suggest that species differences at position 84 between the mouse and rat orthologues largely accounts for the species specific pharmacology of these SUCNR1 agonists.

#6 ATP demonstrates agonism at the platelet P2Y12 receptor

Katie L Brannan, Chan Yi Ang, Eamonn P Kelly, J Lawrence Hutchinson, Stuart J Mundell
University of Bristol

The P2Y12 receptor (P2Y12R) regulates platelet activation, representing a major therapeutic target globally. ATP is regarded as a P2Y12R antagonist in platelets, but this pharmacological characterisation is complicated by platelets expressing multiple purinergic receptors and secreting multiple purinergic ligands.
Here we aim to investigate the pharmacological effects of ATP on P2Y12-mediated platelet activity.

Human platelet responses were assessed using Light Transmission Aggregometry (LTA) and VASP phosphorylation FACS assays. To investigate the molecular basis of these responses, Bioluminescence Resonance Energy Transfer (BRET2) assays were employed to characterise G-protein and arrestin activation.
Platelet studies revealed an inhibitory effect of high dose (100μM) ATP in LTA, consistent with established literature. However, when lower doses (1-10μM) were used, we observed potentiation of aggregation consistent with agonist behaviour. This potentiation was inhibited by ticagrelor, and likewise ATP showed a partial, ticagrelor-dependent reduction in PGE-1-induced VASP phosphorylation. Importantly, an ATP regeneration system confirmed this effect was not due to ATP-derived ADP contamination. BRET studies revealed that while ATP is a full G-protein agonist with similar potency to ADP, it displays only partial agonism with respect to arrestin recruitment.
Together these findings suggest ATP displays agonism at the P2Y12R, with a biphasic signalling profile suggesting pleiotropic effects.

#7 GRK-independent Recruitment of β-arrestin to the P2Y12 Receptor

Chan Yi Ang, J. Lawrence Hutchinson, Carsten Hoffmann, Julie Drube, Stuart J. Mundell, Eamonn Kelly
University of Bristol

G protein-coupled receptor kinases (GRKs) classically phosphorylate activated G protein-coupled receptors (GPCRs), promoting β-arrestin recruitment and receptor desensitisation. While the role of GRKs in μ-opioid receptor (MOR) regulation is well established, their contribution to function of the P2Y12 receptor (P2Y12R), an important platelet receptor, remains unclear. This work aimed to characterise the roles of individual GRK isoforms in β-arrestin recruitment to the MOR and P2Y12R.
Bioluminescence resonance energy transfer (BRET) assays measured agonist-induced β-arrestin recruitment in HEK293 cells lacking endogenous GRKs (ΔQ-GRK).1 Individual GRKs were re-expressed, while pharmacological GRK2/3 inhibition with Cmpd101 and mutations of the P2Y12R C-terminus, including truncation of the PDZ-binding motif (ΔPDZ-P2Y12R), were used to investigate GRK-independent signalling.
Agonist-induced β-arrestin recruitment to the MOR was markedly impaired in ΔQ-GRK cells, confirming its dependence on GRKs. In contrast, the P2Y12R retained robust agonist-induced β-arrestin recruitment in ΔQ-GRK cells and following GRK2/3 inhibition. ΔPDZ-P2Y12R showed reduced, but not abolished, β-arrestin recruitment.
Unlike the MOR, β-arrestin recruitment to the P2Y12R occurs largely independently of GRKs. The PDZ-binding motif contributes to, but is not essential for, β-arrestin recruitment by P2Y12R, suggesting regulation by alternative mechanisms.

1.Drube J, Haider RS, Matthees ESF, Reichel M, Zeiner J, Fritzwanker S, et al. GPCR kinase knockout cells reveal the impact of individual GRKs on arrestin binding and GPCR regulation. Nature Communications. 2022 Jan 27;13(1):540.

#8 Structural and functional consequences of naturally occurring human GPR84 single nucleotide polymorphisms

Eilidh McKay, Abdul-Akim Guseinov, Laura Jenkins and Graeme Milligan
School of Molecular Biosciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, U.K.

GPR84 is a pro-inflammatory Gi/o-coupled GPCR implicated in immune and metabolic regulation. As receptor function is highly dependent on its dynamic structure, naturally occurring human SNPs provide a useful approach to identify structural determinants of receptor maturation, ligand binding and signalling. However, the functional effects of naturally occurring GPR84 variants remain poorly characterised. In this study, we investigated six human GPR84 SNPs located in varying regions of the receptor, using radioligand binding, G-protein activation and arrestin recruitment assays. Fluorescence levels of C-terminally fused eYFP constructs show successful translation of all SNPs. However, several variants - Asp61Ser, Pro163Thr, Gly190Glu and Tyr370His - displayed severe loss-of-function phenotypes and failed to generate detectable radioligand binding. In contrast, the Iso254Thr and Leu58Phe variants retained radioligand binding and signalling function. Iso254Thr exhibited functional properties similar to wildtype while Leu58Phe showed a distinct signalling phenotype characterised by reduced agonist potency but enhanced arrestin-2/3 recruitment efficacy. Collectively, these results demonstrate that residues in distinct regions of GPR84 contribute to separate stages of receptor function, from maturation and membrane localisation to ligand binding and receptor activation.

#9 Dual Channel Kinetic assays for detecting ligand bias at GPCRs

Tobias Pusterla[1], Paul Tewson[2], Scott Martinka[2], Kevin M. Harlen[2], Thom Hughes[2], Anne Marie Quinn[2], Sam R.J. Hoare[3]
[1]BMG LABTECH, Germany
[2]Montana Molecular, Bozeman, MT, US
[3]Pharmechanics, USA

Biased agonism at G protein-coupled receptors (GPCRs) has emerged as an important strategy for developing therapeutics with improved efficacy and reduced adverse effects. Reliable methods that simultaneously monitor multiple signalling pathways are therefore essential for characterising ligand bias. This study highlights a dual-channel kinetic fluorescence assay for the real-time measurement of G-protein signalling and β-arrestin recruitment in living cells. HEK293T cells expressing the angiotensin II type 1 receptor (AT1R) were co-transduced with genetically encoded fluorescent biosensors to monitor β-arrestin recruitment alongside either intracellular calcium or diacylglycerol (DAG) production. Fluorescence signals were recorded simultaneously using the CLARIOstar® microplate reader following stimulation with a panel of AT1R agonists. The multiplex assay generated robust kinetic data, demonstrated excellent assay performance, and identified distinct signalling profiles indicative of ligand bias. Simultaneous monitoring of multiple pathways provides greater insight into the temporal dynamics of GPCR activation than conventional endpoint assays and supports more accurate assessment of biased agonism. This high-throughput approach offers a valuable platform for GPCR pharmacology and has broad applications in drug discovery and ligand screening.

#10 Using label-free cellular impedance to characterise GPR35 regulation in an endogenous cell system.

Alison Porter, Kirsten Deytrikh, Brian McFerran and Stuart McElroy
BioAscent Discovery Ltd, Bo'Ness Road, Newhouse, Scotland, ML1 5UH

During GPCR agonist discovery, receptor desensitisation can significantly affect a compound's efficacy, particularly following repeated dosing. Understanding how agonists influence receptor signalling and desensitisation is therefore critical when selecting and optimising candidate molecules. An attractive drug candidate is one that produces a strong initial response, maintains efficacy after repeated dosing, causes limited receptor downregulation, and does not induce rapid tolerance.
BioAscent scientists used the Agilent xCELLigence® RTCA platform to monitor real-time cellular impedance changes in HT-29 cells, which endogenously express constitutively active GPR35b, in response to the agonist lodoxamide. A series of pharmacological approaches was applied, including agonist pre-treatment, GRK5/6 inhibition, β-arrestin-2 knockdown, and pathway inhibition studies, enabling comprehensive characterisation of the desensitisation profile of endogenous GPR35b in real time.
We were able to identify key regulatory roles for GRK5/6 and β-arrestin-2 and confirm the dominant contribution of G12/13-ROCK signalling in HT-29 cells. The kinetic nature of the assay enabled subtle changes in signalling dynamics to be detected that would likely be missed using conventional endpoint approaches.
This study demonstrates how label-free impedance technology can provide detailed insights into GPCR signalling that are not accessible through conventional endpoint assays. Using an endogenous expression system allowed a physiologically relevant assessment of GPR35b desensitisation, recovery, and signalling pathway utilisation, generating a deeper understanding of receptor behaviour to support agonist discovery programmes.

#11 Computational Modelling of GnRHR Conformational Activation Dynamics

Lily Tosh[1], Valerie Ferro[1], Margaret Cunningham[1], Paul Mulheran[2,3]
[1] Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathclyde, Glasgow, UK
[2] Chemical and Process Engineering, University of Strathclyde, Glasgow, UK
[3] ARCHIE-WeSt, hosted at the University of Strathclyde (archie-west.ac.uk)

G Protein-coupled receptors (GPCR) are dynamic transmembrane proteins in which binding of an extracellular ligand promotes stabilisation of distinct conformations directly influencing downstream responses. The Gonadotropin releasing hormone receptor (GnRHR), a major regulator of mammalian reproduction, is a class A GPCR that has an absent C-terminal tail and substitutions in activation motifs. Although, recent studies have begun to elucidate activation mechanisms of GnRHR, structural data is limited and ligand-induced conformational dynamics remain undetermined. This study aims to develop and deploy a computational pipeline which simulates and analyses GnRHR conformational dynamics.

Initially, predicted pose of GnRH and triptorelin were docked to active state GnRHR homology model and refined using FlexPepDock molecular docking. The lowest energy poses/complexes were subjected to 1 microsecond unbiased molecular dynamic (MD) simulation in a POPC bilayer using GROMACS software. Trajectories were subjected to fundamental MD and transmembrane distance analysis, before principal component analysis (PCA) with K-means clustering was used to determine dominant receptor and peptide backbone motions and conformations.
MD simulations of the Apo-, GnRH- and Triptorelin- GnRHR complexes unveiled the highly dynamic nature of the complexes, where surprisingly high flexibility in the peptide was observed. PCA and clustering revealed distinct receptor and peptide conformational ensembles and differential transition pathways and final stabilisation conformations were observed across the three systems, including deviation from experimentally proposed conformations. Primary correlation analysis may suggest an interdependent relationship between ligand and GnRHR backbone conformations.
This successful computational pipeline provides an initial perspective on both endogenous and synthetic GnRHR peptides modulation of active GnRHR conformational dynamics. Specifically, we have shown potential ligation state-specific conformations and alternative peptide poses which may suggest secondary/allosteric sites of hGnRHR. Therefore, further validation and exploration will provide a deeper insight and potential feed into structure-based drug design.

#12 ICL1 in Opioid Receptor Effector Engagement

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Opioid receptors represent the gold standard target for analgesics in the treatment of moderate to severe pain. Recently, G-protein bias agonists have emerged as a novel and intriguing drugs with reportedly greater safety and side-effect profiles. Though this is still subject to debate, a comprehensive understanding of intracellular GPCR effector engagement may enlighten new and exciting ligand design. Here, we explore the role of ICL1 in opioid effector coupling. Taking an orthogonal approach with a barrage of biosensors and biochemical assays, we present ICL1 as a key coupling determinant with importance in discriminating observed differences in agonist efficacies.

#13 Investigating the spatial properties of EP2 activation by different agonists

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Despite the prostanoid G protein-coupled receptor (GPCR) EP2 being implicated in a range of diseases, the glaucoma medication omidenepag isopropyl remains the only clinically

approved EP2-specific drug. Recent advances in GPCR drug discovery have exploited an enhanced understanding of spatially biased receptor signalling from distinct subcellular compartments. While EP2 is known to undergo constitutive internalisation, the mechanisms underlying its trafficking and the downstream consequences of any compartmentalised signalling remain unclear. We therefore investigated the effect of different agonists, including the endogenous ligand prostaglandin E2 and the clinical drug omidenepag, on EP2 activation throughout the cell, as neglecting this spatial aspect may have contributed to slow therapeutic progress. Utilising enhanced bystander bioluminescence resonance energy transfer-based biosensors transfected into HEK293 cells, we have demonstrated the presence of activated EP2 receptors within the endosomal system and at the Golgi. When used at a sufficient concentration, the agonists demonstrated similar capacities to rapidly activate each pool of EP2 receptors, but different trends in potency and efficacy were observed between the plasma membrane and subcellular locations. Understanding the downstream effects of this differential EP2 activation by different agonists could potentially facilitate improved EP2-targeting approaches in the future.

#14 Identification of Mammalian Fatty Acid Metabolism-Derived Endogenous Agonists Acting at the Orphan Receptor GPR84

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GPR84 is a G-Protein Coupled Receptor (GPCR) known for its role in inflammation. Medium chain fatty acids were originally proposed as endogenous ligands, however GPR84 activation is only seen with these compounds at concentrations above the normal biological range, so GPR84 remains an orphan receptor.
C7-alkylpyrone was serendipitously identified as a potent and selective GPR84 agonist in an untargeted metabolomics study focusing on the human oxidoreductase AKR1C3. We synthesised a series of Cn-alkylpyrones with varying chain lengths and found them to be potent and selective agonists at human GPR84 (3.72pM–219nM).
It has been suggested that Cn-alkylpyrones may be derived from mammalian fatty acid synthase (FAS) enzymes, however this has never been demonstrated in cells. We hypothesised that Cn-alkylpyrones can be biosynthesised by human FAS, during ketoreductase (KR) domain dysfunction.
Human HepG2 cells were treated with an FAS KR inhibitor, and media was analysed by UPLC-MS for secreted metabolites. We confirmed the presence of TAL, C3-, and C5-alkylpyrone under these conditions. This was validated in vitro using the inhibitor in enzyme assays with a murine FAS construct. This work proves that endogenous Cn-alkylpyrones can have biological relevance in mammalian systems and may provide foundation for the deorphanisation of GPR84.

#15 Investigation of the role of Frizzled 6 receptor in diabetic retinopathy

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Frizzled receptors (FzDs) are class F GPCRs comprising 10 receptors (FzD1–FzD10). Although they share canonical GPCR architecture, signalling is primarily mediated by the dishevelled protein rather than classical G protein or β-arrestin pathways. FzDs cooperate with the co-receptors low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6) to mediate Wnt signalling, regulating cellular processes including proliferation, migration and retinal vascular maintenance. Dysregulated Wnt signalling through FzD4/LRP5 is associated with reduced barrier protein expression, increased vascular leakage and compromised vascular integrity, contributing to diabetic retinopathy (DR). Current therapies do not provide sustained vision improvement or long-term stability. While SNZ-413, a bispecific antibody targeting FzD4/LRP5, is currently in clinical trials for DR, this project aims to explore the potential role of alternative FzDs in DR.
To explore transcriptional differences in FzD expression, scRNA-seq analysis of normoglycaemic and diabetic mouse retinas was performed. FzD4 and FzD6 expression, highly expressed in retinal endothelial cells, was dramatically reduced in diabetic mouse retinas. Immunohistochemistry of retinal sections confirmed FzD4/6 reduced expression.
To determine the functional role of FzD6, human retinal microvascular endothelial cells (HRMECs) were transfected with siRNA targeting FzD6. FzD6 knockdown reduced cell proliferation, endothelial tube formation and tube invasion capacity.
To investigate the direct cause of FzD6 downregulation in retinal endothelial cells from diabetic mice, hyperglycemic environment and hypoxia were investigated. Hyperglycaemic incubation of HRMECs revealed no significant expression change. In contrast, immunohistochemistry of oxygen-induced retinopathy (OIR) mouse retinas displayed a reduction in FzD6 expression, suggesting hypoxia as a potential driving factor of FzD6 downregulation.
These findings demonstrate the importance of FzD6 in normal retinal endothelial cell function, suggesting that its downregulation in diabetic and OIR retinas may compromise retinal integrity. Promoting FzD6 activation may help to restore lost cell function observed in DR and represent a new therapeutic avenue.

#16 IPR-PDE1C in Pulmonary Hypertension: Uncovering the Signalosome

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Pulmonary arterial hypertension (PAH) is a severe and progressive disease characterised by remodelling of the pulmonary arteries, leading to an increase in mean pulmonary arterial pressure, ≥ 20 mmHg. There is no cure. Second messenger cyclic AMP (cAMP), which elicits vasodilatory effects, is decreased in PAH-PASMCs. The prostacyclin receptor (IPR), a Gαs-coupled G protein-coupled receptor (GPCR), is a therapeutic target for PAH by increasing levels of cAMP. Phosphodiesterase 1C (PDE1C), by hydrolysing cAMP, plays a crucial role in

shaping cAMP gradients in PASMC. PDE1C is increased in PAH and accounts for decreases in IPR expression limiting the efficacy of IPR-agonists: IPR-PDE1C signalosomes ultimately promote disease progression. We aimed to define the functional role of the IPR-PDE1C signalosome in PAH and how it could be targeted therapeutically. Our data showed PDE1C and IPR expression is inversely correlated. IPR signalling could be restored by 72h treatment of PDE1 inhibitor, validating the signalosome. By peptide array, we have identified amino acid sequences where IPR and PDE1C interact directly: these sites could be targeted to inhibit this protein-protein interaction. We believe disrupting the IPR-PDE1C signalosome by PDE1 inhibitors or peptide protein disruptors could prevent disease progression and improve PAH-patient response to IPR-agonists.

#17 Janelia Fluor® Probes for Live-Cell Imaging of Adenosine A2A and A2B Receptors

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Adenosine A2A and A2B receptors are implicated in inflammation, pulmonary arterial hypertension and cancer, but characterising their pharmacology at endogenous expression levels remains challenging. Existing BODIPY-labelled fluorescent ligands are limited by brightness, photostability and background fluorescence. To address this, we developed ligands incorporating Janelia Fluor® (JF) dyes, which equilibrate between a non-fluorescent lactone and a fluorescent zwitterion and become fluorescent on target engagement, minimising background.
Affinity and selectivity were determined by NanoBRET saturation and competition binding in HEK293G cells expressing NLuc-tagged A2A or A2B receptors, with live-cell confocal microscopy using SNAP-tagged receptors. JF-labelled XAC derivatives (XAC-525, XAC-549) confirmed specific and reversible binding at both subtypes, establishing proof of concept. Systematic fluorophore substitution showed that fluorophore size, lipophilicity and structure determine affinity independently of the pharmacophore: replacing BODIPY 630/650 with 6-TAMRA abolished A2B binding across three ligand series, while the A2A-selective 6-TAMRA ligand NDK-492 retained nanomolar affinity (Kd = 96 ± 30 nM, n = 5). NDK-500, bearing the fluorogenic JF-526 dye [K(L–Z) = 0.005], showed exceptional A2A affinity (Kd = 3.6 ± 0.4 nM, n = 5), confirmed selectivity, and clear membrane fluorescence at 10 nM with full displacement. NDK-500 is a high-affinity, selective tool for imaging A2A receptors in living cells.

#18 Genetically encoded intrabody biosensors to detect activation states of the atypical chemokine receptor 3 (ACKR3)

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The atypical chemokine receptor 3 (ACKR3) is a G protein-coupled receptor (GPCR) important in the regulation of cell migration. In recent years, ACKR3 has emerged as an axiomatic target in inflammatory diseases, metastatic cancers, and neurodegenerative disorders. As an atypical chemokine receptor, ACKR3 fails to trigger conventional G protein signalling. Its primary function is to rapidly internalise bound peptides, sequestering them from the extracellular milieu, limiting local concentrations and generating gradients that guide cell migration. Given this lack of G protein signalling, identification of drugs targeting this receptor has relied on assays of β-arrestin recruitment, although the involvement of this scaffolding protein in ACKR3 internalisation is not yet clear. Previous structural studies have identified CID24 as an antigen-binding fragment (Fab) that stabilises the cytoplasmic face of ACKR3 in the active state.
Here, we report the design of intrabodies based on the CID24 variable domain that are selectively recruited to active ACKR3. These intrabodies can be used as sensors of ACKR3 activation in live cells, and detect the activity of a broad range of ACKR3 ligands including chemokines, small molecule agonists/inverse agonists, inverse agonist nanobodies, and opioid/opioid-based peptides. Membrane-tethering of these intrabodies allows their use in membrane preparations and in miniaturised formats that will facilitate high-throughput screening campaigns. The development of this library of intrabody tools with robust response windows to probe ACKR3 activation represents a significant advancement in the toolset for the identification of ACKR3 ligands, in particular inverse agonists, which are emerging as potential therapeutics in oncology.

#19 CaSR Signalling in Sensory Neurons: A GPCR Mechanism for Visceral Pain in IBS

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Irritable bowel syndrome (IBS) is frequently accompanied by debilitating visceral pain, driven in part by visceral hypersensitivity, yet the underlying sensory signalling mechanisms remain poorly defined. The Calcium Sensing Receptor (CaSR), a class C GPCR expressed on gut innervating dorsal root ganglion (DRG) neurons, has emerged as a candidate mediator of nociceptor sensitisation. This study investigated CaSR dependent signalling mechanisms relevant to visceral pain.
Calcium imaging of mouse DRG neurons showed that the CaSR positive allosteric modulator cinacalcet evoked robust, concentration dependent Ca^{2+} mobilisation ($p = 0.01$). This response was abolished by the CaSR negative allosteric modulator NPS-2143 ($p = 0.03$) and by thapsigargin ($p = 0.01$), confirming receptor specificity and demonstrating that CaSR

activation mobilises Ca^{2+} from endoplasmic reticulum stores. Inhibition of PLC ($p > 0.99$) or IP_3 ($p = 0.11$) receptors did not significantly attenuate responses, suggesting that CaSR signalling in DRG neurons may diverge from canonical Gq-PLC-IP_3 pathways and involve additional G-protein signalling components. Cinacalcet responsive neurons were predominantly small to medium sized (300–600 μm²), consistent with a nociceptive phenotype. Only 22.6% also responded to capsaicin, indicating limited overlap between CaSR- and TRPV1-responsive populations.
These findings identify non canonical CaSR signalling in DRG neurons and provide mechanistic insight into GPCR mediated visceral pain in IBS.

#20 FPR2 regulation by GRKs: implications for pro-resolution pharmacology

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Formyl peptide receptor 2 (FPR2), a GPCR found on immune cells, can regulate the switch between pro-inflammation and pro-resolution phases. To better understand the role of FPR2 in inflammation, we sought to analyse the influence of GRKs on FPR2 regulation and how this impacts immune cell function. We investigated altered human and mouse (h/m)FPR2 regulation using GRK phosphorylation-deficient receptors (hFPR2-ΔABC/mfpr2-ST), measuring β-Arrestin recruitment by BRET in transiently transfected HEK cells and characterising the inflammatory response in stable RAW macrophage cell lines.
β-Arrestin recruitment to the cell membrane and early endosome was decreased in HEK cells expressing the mfpr2-ST mutant compared to mfpr2. Further, β-Arrestin recruitment to hFPR2 and mfpr2 was also impaired in cells CRISPR-Cas9 edited to remove GRKs. Our macrophage lines exhibited altered immune function, including changes to expression levels of inflammatory regulators, nitric oxide response, chemotaxis and migration, in response to overexpression of hFPR2 and hFPR2-ΔABC. Interestingly, hFPR2 and hFPR2-ΔABC overexpression induced different immune response alterations, with RAW-hFPR2 cells exhibiting a blunted nitric oxide response compared to wildtype RAW and RAW-hFPR2-ΔABC. These findings demonstrate the importance of GRKs for FPR2 regulation and the implications for immune cell function and, thus, control of inflammation.

#21 GRK-dependent trafficking of NK1R and its impact on subcellular signalling dynamics

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The neurokinin 1 receptor (NK1R) is a pro-nociceptive G protein-coupled receptor (GPCR) implicated in chronic pain. Substance P-activated NK1R signals via Gαq and Gαs pathways, while phosphorylation by GPCR kinases (GRKs) promotes β-arrestin recruitment and receptor internalisation. Traditionally, GPCR signalling was thought to occur exclusively at the plasma

membrane; however, increasing evidence shows that internalised GPCRs contribute to cellular signalling. While aspects of NK1R internalisation have been studied previously, its subcellular trafficking and signalling remains largely underexplored.
To address this, bioluminescence resonance energy transfer (BRET) was used to investigate NK1R trafficking and signalling in wild-type HEK293 and GRK2/3/5/6 knockout cells. β-arrestins and miniG proteins were also employed to assess NK1R transducer-coupling behaviour at a subcellular resolution.
After confirming that internalised receptors contribute to substance P-induced Ca2+ and cAMP responses, NK1R was found to traffic from the plasma membrane to early, late and recycling endosomes, lysosomes and the Golgi, in a largely GRK-dependent fashion. β-arrestin2 recruitment displayed similar GRK dependency, whereas miniGsq recruitment indicated that NK1R remains active within all compartments irrespective of GRK expression.
These findings demonstrate that NK1R signals from both the plasma membrane and intracellular compartments, highlighting compartment-specific roles of GRKs in receptor trafficking and signalling.

#22 Mapping Muscarinic Circuits: Unravelling mAChR1 and mAChR4 Expression and Connectivity in the Brain

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Understanding how muscarinic acetylcholine receptors (mAChRs) are distributed and function in the brain is key to advancing our knowledge of cholinergic signalling in both healthy and pathological states. Among the five subtypes, mAChR1 and mAChR4 stand out as promising therapeutic targets because they can influence dopaminergic, cholinergic, and glutamatergic pathways. Disruptions in these receptors are linked to neuropsychiatric disorders like schizophrenia and Alzheimer's, underscoring their potential for developing receptor-specific treatments.
To define the cellular and circuit-level organisation of mAChR1 and mAChR4, immunohistochemistry, adeno-associated virus (AAV) tracing, and advanced imaging approaches are combined with novel Cre-loxP mouse models in which receptor-expressing cells are fluorescently labelled with GFP. This strategy enables precise identification and characterisation of receptor-expressing populations and their connectivity.
Recent results show that mAChR1 and mAChR4 are expressed in distinct neuronal populations within the hippocampus and lateral septum, regions heavily involved in neurodegeneration, cognition, and neuropsychiatric disorders. Furthermore, mAChR4 neurons in the lateral septum project to the hypothalamus and ventral tegmental area (VTA), revealing new circuitry through which muscarinic signalling may influence motivation, reward, and behavioural state. This research seeks to clarify the importance of receptor-specific cell populations and their connections to neural activity and disease-related behavioural traits.

#23 TRPing receptors – modulatory effect of TRP channels on GPCRs

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Introduction: Transient receptor potential (TRP) channels are emerging regulators of GPCR signalling. TRPV1 is often co-expressed with the mu-opioid receptor (MOR) in dorsal root ganglion neurons, where it has been proposed to enhance opioid analgesia during acute inflammation [1]. However, the mechanisms underlying MOR/TRPV1 regulation remain unclear. It is also unknown whether this regulatory role is unique to TRPV1 or shared across the TRP family, and whether it extends beyond MOR.
Methods: HEK293T cells were co-transfected with MOR-NanoLuc or CXCR4-NanoLuc, β-arrestin2-mVenus, and WT TRPV1, TRPM8, or TRPA1. Ligand-induced β-arrestin2 recruitment was measured using BRET on a PHERAstar FSX plate reader. Local calcium dynamics around TRPV1 were assessed using novel GCaMP6-based “nanoruler” sensors tethered at defined distances from the receptor.
Results: Activation of TRPV1 or TRPA1 markedly inhibited agonist-induced β-arrestin2 recruitment to MOR, whereas TRPM8 activation had no effect, indicating channel-selective regulation. Nanoruler imaging identified TRPV1-associated Ca^{2+} nanodomains within 30 nm of the channel, which may mediate this effect. TRPV1 activation also inhibited β-arrestin2 recruitment at CXCR4 and other GPCRs, demonstrating broader regulatory activity.
Conclusion: TRP-mediated modulation of GPCR signalling is channel-selective and extends beyond MOR. Localised TRPV1-dependent Ca^{2+} nanodomains may provide a mechanistic basis for this regulation.

[1] Basso et al. 2019, Sci Signal, 12(575).

#24 Exploring Succinate Receptor Signalling in the Mitochondria

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The succinate receptor (SUCNR1) is a G-protein coupled receptor activated by the TCA cycle intermediate, succinate. During metabolic stress, there is an excess accumulation of succinate contributing to a state of chronic inflammation. The role of SUCNR1 in modulating the inflammatory response is currently unclear, however this receptor is expressed in immune cells including dendritic cells and macrophages. Much work remains to distinguish how SUCNR1-mediated mechanisms translate in these immune cells, leading to the activation or resolution of the inflammatory response. It is known that the activation of different signalling pathways via different G-proteins is one mechanism which yields different physiological responses. Furthermore, a growing area of interest in GPCR biology is focused on how signalling from different cellular compartments may also activate different signalling pathways. In this study, we have used a combination of targeting strategies to examine SUCNR1

signalling through Gαi and Gαq at different cellular compartments including the plasma membrane, early endosome, endoplasmic reticulum, and mitochondria. Between compartments we observe markedly different kinetic behaviours, particularly between the plasma membrane and early endosomes versus the intracellular endoplasmic reticulum and mitochondrial compartments. Furthermore, there are notable differences in efficacy for Gαi versus Gαq recruitment at the different compartments where Gαi is heavily favoured at the plasma membrane, while at other compartments the difference in efficacy for Gαi versus Gαq is reduced. These studies highlight how cellular context impacts SUCNR1 signalling, and future studies will examine the downstream physiological effects of SUCNR1 signalling from different compartments.

#25 FFA4 inverse agonism and Piezo1 agonism regulate cellular mechanics of 3T3- L1 adipocytes

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Metabolite-sensing GPCRs, such as FFA4, have emerged as key regulators of cellular physiology, particularly in adipocytes. However, other cellular pathways such as mechanotransduction have now been shown to have a significant role in adipogenesis and adipocyte function. Yoda1, a selective agonist of the mechanosensitive ion channel Piezo1, provides a valuable tool to dissect the role of mechanotransduction in adipocyte biology. In this study, we investigated how investigated inverse agonism of FFA4 and its role in modulating cellular mechanics. This study also looks at Piezo1 agonism and its modulatory role in adipogenesis and GPCR expression. Using 3T3-L1 adipocytes, we demonstrate that Yoda1 treatment throughout differentiation can cause varied phenotypic and genotypic changes which is greatly dependent on the Yoda1 concentration, thus the level of Piezo1 activation, used. These results suggest Yoda1-mediated Piezo1 activation has a significant role in mediating adipogenesis where low or high activation can direct differentiating cells down differing cell lineages. Treatment with AH7614, an inverse agonist of FFA4, showed reduced adipogenesis with increased cell stiffness, suggesting that FFA4 inverse agonism regulates intracellular mechanics. These findings suggest that Piezo1 activation and FFA4 inverse agonism serve a modulatory role in adipocyte signalling, suggesting a possible link between the two signalling pathways.

#26 Measuring Gi/o subtype signalling by metabolite sensing GPCRs with novel BRET-based biosensors

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Metabolic disorders including obesity and type 2 diabetes represent a pressing global health challenge. Because adipose tissue plays a central role in metabolism and development of metabolic disorders, understanding how adipocytes sense and respond to metabolic cues is

essential for identifying new therapeutic strategies. We have identified four metabolitesensing GPCRs that are expressed in adipocytes: the FFA2 and FFA4 free fatty acid receptors, the HCA2 hydroxycarboxylic acid receptor, and the succinate receptor (SUCNR1), which may hold therapeutic potential for metabolic disease. These GPCRs are known to couple to Gαi/o signalling pathways, however the profile of which specific Gαi/o family members they signal through is unclear. Here we describe novel BRET-based biosensors and used these sensors measure metabolite GPCR signalling through different Gαi/o family members. Our results demonstrate the ability of these biosensors to detect Gαi/o subtype selective coupling profiles of metabolite sensing GPCRs. Expressing these sensors in adipocyte models will provide a platform to dissect metabolite sensing GPCR pharmacology and function, helping identify new approaches for the treatment of metabolic disorders.

#27 Dissecting the Subcellular Trafficking and Signalling of PAR2

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The protease-activated receptor 2 (PAR2) is a G protein-coupled receptor (GPCR) activated through proteolytic cleavage of its N-terminus. PAR2 is a key regulator of inflammation and pain signalling in inflammatory bowel disease (IBD). Recent studies suggest that not only membrane-localised, but subcellular PAR2 signalling is essential for these (patho-) physiological functions. We hypothesise that β-arrestins scaffold compartment-specific signalling complexes, thereby regulating PAR2 trafficking and signalling.
To uncover the processes that influence trafficking of PAR2, we utilised proteolytic (via trypsin) and non-proteolytic (via SLIGRL peptide) activation of the receptor. This was assessed using bioluminescence resonance energy transfer (BRET) assays, with NanoLuc-tagged PAR2 or β-arrestin2, which were co-expressed with mNeonGreen-labelled subcellular compartment markers. Trafficking was assessed in wild-type HEK293 cells and cells lacking GPCR kinases (GRKs) or β-arrestins. Our findings indicate that PAR2 trafficking is influenced by both the mode of receptor activation and the availability of key regulatory proteins.
To examine intracellular signalling, we inhibited receptor internalisation using the dominant-negative dynamin mutant DynK44E and measured agonist-induced Ca^{2+} mobilisation with a BRET-based Ca^{2+} biosensor. These studies demonstrate that PAR2 internalisation is required for maximal Ca^{2+} signalling, highlighting the importance of subcellular receptor trafficking in PAR2 function.

#28 GPCRs actively specify the spatiotemporal compartmentalisation of cAMP signalling in cardiac myocytes

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Compartmentalisation of Cyclic 3'-5' adenosine monophosphate (cAMP) signalling underlies signalling specificity downstream of G protein-coupled receptors (GPCRs), but whether GPCRs actively determine where intracellular cAMP is generated remains unclear. Using targeted FRET-based reporters to monitor cAMP and PKA activity in live cardiac myocytes, we demonstrate that GPCRs specify distinct spatial patterns of signalling through three complementary mechanisms. First, receptor identity specifies signalling location, with β1-adrenergic receptors (β1ARs) generating cAMP at the sarcoplasmic reticulum and myofilament, whereas β2AR signalling is restricted to an AKAP79-associated plasma membrane nanodomain. Second, ligand identity further diversifies signalling, as carvedilol acts as a partial agonist yet generates a distinct spatial pattern of cAMP signalling from full agonists isoproterenol and norepinephrine. Finally, activation of the Gi-coupled M2 muscarinic receptor selectively suppresses cAMP in specific compartments while sparing the AKAP79 nanodomain, revealing that inhibitory signalling is also spatially organised. These findings demonstrate that receptor identity, ligand bias, and G protein coupling act together to specify the spatiotemporal organisation of cAMP signalling, extending current concepts of GPCR signalling by highlighting GPCR-dependent specification of cAMP compartmentalisation as an important mechanism for achieving signalling specificity.

#29 Investigating the Interactome of Human Adenosine Receptor A2A at Endogenous Expression Levels

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The human adenosine receptor A2A, a G protein-coupled receptor (GPCR), is expressed in tissues in the immune, cardiovascular, and central nervous systems. As a result, it plays a critical role in immunosuppression, cancer progression, and cardiovascular diseases. Its involvement in these diseases make it an attractive drug target and highlights the need for a deeper understanding of A2A receptor regulation. Through CRISPR/Cas9 technology, we have tagged the C-terminus of endogenous A2A with the ultraID biotin ligase in HEK293T cells. The A2A selective agonist CGS 21680 stimulated cAMP production by the CRISPR-edited A2A cells with a similar potency to unedited wildtype HEK293T controls, demonstrating it as a suitable model to study the receptor. Cells were supplemented with biotin, allowing the ultraID ligase to biotinylate proteins in close proximity to the A2A receptor. Biotinylated proteins were then analysed via mass spectrometry, which identified multiple protein hits from different classes. Validating these proteins for direct interactions with A2A could reveal novel drug targets for A2A-linked diseases.

#30 FFA3 activation engages visceral sensory pathways associated with dysbiosis-mediated abdominal pain

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This work was supported by the Pain Relief Foundation.
Irritable bowel syndrome is characterised by chronic abdominal pain arising from sensitisation of sensory pathways innervating the bowel. Increasing evidence implicates dysbiotic microbial communities and altered production of short-chain fatty acids in this hypersensitive state. These metabolites signal through the G protein-coupled receptors FFA2 and FFA3, but their respective contributions to visceral nociception remain poorly defined.
FFA receptor function was investigated using calcium imaging and immunocytochemistry in cultured mouse dorsal root ganglion neurons, alongside ex vivo lumbosplanchnic afferent nerve recordings.
Selective FFA2 agonism produced weak responses in a small neuronal population, with partial overlap among capsaicin-sensitive nociceptors. By contrast, selective FFA3 activation recruited a substantially larger population, predominantly comprising small- to medium-sized neurons enriched in capsaicin-responsive cells. FFA3 immunoreactivity was detected in NeuN-positive neurons and partially co-localised with TRPV1, supporting its expression within nociceptive sensory populations. Pharmacological inhibition varied between FFA3-targeting compounds. In ex vivo colonic preparations, FFA3 agonism increased lumbosplanchnic afferent firing, extending the cellular observations to functional visceral sensory activity.
These data identify FFA3 as a candidate neuronal pathway linking microbial metabolite signalling to visceral hypersensitivity in IBS.

#31 Pharmacological characterisation of novel FPR2 ligands reveals distinct receptor subtype and species selectivity

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The Formyl Peptide Receptor 2 (FPR2) is a class A G protein-coupled receptor (GPCR) and a key switch in pro-resolution signalling, emerging as a leading target in “resolution pharmacology”. Despite this, FPR2-agonists ACT-389949 and BMS-986235 both failed in phase 1 trials due to receptor desensitisation and pharmacological tolerance, highlighting the need for improved understanding of FPR2 ligand pharmacology. Here, we pharmacologically characterised four novel FPR2 ligands using competition binding, BRET-based G-protein and β-arrestin 2 recruitment, internalisation, and ERK1/2 phosphorylation assays in HEK293 and RAW264.7 cells stably expressing human and mouse FPR1 and FPR2. Lower relative binding affinity corresponded to reduced G-protein and β-arrestin 2 recruitment across compounds. Compounds 1 and 2 showed efficacy, relative affinity and potency comparable to WKYMVm at human FPR2, while compounds 3 and 4 were less potent. Receptor subtype specificity varied: compound 1 exhibited dual FPR1/FPR2 agonism, compound 2 was selective for hFPR2 over hFPR1, compound 3 was selective for hFPR1, and compound 4 was mFPR2-specific. These profiles were further recapitulated in human neutrophils using ROS chemiluminescence with subtype-selective inhibitors. Compound 2 emerged as a high-affinity,

potent, FPR2-selective ligand, offering a valuable potential therapeutic candidate for resolution pharmacology.

#32 GPR75 – Cracking the Function Code

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Orphan G protein-coupled receptors represent a substantial untapped resource for drug discovery. GPR75 has emerged as a promising target for metabolic disease, with rare loss-of-function variants conferring protection against obesity and type 2 diabetes.
With no current validated pharmacological tools, GPR75 has remained largely intractable within conventional drug discovery. Recent cryo-EM studies have shown that GPR75 adopts an active-like conformation, lacking several canonical class A 'inactivating' motifs and possessing a long, disordered C-terminus and a backfolded ECL2 which occludes the orthosteric binding pocket. We aim to identify the functional significance of these unique structural features and investigate alternative methods of receptor modulation aside from classical ligand-based approaches.
Using BRET-based biosensors, we have demonstrated GPR75 constitutively couples to Gαi and recruits β-arrestin in a GRK2/3-dependent manner. To define the structural basis of this behaviour, we have generated a panel of GPR75 mutants, comprised of progressive C-terminal truncations, restoration of absent canonical motifs, alteration of the backfolded ECL2, and substitution of intracellular phosphorylation sites. Profiling of these constructs aims to identify tractable pharmacological intervention points and inform the development of ligand screening platforms.

#33 Developing novel bifunctional compounds for targeted proteolytic degradation of CXC Chemokine Receptor 2 (CXCR2)

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CXCR2-negative allosteric modulators (CXCR2 NAMs) show promise as cancer immunotherapy adjuncts, as they combat tumour-mediated neutrophil polarization. However, CXCR2 NAMs may have limited therapeutic effect because of the complexity of the TME and high concentrations of endogenous chemokine (for example, CXCL8). Combining non-competitive, negative intracellular allosteric modulation with induced downregulation of receptor expression via degrader-ligase recruitment is one strategy to achieve longer-lasting, non-surmountable inhibition of the neutrophil response. Our previously published cell-permeable CXCR2 fluorescent ligand demonstrates that the intracellular binding site of CXCR2 tolerates binding of heterobifunctional molecules. So, in this project, we have adapted the synthesis route of the CXCR2 fluorescent ligand to synthesize two novel CXCR2 bivalent compounds consisting of a NAM linked to a substrate recognition subunit of von Hippel-Lindau

(VHL) Cullin Ring E3 ubiquitin ligase. Accordingly, these compounds were demonstrated to retain high affinity (Ki = 1.7nM and 9.5nM) for the intracellular binding site of the CXCR2 receptor and to engage the VHL E3 ligase target using NanoBRET-based assays. We are now optimizing assays to assess induced CXCR2 degradation and the formation of the ternary complex (between CXCR2 and the VHL E3 Ligase), as well as to understand the degradation mechanism. This proof-of-concept project could provide a platform for a different approach to target the immunosuppressive TME that could be combined with immunotherapy to yield improved clinical outcomes.

#34 Investigating the Ability of NanoLuciferase and mGOLD-tagged Nanobody AD101 to Target Active-State A2A Receptors in Living Cell

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Upon adenosine binding, A2A, a subtype of adenosine receptor, results in elevated intracellular cAMP signalling via Gs/olf-protein coupling. Over-activation of this pathway can promote cancer progression and immune-suppression, making A2A an attractive therapeutic target. Nanobodies are ~12-15kDa antigen-binding fragments derived from heavy-chain-only antibodies that be used for studying GPCR pharmacology. In this study, we investigated AD101, a nanobody reported to bind to active-state A2AR (McMahon et al., 2018).
HEK293T cells were transfected with plasmids that encoded for A2AR and intracellular AD101 with either C-terminal NanoLuciferase or mGOLD tags. Following (1:400) furimazine addition, kinetic Bioluminescent Resonant Energy Transfer (BRET) measurements with 10-4-10-10M NECA/CGS21680 were taken for 50 cycles. BRET ratios were determined by luminescence (460-480nm bandpass filter) and fluorescence emissions (535±40nm filter) measured for ~30min in a PHERAstar-FSx plate-reader (BMG LabTech).
NECA/CGS21680 treatment of HEK293Ts expressing A2A-NLuc and AD101-mGOLD showed a concentration-dependent increase in BRET ratio over time. Concentration-response curves yielded Log10EC50 values of NECA=6.51±0.23, CGS21680=-6.25± 0.13. Reversing the NLuc/mGOLD tags produced similar Log10EC50 values (NECA=-6.20±0.14, CGS21680=-6.10±0.33). Repeating these experiments in HEK293Ts expressing other AR subtypes and CXCR4 did not show any AD101 recruitment. These results demonstrate AD101's ability to specifically recognise activated A2AR in live HEK293T cells.

#35 Spatial Biology, a Novel Strategy to Better Understand Opioid Addiction Pathology

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Dopaminergic neurotransmission from the ventral tegmental area (VTA), a key mesolimbic structure, to the nucleus accumbens (NAc), the pleasure centre of the brain, is critical for

biological motivation while simultaneously facilitating addiction pathology. Neuroplastic, structural and functional alterations within the mesolimbic system form the basis for the addiction phenotype, presenting a complex challenge for pharmacological treatment. The M_5 muscarinic acetyl choline receptor (M_5-mAChR) has been researched as a potential target. The anatomical localisation of M_5-mAChR within the mesocorticolimbic neurocircuitry has raised questions around the role of this receptor in neurological diseases and substance abuse disorders. This project aims firstly to map the expression profile of M_5-mAChR in the mesolimbic system followed by measuring changes in M_5-mAChR expression during various stages of addiction.

#36 Evaluating AI-Based Predictions of Ligand Binding to the P2Y12 Receptor

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AI-based protein–ligand structure prediction has emerged as a promising approach for predicting molecular interactions and ligand-binding poses, particularly with recent methods capable of predicting biomolecular complexes containing small-molecule ligands [1]. However, the reliability of these approaches for G protein-coupled receptors (GPCRs) remains to be evaluated.
In this study, we assessed the performance of AI-based modeling in predicting ligand-binding poses and interaction patterns within the P2Y12 receptor, a clinically important GPCR involved in platelet aggregation. Experimentally determined P2Y12 structures bound to the antagonist AZD1283, the agonist 2MeSADP, and the inverse agonist selatogrel have revealed distinct ligand-binding modes and receptor conformations, providing suitable structural references for evaluating AI-generated predictions [2–4]. Chai-1 and Boltz-2 were used to predict P2Y12–ligand complexes and benchmark binding poses against experimental structures. Predictions were extended to multiple ligands to assess model generalisability. Experimental and predicted AZD1283 and selatogrel complexes were further compared using GROMACS molecular dynamics simulations. Dual-ligand modelling was also performed to explore additional binding regions. Notably, ticagrelor predictions suggested a potential secondary binding site beyond the orthosteric pocket, although its stability and biological relevance require further computational and experimental validation.

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#37 Novel Ligand to explore the function of GPR84

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GPR84 is a Gi-coupled G protein-coupled receptor predominantly expressed in immune cells and has emerged as a promising therapeutic target in inflammatory diseases. However, the limited availability of selective antagonists has restricted detailed pharmacological investigation. Here, we characterised a novel series of GPR84 antagonists identified from a high-throughput screening campaign using functional pharmacological assays and structure-guided analysis. Antagonist activity was evaluated using [^{35}S]GTPγS binding assays, while receptor–ligand interactions were investigated by molecular docking and site-directed mutagenesis. Enantiomeric separation revealed marked stereoselectivity, with only the E2 enantiomers displaying potent antagonist activity. Compound 494E2 showed the highest potency (pIC_{50} ≈ 6.6; pA_2 ≈ 8.4). Mutational analysis demonstrated that substitution of Y69 markedly reduced antagonist potency, whereas mutations of other predicted binding residues produced only minor effects, identifying Y69 as a key determinant of antagonist recognition. These findings provide new insights into the molecular basis of GPR84 antagonist binding and establish a novel antagonist scaffold for future pharmacological studies and ligand optimisation.

#38 Sensitisation Drives Dendritic Localisation of TrpA1 mRNA and Enhances Nociceptive Behaviour in Drosophila

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Sensory neurons detect and respond to noxious stimuli. Inflammation and tissue damage heighten their responsiveness, a process known as sensitisation. Although sensitisation involves changes in gene expression and post-translational regulation, the spatial and temporal mechanisms controlling these adaptations remain unclear. For example, inflammation upregulates the evolutionarily conserved pain-associated ion channel TrpA1 and increases its surface abundance, but it is unknown whether this occurs via long range protein transport or local translation of dendritically localised transcripts. More broadly, how sensitisation modifies RNA synthesis and trafficking in sensory neurons is poorly understood. The compartmentalised architecture of nociceptors, together with evidence for RNA localisation and local translation at neuronal terminals, led us to examine whether these processes contribute to sensitisation. Specifically, we asked whether sensitisation drives TrpA1 mRNA into peripheral dendrites.
Using Drosophila larvae, a well-established model for nociceptive sensitisation, we combined behavioural assays with in situ hybridisation techniques capable of detecting single molecules o RNA to quantify TrpA1 mRNA abundance and subcellular localisation in vivo. Under basal conditions, TrpA1 transcripts were detectable within cell bodies and dendrites. Following sensitisation with copper sulphate, the number of dendritically localised TrpA1 transcripts was selectively increased, while signal in the cell body remained unchanged. This redistribution

correlated with enhanced nociceptive behaviours evoked by both mechanical and chemical noxious stimuli.
Together, these findings support a model in which sensitisation regulates TrpA1 expression, revealing an RNA based mechanism that may enable nociceptors to rapidly and spatially fine-tune their sensitivity during inflammatory pain.

#39 Effects of altered gravity on β-adrenergic-mediated cAMP signalling in intact cells

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Spaceflight-induced cardiac atrophy and rhythm disorders are linked to dysregulation of the adrenergic-cAMP-PKA pathway. Gravity-dependent alterations in adrenergic signaling, particularly cAMP dynamics, remain poorly understood. Using fluorescence biosensors, we studied intact cells under simulated microgravity and hypergravity. We observed shifts in the EC50 of cAMP production: leftward under hypergravity and rightward in microgravity, with faster cAMP accumulation kinetics in hypergravity. Cytoskeletal remodeling, hypothesized to be a determinant of such changes, was negligible, suggesting alternative mechanisms. These findings highlight significant gravity-induced offsets in the pharmacology of a prototypical G protein-coupled receptor, with implications not only for adrenergic signaling but also for other pathways of pharmacological interest, potentially informing countermeasures for astronaut health and pharmacology in altered gravity settings.

#40 Structural Investigation of FFAR1 Activation Using Cryo-Electron Microscopy

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Free fatty acid receptor 1 (FFAR1/GPR40) is a class A G protein-coupled receptor involved in glucose-dependent insulin secretion and is an attractive therapeutic target for metabolic disease. This MSCA-funded project investigates the structural basis of FFAR1 activation by extracellular allosteric agonists using single-particle cryo-electron microscopy.
To facilitate structural analysis of this small and flexible receptor, FFAR1 constructs containing BRIL fusion partners were assembled with stabilising antibody fragments, nanobodies and mini-Gs. Following extensive optimisation and cryo-EM data collection, image processing identified intact receptor complexes and enabled the isolation of distinct three-dimensional particle populations.
Classification revealed straight and kinked BRIL-associated conformations within the agonist-bound complexes. Although these differences may partly reflect flexibility within the engineered assembly, they also raise the possibility that the ligand stabilises distinct receptor conformational states. Current work focuses on refining these reconstructions, improving

receptor-region resolution and determining whether the observed populations represent structurally and functionally distinct activation states.
These findings provide new insight into the conformational landscape of FFAR1 and may contribute to understanding its activation.

#41 G protein coupling class dictates GPCR susceptibility to ERAD

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Class A G protein-coupled receptors (GPCRs) are functionally diverse and clinically important membrane proteins, each of which have a highly conserved transmembrane (TM) core that directly mediates ligand binding and receptor activation. Bioinformatic predictions and analysis of human disease databases indicate that mutations within this TM core are highly pathogenic, yet how such variants are recognised and triaged by cellular quality control is unclear. Here, we demonstrate that mutation of conserved TM residues selectively destabilises Gαs-coupled receptors but not Gαi-coupled counterparts. Our findings suggest that G protein coupling class influences susceptibility to endoplasmic reticulum-associated degradation (ERAD), revealing a previously unrecognised quality control checkpoint. Given that the molecular mechanisms underlying ERAD of membrane proteins remain ill defined, these insights provide a route for its fundamental characterisation and offers tractable pathways for rescuing misfolded Class A GPCRs implicated in cancer and neurological disorders.

#42 A NanoBRET Conformational Biosensor for the Glucagon-Like Peptide 1 Receptor

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Here, we present a Bioluminescence resonance energy transfer (BRET)-based biosensor of the Glucagon-Like Peptide-1 Receptor (GLP-1R), capable of identifying receptor conformational changes in live cells. Biased agonists of the GLP-1R are reported to have better efficacy in reducing blood glucose and promoting weight loss1–3, and as such presents a relevant target for identifying different conformational states in vitro. We screened a range of ligands of the GLP-1R, to measure ligand affinity, and efficacy across different stages of the GPCR-G protein-second messenger axis. We correlated receptor activation, with ligand affinity and efficacy in cAMP accumulation. GLP-1 (7-36), Exendin-4, oxyntomodulin, and tirzepatide displayed predictable behaviour with a linear increase in potency in at biosensor relative their binding affinity, and again between the biosensor and cAMP accumulation. The partial agonist orforglipron displayed no amplification in potency, with pKi ≈ pEC50 (biosensor) ≈ pEC50 (cAMP). Semaglutide and danuglipron displayed roughly equivalent affinities and potencies in the biosensor assay, but a large increase in the cAMP assay. By screening a range of agonists with different potencies and efficacies, we were able to demonstrate that our sensor does not mirror either affinity or potency in a downstream assay, providing additional information to traditional signalling assays.

#43 What β-arrestin2 conformational change fingerprints can teach us: a cross-receptor comparison

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Upon recruitment to a GPCR, β-arrestins adopt distinct conformations, which are thought to dictate functional outcomes. The pattern of receptor phosphorylation imprinted by GRKs is a major determinant of these conformations. Previously, we established a panel of eight intramolecular BRET-based β-arrestin2 (βarr2) conformational sensors, distributed across the N- and C-domain, to monitor βarr2 conformational changes. If performed with all eight sensors, live-cell measurements yield a conformational "fingerprint". While individual fingerprints have previously provided receptor-specific insights, a systematic cross-receptor comparison promises to reveal general principles of βarr2 activation and functionality.
We compiled published and unpublished BRET datasets utilising this sensor panel across eleven GPCRs, eight of which were also measured in GRK2/3/5/6-knockout cells (Drube et al. 2022). Two N-domain sensors (F3, F5) consistently showed a concentration-dependent response to GPCR stimulation across all receptors, indicating that these sites report on βarr2 activation during GPCR engagement. In contrast, the remaining sites varied considerably between receptors, serving as reporters of GPCR identity. Interestingly, the signal of C-domain, but not N-domain, sensors correlated with GPCR classification (Oakley et al., 2000): with Class-A GPCRs (n=3) inducing significantly lower C-domain signals than Class-B GPCRs (n=8). GRK depletion reduced signal intensity across most sensors, yet, this reduction was not uniform across the sensor panel: two of the three tested Class-A GPCRs lost the ability to activate βarr2, while conformational changes in response to Class-B GPCRs were significantly more decreased, especially for C-domain sensors in comparison to N-domain FlAsH insertion sites. Therefore, their βarr2 conformational change fingerprint shifted towards a more "Class-A-like" profile in the absence of GRKs.
By systematically comparing βarr2 conformational fingerprints across GPCRs, this study disentangles the contributions of GPCR identity, receptor class, and GRK availability to βarr2 conformations, providing a framework for correlating βarr2 activation states with receptor and arrestin interaction modes.

#44 Single-molecule analysis reveals long-lived RAMP1–GPCR complexes in living cells

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G protein-coupled receptor (GPCR) interactions are increasingly recognized as highly dynamic, with receptor complexes undergoing rapid association and dissociation at the plasma membrane. Here, we combined single-molecule tracking, covalent surface immobilization, and fluorescence recovery after photobleaching (FRAP) to investigate receptor activity-modifying protein 1 (RAMP1)-dependent complexes in living cells.
Single-molecule tracking revealed robust co-diffusion of RAMP1 with both calcitonin receptor-like receptor (CLR) and calcitonin receptor (CTR). Quantitative interaction analysis showed no detectable dissociation of either complex within experimentally accessible timescales, with apparent interaction lifetimes limited by fluorophore photophysics. To overcome this limitation, we developed a SNAP-tag-based surface immobilization strategy that enables interrogation of dimer lifetime independently of long-term tracking. Immobilized RAMP1 efficiently trapped CLR, while FRAP experiments failed to reveal significant CLR rebinding or subunit exchange. Together, these approaches provide direct evidence that the canonical CGRP receptor exists as a long-lived, preassembled RAMP1–CLR complex at the plasma membrane. Similar interaction behaviour was detected for RAMP1–CTR, although the number of observable dimers was lower, indicating the presence of CTR monomers alongside dimers. These findings identify an important exception to the emerging model of dynamic GPCR organization and establish a framework for studying exceptionally stable membrane protein complexes in living cells.

#45 The Novel Synthetic Opioid Cychlorphine is a highly potent agonist at the μ opioid receptor

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Novel Synthetic Opioids (NSOs), including fentanyls, nitazenes and orphine-derived compounds pose an increasing threat to human health because of their high potency at the μ opioid receptor (MOR) and their potential to cause fatal respiratory depression [1,2]. Cychlorphine is a recently identified NSO for which the molecular and pharmacological basis of its potency remains poorly understood.[3]
Here we investigated cychlorphine alongside chlorphine, etonitazene, DAMGO and morphine using a combination of molecular modelling, in vitro cell signalling assays, ex vivo brain slice electrophysiology, and in vivo mouse respiration studies. Molecular modelling identified distinct interactions between cychlorphine and the active MOR–Gi complex, while signalling and in vivo assays reveal cychlorphine to have high potency at MOR.
Together, our results define structural and functional determinants of cychlorphine activity at MOR and link receptor-level pharmacology to neuronal and physiological opioid responses. These findings provide mechanistic insight into the exceptional potency and potentially high toxicity of this emerging synthetic opioid.

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#46 Free fatty acid receptor 4 (FFAR4) variants associated with disease have distinct signalling profiles

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Free fatty acid receptor 4 (FFAR4) is activated by medium- to long-chain fatty acids and regulates metabolic processes. Two FFAR4 variants, R67C and R254H/R270H, have been associated with increased obesity and diabetes risk, but their signalling profiles remain poorly defined. This study characterised variant signalling in model and adipocyte cell lines.
HEK293T cells and immortalised brown adipocytes were transfected with wild-type (WT), R67C, or R254H FFAR4-Nluc constructs, together with Venus-tagged mini-G proteins (mG12, mGq, mGo, mGs, and mGi) or β-arrestin 2. Recruitment induced by TUG891 or α-linolenic acid (αLA) was quantified using BRET. Additional assays examined responses to intracellular free fatty acids generated by lipolysis. Receptor localisation was assessed using immunofluorescence and HILO microscopy.
In HEK293T cells, R67C showed reduced mGq, mGo, and mGi recruitment, whereas R254H showed decreased mGq and mGo but increased mGi and mGs recruitment. In brown adipocytes, R254H showed reduced mGq and mGo recruitment but enhanced mGi and β-arrestin 2 coupling in response to lipolysis-derived fatty acids. Both variants localised predominantly intracellularly near lipid droplets.
These variants display distinct, context-dependent signalling profiles. Enhanced inhibitory coupling by R254H may attenuate stimulated lipolysis, providing a potential mechanistic link to obesity-related lipid dysregulation in metabolically relevant adipocyte cellular contexts.

#47 Mechanisms of GPR35 Signalling in Protection Against Hepatic Steatosis

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Introduction. Hepatic steatosis is a prevalent chronic liver disease characterised by excessive lipid accumulation in hepatocytes and progression to cirrhosis and hepatocellular carcinoma. G protein-coupled receptor 35 (GPR35) has emerged as a modulator of hepatic lipid accumulation and a potential therapeutic target; however, the underlying signalling mechanisms remain poorly defined. Here, we investigated the pathways through which GPR35 suppresses lipid accumulation.

Methods. Lipid accumulation was assessed using Oil Red O staining following treatment with oleic acid (OA) in the presence or absence of the high-potency GPR35 agonist lodoxamide. Experiments were performed in parental HEK293 cells, as well as cells lacking Gα proteins (ΔGall) or β-arrestin 1/2 (ΔβARR). GPR35-specific signalling was evaluated using the competitive antagonist compound 38 in xCELLigence impedance assays. Receptor internalisation was assessed using BRET-based assays in the presence or absence of a GRK5/6 inhibitor (compound 18) to disrupt β-arrestin recruitment.
Results. OA significantly increased lipid accumulation in HEK293 cells expressing GPR35. Lodoxamide markedly reduced OA-induced lipid accumulation in parental and ΔGall cells, but this effect was abolished in ΔβARR cells. Consistent with this, xCELLigence assays demonstrated robust lodoxamide-induced responses in parental and ΔGall cells, which were attenuated by compound 38 and absent in ΔβARR cells. Receptor internalisation studies revealed concentration-dependent GPR35 internalisation in response to lodoxamide, which was preserved in ΔGall cells but significantly reduced in ΔβARR cells. Inhibition of GRK5/6 similarly reduced receptor internalisation to levels observed in β-arrestin-deficient cells.

#48 Differential regulation underlies unappreciated complexity of pH-sensing GPCR signalling

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Acidosis is a hallmark feature of many clinically relevant pathologies such as cancer and inflammatory disorders. GPR4, GPR68 and GPR65 are a subset of G protein-coupled receptors (GPCRs) activated by an increase in proton concentration. A recent study demonstrated constitutive subcellular signalling of GPR65. To build on this observation, we combined bioluminescence resonance energy transfer (BRET) assays and live cell confocal microscopy to provide the first comprehensive side-by-side comparison of signalling, localisation, trafficking and receptor regulation for each of GPR4, GPR65 and GPR68 receptor. The results reveal distinct proton sensitivity, unique subcellular localisation and trafficking of each receptor. Distinct regulatory processed govern the signalling of each receptor. These findings give insight into the signalling and regulation of these receptors from neutral to acidic conditions and can inform future drug discovery and development efforts at this fascinating subclass of GPCRs.

#49 Impact on Kisspeptin Receptor (KISS1R) Regulation from its Interaction with G Protein-Coupled Estrogen Receptor (GPER)

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Introduction: The kisspeptin receptor (KISS1R) and G-protein-coupled estrogen receptor (GPER) are both pivotal in the endocrine regulation of reproductive health. KISS1R is essential for pubertal onset and regulating gonadotropin secretion via GnRH neurons, while GPER mediates non-genomic estrogen signalling and has been shown to dimerize with KISS1R, potentially playing an important role in distinct processes including endocrine feedback. We have previously shown that pretreatment with estrogen potentiates gonadotropin secretion after administration of the potent kisspeptin receptor agonist TAK-448. To date, there is limited data exploring the impact of estrogen and GPER on KISS1R activity activated by distinct KISS1R ligands.
Aim: To understand the role of the interaction between KISS1R and GPER on KISS1R cell surface expression, signalling and internalization.
Methods and Results: HEK293 cells were transfected with human KISS1R and/or human GPER. Bioluminescence Resonance Energy Transfer (BRET) assays confirmed that KISS1R and GPER form heteromers, while KISS1R also forms homomers. Heteromer formation was not influenced by KISS1R ligand treatment with either kisspeptin-10 (KP10, 10nM), kisspeptin-54 (KP54, 10nM), or TAK-448 (1nM). Flow cytometry revealed that KISS1R surface expression was reduced when co-expressed with GPER, and this reduction was not reversed upon treatment with KISS1R ligands. KISS1R internalization in response to KP10, KP54, and TAK-448 was analysed between 0 and 60 minutes, with maximum internalization observed 10 minutes post ligand treatment. Intriguingly, there was a different internalization profile observed across all ligands with distinct kinetics and degree of receptor internalized. TAK-448 induced the greatest level of KISS1R internalization (20.5%), however, when receptors were co-expressed, internalization was reduced to 14%. In contrast, the transient KISS1R internalization profile induced by KP54, was sustained in cells co-expressing GPER. To investigate the influence of dimerization on receptor function, we measured intracellular inositol phosphate-1 (IP1) accumulation following ligand treatment. Cells that expressed GPER exhibited a high basal level of IP1. Co-expression of KISS1R and GPER decreased basal levels of IP1, however, there was no further increase following treatment with any of the KISS1R ligands, including pre-treatment with estrogen (50 pg/ml).
Conclusion: These findings demonstrate that KISS1R and GPER heteromerization may not only influence KISS1R surface expression and agonist-induced Gq signal activity but could differentially modulate the agonist-induced endocytic trafficking profiles of KISS1R. These data highlight the regulatory role of receptor heterodimerization and trafficking in modulating KISS1R activity, providing insights for its potential use to treat endocrine disorders.

#50 Ortholog Selective Agonism at Succinate Receptor 1 Reveals Species-Dependent Differences in G Protein Activation

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Succinate receptor (SUCNR1) is a GPCR activated by the tricarboxylic acid (TCA) cycle metabolite succinate. Differences in SUCNR1 pharmacology across species may influence the interpretation of preclinical data and the selection of appropriate experimental models. In

this study, HEK293 cells were transiently transfected with human (h), mouse (m), or rat (r) SUCNR1 together with a BRET G(o) protein dissociation biosensor. Six agonists were evaluated, including the endogenous ligand succinate, and synthetic agonists cis-epoxysuccinate (cESA), compound 130(S), TUG-1688, TUG-1689, and compound 131(S). SUCNR1 agonists show clear species-dependent differences in activity across human, mouse and rat receptors. Compound 130(S),TUG-1688 and TUG-1689 showed strong activation at mSUCNR1 but no measurable activity at rSUCNR1. In contrast, compound 131(S) was inactive at mSUCNR1 but active at rSUCNR1. Succinate and CES were active across all species orthologues. These results have implications for the translation of SUCNR1 pharmacology in the treatment of disease.

#51 PD-PALM single-molecule microscopy as a tool to study membrane receptor oligomerization

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Membrane receptor dimerization and oligomerization are fundamental processes that diversify signalling responses and regulate downstream signalling. Bulk proximity-based approaches commonly used to investigate receptor oligomerization lack the ability to distinguish between complex types or resolve stoichiometry. However, PD-PALM (photoactivated localization microscopy with photoactivatable dyes), a single-molecule super-resolution microscopy approach, allows membrane localised receptors to be quantified with high precision. By using an antibody conjugated to a photoactivable dye in a 1:1 ratio, receptor oligomerization and complex stoichiometry can be determined, providing a level of detail beyond bulk monomer/oligomer levels.
Here we demonstrate that PD-PALM can be used to quantify membrane receptor oligomerisation, in both heterologous tagged-receptor expression systems and native receptor contexts. Using the GPCRs D2 dopamine receptor (D2R), and luteinising hormone receptor (LHR), we show that this approach can detected ligand-induced, mutation-dependent and peptide-perturbed changes in receptor oligomerisation profiles. Additionally, we quantify endogenous oligomerisation of the receptor tyrosine kinase, EGFR in non-small cell lung cancer cell lines, with mutant-specific changes in oligomerisation profiles detected following drug treatment.
Collectively, these findings establish PD-PALM as a broadly applicable, high-precision tool for quantifying membrane receptor oligomerization, with implications for understanding receptor signalling and therapeutic targeting.

#52 Complex Formation Between A2A and A2B Adenosine Receptors in Live Cells.

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A2A and A2B are Gs-coupled receptors that regulate adenosine signalling, with reported roles in inflammation, fibrosis, angiogenesis, and tumour progression (1). A2A has been shown to dimerise with other GPCRs (2), but whether A2A and A2B associate with each other remains unclear. This study assessed homo- and heterodimer formation between A2A and A2B using Bioluminescence Resonance Energy Transfer (NanoBRET). HEK293T cells were co-transfected with C-terminal NLuc- or mGOLD-tagged A2A and A2B receptors, with the NLuc-tagged donor kept constant (10ng) and the mGOLD-tagged acceptor increased (0-90ng). This was performed for A2A-A2A and A2B-A2B homodimer pairs, the A2A-A2B heterodimer pair. With C-terminal tagging, both homodimer and heterodimer BRET pairs showed a linear increase in FI with increasing mGold expression, while the BRET ratio reached saturation for A2A_Nluc–A2A_mGold; A2B_Nluc–A2B_mGold; for A2A_Nluc–A2B_mGold and for A2B_Nluc–A2A_mGold, suggesting close proximity between donor and acceptor. With N-terminally tagged receptors, the BRET ratio was saturable for A2A-A2B and A2A-A2A pairs but not A2B-A2B. These NanoBRET results indicate dimer formation for A2A homodimers and A2A-A2B heterodimers, with tag-dependent evidence for A2B homodimer formation.

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#53 Spatiotemporal APEX2 proximity proteomics reveals potential novel protein interactions of the ghrelin receptor (GHSR)

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The ghrelin receptor (GHSR) is a class A GPCR that regulates appetite and energy homeostasis, yet the molecular mechanisms governing its trafficking and signalling remain incompletely understood. Here, we combined APEX2 peroxidase-catalysed proximity labelling with functional assays to define the dynamic GHSR interactome following agonist stimulation. C-terminal APEX2 tagging preserved GHSR signalling, expression and trafficking while enabling rapid biotinylation of proximal proteins.

Following agonist treatment, biotinylated proteins were captured by streptavidin and temporally identified via proteomics. Temporal proteomic analysis identified distinct protein interaction networks associated with receptor activation, endocytosis and intracellular trafficking. Two GPCRs were also identified to be novel interactors to GHSR. We validated the knockdown of GPCR A and GPCR B and tested their effects on GHSR signalling surface expression and internalisation. Functional validation demonstrated that GPCR A and GPCR B did not substantially alter GHSR signalling, surface expression or internalisation, indicating that these interactions are unlikely to directly regulate receptor function under the conditions tested.

Our study provides the first temporal map of the GHSR interactome and identifies multiple candidate regulators of receptor trafficking and signalling, establishing a valuable resource for future mechanistic studies.

#54 Phosphokinome analysis of spatially restricted, ligand specific and temporal GPCR signalling.

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Follicle-stimulating hormone receptor (FSHR) is a Class A GPCR essential reproductive function, mediated via Gs/cAMP/PKA and β-arrestin-dependent signalling. For many GPCRs, the subcellular compartment localisation has emerged as a key determinant of signal propagation. Yet how FSHR localisation shapes its signalling output remains poorly understood.
To understand how spatial modulation impacts FSH ligand signal activation, we performed an unbiased kinome-wide profiling using PamChip® peptide microarray. Primary mouse ovarian granulosa cells were isolated from postpubertal mice and stimulated for 5 or 30 minutes with the full agonist, FSH, or the β-arrestin-biased agonist dg-eLHt, ± the dynamin inhibitor Dyngo-4a to restrict FSHR internalisation and thus FSH/FSHR signalling at the plasma membrane. This generated a ligand-, time- and space-resolved map of FSHR-driven kinase activity.
Of the 340 kinases analysed, 35 displayed novel activation/inhibition when spatially restricted. 29 were ligand biased in activation. 48 showed distinct temporal kinetics with the full agonist and biased agonist.
Together, these data establish a framework of how FSHR location modulates FSH ligand-dependent signalling signatures and the impact of acute versus chronic signalling in native tissues, with ramification for ovarian function.

#55 GPR84: from structure to pharmacology and bias

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GPR84 is a pro-inflammatory class A GPCR expressed predominantly in innate immune cells and responding to high concentrations of medium-chain fatty acids [1]. GPR84 is targeted in drug development against inflammatory conditions [2] and in bioimaging probe development for monitoring and mapping inflammation responses [3].
We recently reported the binding modes and mechanisms of action for orthosteric and allosteric agonists of GPR84 [4,5]. Guided by our cryo-EM structures and molecular simulations, we elucidated the structural basis of GPR84 activation, inactivation and biased signalling, informing the future drug discovery efforts against this receptor.

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#56 pHSense Eu Reagents for Time-Resolved Fluorescence-based monitoring of GPCR internalization

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Revvity

Monitoring the internalization of GPCRs in overexpressed and native conditions has become a major area of interest over the past decade. Revvity has developed pHSense Eu reagents from novel pH-sensitive Europium probes enabling no-wash and plate-based reader assays supporting both kinetic recording and end-point time-resolved fluorescence (TRF) detection to monitor the internalization efficacy and potency of many types of GPCR binders (small molecule, peptides, antibodies) in live cells. These fluorescent probes exhibit dual pH sensitivity, with both brightness and fluorescence lifetimes increasing in acidic environments, thereby significantly enhancing the signal-to-background ratio. pHSense Eu assays are performed with tag-based detection systems by using anti-FLAG antibody and SNAP labeling reagent, or without the need of any tag by using Fab anti-Human IgG or Fab anti-Mouse IgG2. All reagents are covalently labeled with pHSense Eu probes, and display nanomolar-range of affinity that remains stable under both alkaline and acidic conditions. These assays are conducted under physiological conditions in buffer or cell culture medium at room temperature or 37°C using either adherent or suspension cells.

#57 Seeing β-arrestin recruitment in real time

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Biased agonism is time-dependent, so endpoint assays can make pharmacologically distinct ligands look identical. Resolving how and when β-arrestin is recruited to a receptor requires measurement in living cells, in real time. It also requires an assay that is both reversible and sensitive enough to follow recruitment kinetics, separate transient from sustained recruitment, and report response amplitude. Here we show how NanoBiT® protein:protein interaction assays for GPCR:β-arrestin-1/2 recruitment provide a kinetic, reversible readout in live cells at low expression levels, in dual and single vector systems. Using CX3CR1 and fractalkine, continuous plate-reader traces resolve sustained recruitment, while bioluminescence time-lapse imaging on the GloMax® Galaxy resolves the same event at single-cell resolution, distinguishing responding from non-responding cells that a population average obscures.