Welcome to the 8th GPCR Workshop

We hope you will be inspired by the great science and collegial interactions.

Enjoy Hawaii. Aloha!!

Organizers:

Aashish Manglik
Mark von Zastrow
Jurgen Wess
Sébastien Granier
Brian Kobilka
Tong Sun Kobilka

A Note From Organizers

Dear Participants in the 2025 GPCR Workshop,

We're excited to see you soon at the 2025 Workshop and look forward to a terrific conference. We need to apprise you of a significant practical issue that has arisen in this year's planning...

The cost of supporting the Workshop has turned out to be significantly greater this year than in previous years, and we have less flexibility in how we do it. This has to do with increased costs for items generally, and with the implementation of union-related rules that make it not possible to add guests or extra food / drinks without incurring very substantial "up-charges." The bottom line is that, for this year, we need to be extra-careful to stay within budget.

Accordingly, we will strictly limit access to registered attendees, require name tags for access to all meals and talks, and require tickets for access to the banquet for the guests you bring. You can still purchase the tickets through PayPal. A cash bar will be available to our group when beverages are served.

The good news is that this meeting site has many excellent food and drink options nearby but separate from the Workshop. Please use them for any extras to avoid sinking our budget.

Aloha and Enjoy the workshop!

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GPCR Workshop 2025 Agenda

Day 1: Dec. 12, 2025

8:00am - 9:00am Breakfast - *Paniolo Ocean Terrace*

9:00am - 12:00pm Brian's Birthday Celebration - ***invitation only***

12:00pm - 1:00pm Box Lunch - *Paniolo Ocean Terrace*

2:00pm - 4:00pm REGISTRATION - *Paniolo Ocean Terrace*

Session 1

Chair: Brian Kobilka *Stanford University*

Paniolo Ballroom

4:00pm - 4:10pm
Welcome: Brian Kobilka

Brian Kobilka
Stanford University

4:10pm - 4:50pm
Uncovering unconventional GPCR stimuli

Aashish Manglik
UCSF

4:50pm - 5:30pm
Translational mechanisms for GPR75 loss-of-function driving protection from obesity

Judith Altarejos
Regeneron Pharmaceuticals

5:30pm - 6:00pm
Kidney Exchange

Alvin Roth
Stanford University

6:00pm – onward Reception

Day 2: Dec. 13, 2025

7:30am - 8:20am Breakfast - *Naupaka Lanai*

Session 2

Chair: Brian Kobilka *Stanford University*

Naupaka Ballroom

8:20am - 9:10am
Higher Order Transient Structures and the Principle of Dynamic Connectivity in Membrane Signaling

Rod MacKinnon
The Rockefeller University

9:10am - 9:50am
Exploring the Subcellular Organization of GPCR Signaling

Mark von Zastrow
UCSF

9:50am - 10:05am SHORT TALK #1
Identifying the role of the extracellular vestibule in ligand-dependent b_2-adrenergic receptor endocytosis

Nicole McIntosh
University of California, San Diego

10:05am - 10:25am Break- *Naupaka Lanai*

10:25am - 11:05am
Arrestin binding to GPCRs lacking the C-tail

Irene Coin
Leipzig University

11:05am - 11:45am
Dopamine D2 receptors bypass canonical signaling to directly tune NMDA receptor function and aversive learning

Jonathan Javitch
Columbia University

11:45am - 12:25pm
Computational reprogramming of GPCR signal transduction

Patrick Barth
École Polytechnique Fédérale de Lausanne

12:25pm - 12:40pm SHORT TALK #2
Compartmentalized autonomic signaling reveals distinct sympathetic and parasympathetic domains in cardiac myocytes

Alexander Demby
University of Oxford

12:40pm - 3:00pm Lunch and Poster Viewing *Naupaka Lanai*

Session 3

Chair: Nevin Lambert *Augusta University*

Naupaka Ballroom

3:00pm - 3:40pm
Structure and function of non-canonical GPCR signaling complexes

Kirill Martemyanov
University of Miami

3:40pm - 4:20pm
Mechanistic insights into G-protein coupling with an agonist-bound GPCR: implications for coupling specificity

Peter Hildebrand
Leipzig University

4:20pm - 5:00pm
A new type of biosensor to dissect GPCR conformational equilibria in intact cells

Andreas Bock
University Medical Center Mainz

5:00pm - 5:15pm SHORT TALK #3
Structural basis of a stepwise Gq activation by a chemokine receptor US28

Naotaka Tsutsumi
Institute of Science Tokyo

5:15pm - 5:30pm SHORT TALK #4
Fluorogenic-Based Biosensors Reveal Specific and Universal Regulators of Endosomal GPCRs

Braden Lobinger
Oregon Health Sciences University

5:30pm - 6:30pm Poster Session

6:30pm - 8:00pm Dinner – *On your own*

8:00pm - 10:00pm INTERACTIVE SESSION #1 – *Naupaka Ballroom*

Roger Sunahara
UCSD

Day 3: Dec. 14, 2025

7:30am - 8:30am Breakfast - *Naupaka Lanai*

Session 4

Chair: Aashish Manglik *UCSF*

Naupaka Ballroom

8:30am - 9:10am
Leveraging Computational and Experimental Data to Advance Knowledge of GPCR Signaling and Drug Discovery

Marta Filizola
Icahn School of Medicine at Mount Sinai

9:10am - 9:25am SHORT TALK #5
The mechanisms of PLC3 activation by Gαq and Gβγ

Nevin Lambert
Medical College of Georgia, Augusta University

9:25am - 10:05am
New insights into the spatiotemporal organisation of GPCR signalling

Davide Calebiro
University of Birmingham

10:05am - 10:25am Break - *Naupaka Lanai*

10:25am - 11:05am
How to transduce force into biochemical commands – Mechanosensing by adhesion GPCRs

Tobias Langenhan
Leipzig University

11:05am - 11:45am
Structure and function of GPCR-toxin complexes

Sébastien Granier
Institut de Génomique Fonctionnelle, CNRS/INSERM/Université de Montpellier

11:45am - 12:00pm SHORT TALK #6
Positive allosteric modulation of CCK1R through the third intracellular loop

Fredrik Sadler
University of Minnesota

12:00pm - 12:30pm
Targeting a cryptic pocket in cannabinoid receptors drives peripheral and functional selectivity

Susruta Majumdar
Washinton University St. Louis

12:30pm - 12:45pm SHORT TALK #7
Ligand-dependent activation trajectories dictate GPCR signaling efficacy in intact cells

Romy Thomas
Stanford University

12:45pm - 3:00PM Lunch and Poster Viewing - *Naupaka Lanai*

Session 5

Chair: Sebastien Granier

Naupaka Ballroom

Institut de Génomique Fonctionnelle, CNRS/INSERM/Université de Montpellie

3:00pm – 3:40pm
Unconventional GPCR Signaling in the Hedgehog Pathway and Beyond

Ben Myers
Huntsman Cancer Institute, University of Utah

3:40pm - 4:20pm
Exploring non-canonical signaling mechanisms across diverse GPCRs

Ruth Huttenhain
Stanford University

4:20pm - 5:00pm
Conformational coupling between extracellular and transmembrane domains modulates holo-adhesion GPCR function

Demet Arac
University of Chicago

5:00pm - 5:15pm SHORT TALK #8
Unnatural Amino Acid Labeling and smFRET Reveal Intermediate States in Glucagon Receptor Signaling

Shi Feng
Stanford University

5:15pm - 5:30pm SHORT TALK #9
Single G Protein Turnover Defines DRD2's Coupling Efficiency

Desye Ambaw
The University of Queensland

5:30pm - 6:30pm Poster Session

6:30pm - 8:00pm Dinner – *On your own*

8:00pm - 10:00pm INTERACTIVE SESSION #2 – *Naupaka Ballroom*

Nevin and Sivaraj

Day 4: Dec. 15, 2025

7:30am - 8:30am Breakfast - *Naupaka Lanai*

Session 6

Chair: Mark von Zastrow *UCSF*

Naupaka Ballroom

8:30am - 9:10am
Exploring Intracellular GPCRs and Their Role in Pathway Crosstalk

Nina Tsvetanova
Duke University

9:10am - 9:25am SHORT TALK #10
Age-dependent GPCR expression dynamics guides precision medicine for fetal, pediatric, and geriatric populations

Katarina Nemec
St. Jude Children's Research Hospital

9:25am - 10:05am
A Survival Pressure Selection method for GPCR drug screening

Xiangyu Liu
Tsinghua University

10:05am - 10:25am Break - *Naupaka Lanai*

10:25am - 11:05am
Using nanobodies as tools to interrogate and control GPCR signaling

Andrew Kruse
Harvard Medical School

11:05am - 11:45am
Assembly required – how cells build functional ion channel complexes

Tino Pleiner
Stanford University

11:45am - 12:00pm SHORT TALK #11
Unravelling non-canonical interactions at GPCR intrinsically disordered regions.

Mia Greeson
Stanford University

12:00pm - 12:30pm
Multimodal β-arrestin functions through membrane nanodomain formation

Asuka Inoue
Kyoto University

12:30pm - 3:00pm
Lunch and Poster Viewing *(Poster takedown at end of this session) - Naupaka Lanai*

Session 7

Chair: Aashish Manglik *UCSF*

Naupaka Ballroom

3:00pm - 3:40pm
Application of genetically encoded dopamine and 2nd messenger sensors challenge paradigms of dopamine receptor signaling in the striatum

Ulrik Gether
University of Copenhagen

3:40pm - 4:20pm
The role of protein dynamics in GPCR signaling / Closing Remarks

Brian Kobilka
Stanford University

4:20pm - 5:00pm
Conference Discussion / Closeout

Banquet Dinner

Paniolo Ocean Terrace
6:00pm - 10:00pm

Speaker Abstracts (presenting order)

Uncovering unconventional GPCR stimuli

Aashish Manglik, UCSF

Many of us are enamored with GPCRs because of their incredible versatility as biological sensors. The structural plasticity of the GPCR fold has enabled this large family of proteins to detect and discriminate stimuli ranging from individual photons and ions to millions of chemicals, peptides, and proteins. We have a particular interest in edge cases of GPCR molecular recognition - how does this family evolve to recognize individual protons or the immense diversity of odorous small molecules? I will describe how we are combining structural interrogation with massively parallel mutagenesis strategies to uncover the function of such GPCRs. These approaches allow us to systematically connect atomic-level biophysical mechanisms of molecular recognition and allostery with evolutionary diversification across the GPCR superfamily. By linking structural dynamics, sequence space, and sensory function, we aim to define general principles governing how evolution repurposes the GPCR fold to meet new biological challenges.

Higher-order transient structures and their potential for membrane signaling.

Roderick MacKinnon, Howard Hughes Medical Institute and The Rockefeller University

We have found that some membrane proteins, including GPCRs and ion channels, spontaneously organize into spatial patterns on the plasma membrane. These patterns arise from weak, transient, yet specific interactions that are predominantly homotypic, meaning proteins preferentially associate with themselves. Such homotypic interactions result in the formation of what we call higher-order transient structures (HOTS). In some cases, heterotypic interactions bring into proximity different HOTS made of different proteins. Thus, the patterns we observe reflect a spontaneous sorting of proteins in the cell membrane mediated by weak protein-protein interactions.

I will discuss the thermodynamic principles of HOTS formation and how HOTS can give rise to a biomolecular condensate phase. While these two states of aggregation matter are closely related, they have very different physical properties and likely fulfill unique roles in biology.

Finally, and most importantly, I wish to discuss our hypothesis that the spontaneous patterns of membrane proteins underlie the connectivity of signal pathway components. What do the arrows connecting signal transduction components in textbook figures really mean? At one extreme, localization of components might be unimportant for some pathways in which, for example, a second messenger is spread uniformly throughout the cell, while at the other extreme proteins at the two ends of the arrow might be permanently bound to each other to achieve highly focused localization. We suspect that life may utilize different levels of proximity among pathway components, mediated by a range of interactions from non-existent to strong. The existence of HOTS formed by membrane proteins, and their roots in general principles of thermodynamics applied to weak but specific molecular interactions, suggest that a dynamic form of connectivity mediated by HOTS might be ubiquitous. I very much look forward to a discussion of this idea.

Exploring the subcellular organization of GPCR signaling

Mark von Zastrow, UCSF

Cellular GPCR signaling is now recognized to be exquisitely spatially organized, not only in the plasma membrane but also across various endomembrane compartments. I will discuss recent progress toward understanding the mechanistic basis of GPCR-mediated activation of cognate G proteins on endosomes and the functional significance of such localized G protein activation.

Identifying the role of the extracellular vestibule in ligand-dependent β2-adrenergic receptor endocytosis

Nicole McIntosh, UCSD

G protein-coupled receptors (GPCRs) are a large family of cell-surface proteins that act as cellular sensors for external signals. At the plasma membrane, GPCRs interact with a diverse array of extracellular ligands, such as circulating hormones or diffusing neurotransmitters, and translate extracellular signals into the cell by coupling to intracellular G proteins, which regulate effectors to ultimately yield a cellular response. GPCR signaling is canonically thought to occur at the plasma membrane, where it can be stopped by beta-arrestin, the binding of which desensitizes receptors by occluding G protein coupling and triggering receptor endocytosis. However, there is an emerging body of literature demonstrating that GPCRs engage G proteins from within the cell to regulate discrete, spatially- and temporally- compartmentalized signaling pathways. This discovery presents a new frontier of pharmacological opportunity, and the study of organelle-localized GPCR signaling is currently one of the areas in GPCR biology that is gaining the most traction. Consequently, ligand signaling bias, or the ability of ligands to stabilize distinct receptor conformations that differentially engage G proteins or beta-arrestin, takes on a new significance, because it is not limited simply to differential engagement of transducers, but also encompasses the ability of ligands to induce receptor trafficking to and participation in distinct signaling hubs, thereby engendering spatially encoded signaling outcomes. Intriguingly, dose response curves for many receptors routinely reveal lower agonist potency and positive cooperativity (Hill slopes >1) towards beta-arrestin recruitment compared to that of cognate G proteins. We hypothesize that the most parsimonious explanation for these differences is the existence of a second, low-affinity binding site that plays an important role in receptor endocytosis. Additionally, we hypothesize that the most likely location for this secondary binding site is in the extracellular vestibule of the receptor.

Arrestin binding to a GPCR lacking the C-tail

Irene Coin, Leipzig University

Signaling of most GPCRs is regulated by β-arrestins, cytosolic proteins that recognize active, phosphorylated GPCRs, dock to the intracellular receptor side, and drive the receptor to endosomes, from where it can either recycle to the cell surface or be targeted for degradation. At the molecular level, the most widely discussed pattern of GPCR–arrestin interaction involves two major interfaces: docking of the phosphorylated receptor tail to the arrestin N-domain, and binding of the arrestin finger loop to the receptor core (fully engaged conformation). In addition, several experimental findings have revealed the existence of a so-called “tail conformation,” in which only the interaction of arrestin with the receptor tail is present.

On the other hand, many GPCRs lack a phosphorylatable C-tail. Instead, many of them feature a long third intracellular loop (ICL3) that becomes phosphorylated upon receptor activation and serves as a docking site for arrestin. However, structural information for such complexes is very limited, and most molecular details of the interaction remain unknown.

Here, we apply our platform for systematic crosslinking using genetically encoded crosslinkers, combined with extensive energy-based molecular modeling and molecular dynamics simulation, to dissect the interaction between β-arrestin and the muscarinic acetylcholine M2 receptor (M2R), a GPCR that lacks a C-tail and recruits arrestin through ICL3. Based on 67 experimental intermolecular spatial constraints, we generated an accurate molecular model of the M2R–arrestin complex, which reveals the path of the long receptor ICL3 on the arrestin N-domain and the overall topology of the complex. Our results show that the conformation detected in live cells is a fully-engaged core conformation, albeit featuring a large degree of flexibility. Furthermore, biochemical data suggest that positioning the key phosphorylation sites within ICL3, rather than on the receptor tail, has functional relevance for the extent and kinetics of arrestin recruitment.

Dopamine D2 receptors bypass canonical signaling to directly tune NMDA receptor function and aversive learning

Jonathan Javitch, Columbia University

Dopamine D2-receptors (D2Rs) modulate reward learning and aversive behaviors, with dysfunction linked to addiction and psychiatric disorders. D2Rs regulate behavior through modulation of striatal glutamatergic transmission, yet how D2Rs control postsynaptic glutamate signaling remains poorly understood. Using novel molecular tools to selectively disrupt heteromeric interactions while preserving canonical signaling, here we show that direct physical coupling between D2Rs and GluN2B-containing NMDA receptors in nucleus accumbens medium spiny neurons enables D2Rs to suppress NMDA receptor function independent of canonical G protein and arrestin signaling. This modulation was input-specific, occurring at thalamic but not cortical synapses converging on the same neurons, constrained long-term potentiation, and within the medioventral nucleus accumbens facilitated aversive learning. These findings reveal that D2Rs can bypass second messenger systems to directly tune glutamatergic transmission through receptor-receptor interactions, providing a mechanism by which dopamine selectively gates specific glutamatergic inputs to control striatal plasticity and behavioral adaptation.

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Computational design of allosteric pathways reprograms ligand-selective GPCR signaling

Patrick Barth, École Polytechnique Fédérale de Lausanne

G-protein–coupled receptors (GPCRs) are the largest family of signaling receptors and major drug targets. Yet, linking variations in receptor sequence, ligand chemistry, and binding to specific signaling outcomes remains challenging. To address this, we develop computational approaches combining protein structure, dynamics, and design to predict and reprogram GPCR responses to diverse ligands. Using these methods, we engineered 32 dopamine D1 and D2 receptor variants with broadly reprogrammed agonist-induced signaling profiles. I will discuss how subtle natural and designed sequence variations, predicted to modulate distinct structural and dynamic mechanisms, profoundly altered ligand potency and efficacy in agreement with our models. I will also highlight emerging AI-physics hybrid methods enabling efficient generative modeling of GPCR conformational ensembles. Ultimately, this work establishes a rational framework for computing the effects of sequence polymorphisms on ligand-selective signaling and for designing receptors with programmable signaling properties.

Compartmentalised autonomic signalling reveals distinct sympathetic and parasympathetic domains in cardiac myocytes

Alexander Demby, University of Oxford

Cyclic 3'-5' adenosine monophosphate (cAMP) plays a key role in the regulation of cardiac function, including rate and force of contraction and relaxation. In cardiac myocytes, cAMP is modulated by sympathetic stimulation via Gαs-coupled beta-adrenergic receptors (β-AR) and by parasympathetic stimulation via Gαi-coupled muscarinic acetylcholine receptors. Spatiotemporal compartmentalisation of cAMP signalling is critical for hormonal specificity, and it is well established that stimulation of cAMP generation driven by GsPCRs is compartmentalised. What is not clear is whether inhibition of cAMP generation by GiPCRs is homogenous or similarly compartmentalised. cAMP signals were measured in live cells at adrenergic targets using targeted FRET-based cAMP reporters in neonatal rat ventricular myocytes. β1-AR stimulation but not β2AR stimulation increased cAMP locally at the sarcoplasmic reticulum and at the myofilament but not at the L-type calcium channel whilst β2-AR stimulation alone elevated cAMP locally at the L-type calcium channel only. During simultaneous stimulation of muscarinic and adrenergic receptors, cAMP elevation was attenuated at the sarcoplasmic reticulum and the myofilament but not at the L-type calcium channel. This suggests that cholinergic stimulation opposes cAMP elevation driven by β1-AR but not β2-AR. Furthermore, it suggests that the regulation of the L-type calcium current by muscarinic receptors is cAMP-independent, and that crosstalk between adrenergic and cholinergic signalling in regulation of cardiac function is more complex than having opposing effects on cAMP generation.

Structure and function of non-canonical GPCR signaling complexes

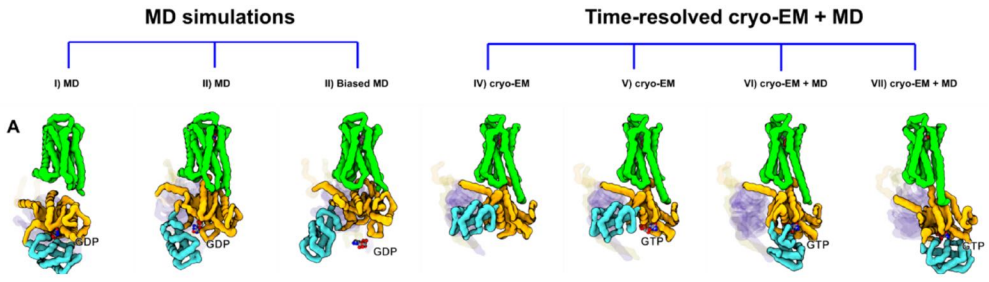
Kirill Martemyanov, University of Miami

Canonically, GPCRs function to activate heterotrimeric G proteins by catalyzing binding of GTP to Ga subunits. Their function is opposed by the Regulator of G protein Signaling (RGS) proteins that catalyze the reverse reaction, speeding up GTP hydrolysis by the Gα proteins to accelerate signal termination. We have discovered that an orphan receptor GPR158 directly binds RGS protein RGS7 and solved the structure of the complex by CryoEM. The structure reveals that the non-catalytic domain of RGS7 interacts with the intracellular region of the receptor typically involved in the Gα binding. We further found that an amino acid glycine serves as an endogenous ligand of GPR158 and its binding to the receptor influences the activity of RGS7 suggesting an alternative method of signal transduction by this receptor. We further developed a nanobody that selectively interacts with the glycine binding domain in GPR158. CryoEM structure of the nanobody bound GPR158 shows concerted conformational changes in the receptor and the associated RGS7 complex suggesting the mechanism for ligand induced regulation of the RGS activity. Together, these findings illustrate a non-canonical mechanism for the transduction signals employed by this metabotropic glycine receptor system.

Mechanistic insights into G-protein coupling with an agonist-bound G protein-coupled receptor

Peter W.Hildebrand, Leipzig University

G protein-coupled receptors (GPCRs) activate heterotrimeric G proteins by promoting guanine nucleotide exchange. Here we use classical µ-second MD simulations to investigate the coupling of Gs proteins with the β2-adrenoceptor (β2AR) and describe the structural events that ultimately lead to the ejection of GDP from its binding pocket in the Gα subunit, the rate-limiting step during G-protein activation. Signal propagation from the receptor to the G protein occurs via an extended receptor interface, involving a lysine-rich motif at the intracellular end of a kinked transmembrane-helix 6, confirmed by site-directed mutagenesis and functional assays. From this β2AR-GsGDP intermediate, Gs undergoes an in-plane rotation along the receptor axis to approach the β2AR-Gsempty state[(1)]. Notably, in a separate time-resolved cryo-EM study (Skiniotis lab) combined with MD simulations to examine the stepwise mechanism of G-protein activation in response to GTP loading, we find that the G protein undergoes an in-plane rotation against the receptor in the opposite direction during the dissociation process[(2)]. When comparing the two processes, other similarities emerge: the individual structural changes (such as breakage of contacts of the switchI region with the nucleotide, opening of α1-Gα and opening of the all- helical domain) induced in Gα by binding to the receptor, which ultimately lead to the ejection of GDP, are reversed in order during G protein dissociation following GTP binding. In the case of the system studied here, the corkscrew-like binding and unbinding motion results in the interrogation of almost the entire receptor interface by the G protein, providing multiple opportunities for receptor discrimination between cognate and non-cognate interactions. Central to the G protein selection process are flexible receptor elements, including TM6 and ICL2, that tune the receptor's ability to bind and activate G proteins. Our analysis sheds new light on the steps of receptor-mediated G protein activation and extends the current limited view of nucleotide-free snapshots to include additional states and structural features responsible for signal transduction and specificity of G protein coupling.



[1] Batebi H, Pérez-Hernández G, Rahman SN, Lan B, Kamprad A, Shi M, Speck D, Tiemann JKS Guixà-González R, Reinhardt F, Stadler PF, Papasergi-Scott MM, Skiniotis G, Scheerer P, Kobilka BK, Mathiesen JM, Liu X, Hildebrand PW. Mechanistic insights into G protein association with a G protein-coupled receptor. Nat Struct Mol Biol 2024

[2] Papasergi-Scott MM, Pérez-Hernández G, Batebi H, Gao Y, Eskici G, Seven AB, Panova O,Hilger D, Casiraghi M, He F, Maul L, Gmeiner P, Kobilka BK, Hildebrand PW, Skiniotis G. Time-resolved cryo-EM of G-protein activation by a GPCR. Nature 2024

A new type of biosensor to dissect GPCR conformational equilibria in intact cells

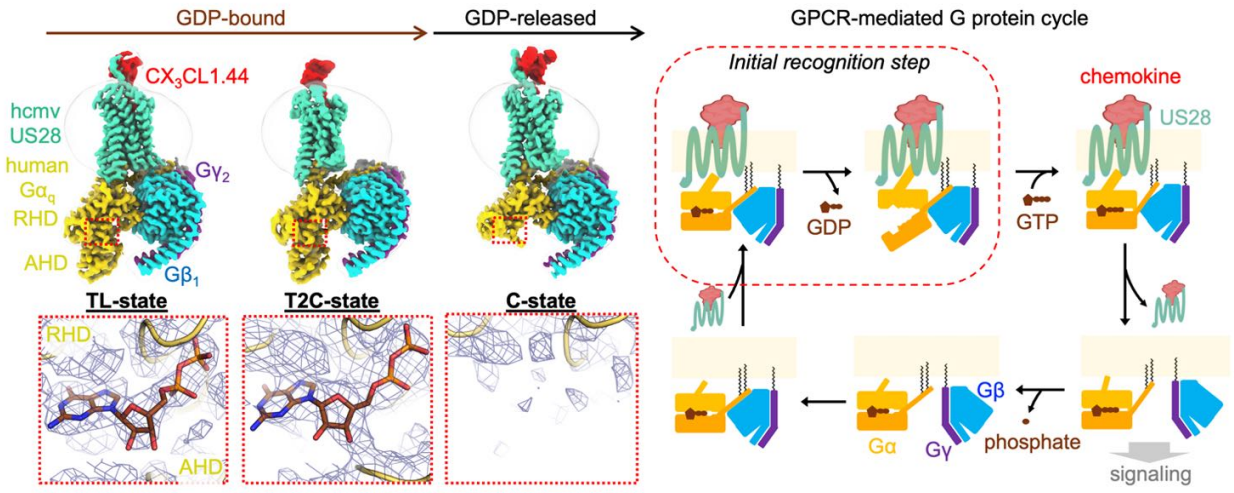
Andreas Bock, University Medical Center of the Johannes Gutenberg-University

G protein–coupled receptors (GPCRs) are central hubs of cellular communication and remain the most widely exploited class of therapeutic drug targets. Biophysical studies with purified receptors have revealed that GPCRs can sample multiple conformational states, but how this complexity translates into ligand efficacy and signaling outcomes in living cells is still unclear. We introduce a new biosensor technology to monitor rapid, ligand-mediated conformational changes of a prototypical GPCR directly in intact cells. By combining genetic code expansion and bioorthogonal labeling with fluorescence-based readouts, we uncover previously hidden activation trajectories that receptors follow upon ligand binding. These trajectories differ between agonists and appear to guide the assembly of receptor–G protein complexes with distinct signaling capacities. Our findings suggest that ligand efficacy in cells does not arise simply from stabilizing an “active” receptor state, but from shaping dynamic, ligand-specific activation routes. This framework offers a refined view of GPCR activation in its native environment and may open new possibilities for designing drugs that selectively exploit distinct activation mechanisms.

Structural basis of a stepwise Gq activation by a chemokine receptor US28

Naotaka Tsutsumi, Institute of Science, Tokyo

G protein-coupled receptors (GPCRs) govern diverse cellular responses and are crucial drug targets. However, the structural events leading from G protein recognition to GDP release have been poorly understood. Here, we utilized the viral GPCR US28, which forms metastable signaling intermediates, to overcome this limitation. Using cryo-electron microscopy and an engineered chemokine superagonist, we determined the structures of the US28-Gq complex representing the initial GDPbound state (TL-state), the nucleotide-free state (C-state), and a previously unseen intermediate (T2Cstate). These structures provide unprecedented snapshots of the G protein activation trajectory, revealing a stepwise mechanism of conformational rearrangements. This activation cascade shows marked similarity to that suggested during G protein activation catalyzed by human GPCRs, suggesting a conserved mechanism for GPCR signaling.



Fluorogenic-Based Biosensors Reveal Specific and Universal Regulators of Endosomal GPCRs

Braden Lobingier, Oregon Health & Sciences University

G protein-coupled receptor (GPCR) trafficking through the endosomal-lysosomal pathway is an important cellular mechanism to regulate GPCR signaling and expression. However, identifying the proteins involved in regulating GPCR trafficking at endosomes is challenging due to the small size and dynamic nature of endosomes as well as the complexity of its physically adjacent yet functionally opposed sorting operations. We have developed a highly sensitive fluorogenic-based biosensor for GPCR expression and trafficking through the endosomal-lysosomal pathway by leveraging the ability of the engineered enzyme APEX2 to activate the fluorogenic substrate Amplex UltraRed (AUR). We have combined the GPCR-APEX2/AUR reporter with CRISPR-based functional genomics to create a genetic map of universal and receptor-specific regulators of GPCR trafficking. This genetic map and our subsequent studies have revealed proteins critical for GPCR sorting at endosomes including DNAJC13 as a master regulator of GPCR endosomal-lysosomal trafficking and the Retromer complex as a selective regulator of endosomal GPCRs and other membrane proteins carrying a novel class of bileucine-based recycling motifs. Together, this genetic map—the product of multiple genome-wide CRISPR screens— has identified novel points of genetic intervention to control GPCR signaling and trafficking along the endosomal-lysosomal pathway.

Leveraging Computational and Experimental Data to Advance Knowledge of G Protein-Coupled Receptor Signaling and Drug Discovery

Marta Filizola, Icahn School of Medicine at Mount Sinai

Understanding G Protein-Coupled Receptor (GPCR) signaling requires more than static structures: it demands atomic-level views of the dynamic and kinetic processes that shape ligand-specific activation. No single approach can capture this complexity; progress depends on integrating experimental and computational data. At the same time, the discovery of chemical probes with precise pharmacological profiles is essential to both dissect receptor function and enable safer drug development. In this presentation, I will discuss recent advances toward these goals. Highlights include: (a) a Bayesian framework that integrates enhanced molecular dynamics with single-molecule imaging to refine ensembles of ligand-bound GPCR–G protein complexes; (b) long-timescale simulations of experimentally derived GPCR-G protein complex intermediates that illuminate how ligands influence G protein activation pathways; (c) robust analyses that trace allosteric communication and suggest mechanisms underlying drug safety; and (d) AI-driven approaches that leverage high-resolution structural information and large chemical libraries to accelerate therapeutic discovery.

The mechanisms of PLCβ3 activation by $G_{\alpha q}$ and $G_{\beta\gamma}$

Nevin Lambert, Augusta University

Phospholipase Cβ enzymes catalyze PIP2 hydrolysis at the plasma membrane and are the primary effectors downstream of G_q heterotrimers. Structural studies have identified two binding sites on PLCβ3 for $G\alpha_q$ and two binding sites for Gβγ dimers, suggesting that synergy between G protein subunits may be a regular part of G_q-mediated activation. We have studied how each of these sites contributes to enzyme activation and translocation of PLCβ3 from the cytosol to the plasma membrane using bioluminescence resonance energy transfer (BRET) assays in living cells. Activation of AT_1 angiotensin receptors results in PIP2 hydrolysis that is abolished by sequestration of $G\alpha_q$ and inhibited by sequestration of Gβγ. Site-directed mutagenesis indicates that $G\alpha_q$ binding to both the catalytic domain and distal C terminal domain (dCTD) of PLCβ3 is required for activation downstream of AT_1, and binding of Gβγ to both catalytic domain binding sites is required for maximal activation. Binding to active $G\alpha_q$ promotes translocation of a fraction of PLCβ3 from the cytosol to the plasma membrane after activation of AT_1, whereas binding to Gβγ does not contribute to translocation. When only endogenous G proteins are present we find that most of the free Gβγ released downstream of AT1 and several other G_q-coupled receptors is derived from secondary activation of $G_{i/o}$ heterotrimers. Together these results suggest that efficient activation of PLCβ3 requires both $G\alpha_q$ and $G\beta_\gamma$ and may involve activation of both G_q and $G_{i/o}$ heterotrimers.

Supported by NIH grants R35GM145284 to N.A.L. and R01GM152701 to A.M.L.

Untangling the GPCR puzzle with light: from arrestin to intracrine signalling

Davide Calebiro, University of Birmingham

Whereas structural studies have provided major insights into the activation mechanisms of GPCRs, how these receptors operate in our cells to produce specific biological responses remains insufficiently understood. Davide's group develops innovative optical methods based on FRET/BRET and single-molecule microscopy that enable studying GPCR signalling in living cells with high spatiotemporal resolution. Using these approaches, they were among the first to demonstrate that GPCRs are not only active at the plasma membrane but also in the endosomal compartment, which has challenged the classical model of GPCR signalling. Ongoing work in the Calebiro lab is dedicated to further clarifying the physiological and pharmacological implications of GPCR signalling at intracellular sites, with a particular focus on metabolically relevant GPCRs. In parallel, they have further developed innovative single-molecule approaches, which previously led to the discovery of receptor-G protein signalling nanodomains at the plasma membrane, to study other fundamental aspects of GPCR signalling such as the mechanisms of β-arrestin recruitment and activation. Altogether, Davide's work has revealed that the molecular events governing GPCR signalling are much more complex and dynamic than previously thought, which has important implications for our understanding of GPCR signalling and the development of 'intelligent' drugs targeting this important family of membrane receptors.

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How to transduce force into biochemical commands - Mechanosensing by adhesion GPCRs

Tobias Langenhan, Leipzig University

In stark contrast to classically targeted GPCRs, adhesion GPCRs have been largely neglected by biologists, pharmacologists and clinician scientists for decades. Only recently their physiological and signaling properties are being unravelled and show exciting features including their roles in tissue architecture, signaling through an intramolecular agonist, cell-autonomous and non-autonomous effects, and their unusual activation through mechanical stimuli. I will discuss the recent developments on adhesion GPCR signaling and introduce the receptor family as a topical area in neuroscience, immunology and cancer research.

Structure and function of GPCR-toxin complexes

Sébastien Granier, Institut de Génomique Fonctionnelle

G protein-coupled receptors (GPCRs) are central to numerous physiological processes and remain major pharmacological targets. Remarkably, many natural products, including toxins from animal venoms and bacterial metabolites, have evolved to hijack GPCR signaling for their own advantage. In this presentation, I will discuss ongoing work in my laboratory exploring how these natural compounds interact with and modulate GPCR activity. Using integrative approaches combining molecular pharmacology, structural biology, and computational modeling, we aim to elucidate the mechanisms by which toxins and microbial effectors reprogram GPCR signaling. Understanding these interactions not only deepens our insight into host-pathogen and predator-prey communication but also inspires the development of novel bio-based therapeutics.

Nanobodies targeting the third intracellular loop (ICL3) are receptor subtype-selective allosteric modulators

Fredrik Sadler, University of Minnesota

Allosteric modulation is an attractive drug discovery strategy for many GPCR targets, in part due to the inherent receptor subtype specificity of allosteric sites relative to the orthosteric ligand binding pocket. Our group recently characterized allosteric properties of the third intracellular loop (ICL3) that regulate signaling effector coupling to the receptor. It would be advantageous to leverage these allosteric properties with allosteric modulators due to the unique sequence composition of ICL3 for individual GPCR subtypes. However, the lack of protein structure in ICL3 makes this premise difficult. We devised a strategy to discover nanobodies from a naïve yeast surface displayed library that stabilize the ICL3 conformational ensemble. Here, we demonstrate the utility of this strategy for a panel of GPCRs with heterogenous sequence properties. We describe ICL3-binding nanobodies with a range of conformational properties, ranging from stabilization of states that mediate receptor signaling (positive allosteric modulators (PAM)) or block receptor signaling (negative allosteric modulators). Interestingly, we found that nanobodies specific to different GPCRs with unique ICL3s act through similar mechanisms of action, mediated by proximity of the nanobody binding site to the cytoplasm-exposed ends of transmembrane helices 5 and 6. As proof-of-principle of this approach for allosteric modulator development, we derived a PAM nanobody that acts on the beta 3 adrenergic receptor (β3AR), a historical target for obesity. Unlike orthosteric agonists that target β3AR[3], this nanobody enhances β3AR signaling without affecting β1AR or β2AR signaling. These results demonstrate the potential of ICL3 as a highly specific site for GPCR allosteric modulation. We anticipate that ICL3-targeted allosteric modulators will be transformative for use cases that demand a low side effect burden.

Targeting a cryptic pocket in cannabinoid receptor 1 drives peripheral and functional selectivity

Susruta Majumdar, Washington University School of Medicine

The current opioid overdose epidemic highlights the urgent need to develop safer, more effective treatments for chronic pain. The cannabinoid receptor type 1 (CB1) is a promising non-opioid target for pain relief, but the therapeutic utility of CB1 agonists has been limited by centrally mediated side effects like psychoactivity and analgesic tolerance when dosed repeatedly. We hypothesized that we could overcome both issues by designing peripherally restricted CB1 agonists that minimize arrestin recruitment. We achieved these goals by computationally designing positively charged derivatives of the potent CB1 agonist MDMB-Fubinaca (FUB). We designed these ligands to occupy a cryptic pocket identified through molecular dynamics (MD) simulations—an extended binding pocket that opens rarely and leads to the conserved signaling residue D2.50. Previous work from our laboratory has shown that targeting this residue in opioid receptors leads to biased signaling (Nature 2023;613(7945):767-774, ACS Cent Sci. 2024;10(8):1490-1503 and Nat Commun 2025 (accepted)). We used structure determination, pharmacological assays, and MD simulations to verify binding modes of the CB1bitopic ligands and to determine the molecular mechanism by which they achieve dampening of arrestin recruitment. Our lead ligand, VIP36, is highly peripherally restricted and demonstrates remarkable efficacy in multiple animal pain models, with 100-fold dose separation between analgesic efficacy and centrally mediated side effects. VIP36 exerts analgesic efficacy through peripheral CB1 receptors and shows limited analgesic tolerance. These results show how targeting a cryptic pocket in a G-protein-coupled receptor (GPCR) can lead to enhanced peripheral selectivity, biased signaling, desired in-vivo pharmacology and reduced adverse effects. This has significant implications for chronic pain treatment via peripheral CB1 activation but also could revolutionize the design of drugs targeting other GPCRs (Nature 2025, doi: 10.1038/s41586-025-08618-7).

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Ligand-dependent activation trajectories dictate GPCR signaling efficacy in intact cells

Romy Thomas, Stanford University

G protein–coupled receptors (GPCRs) form the largest class of membrane receptors and represent one of the most important class of drug targets. Ligand binding to GPCRs does not act as a simple ON–OFF switch, but rather is shaped by dynamic structural interactions between extracellular and intracellular receptor domains but also bound transducers (G proteins, arrestins). These connections, known as allosteric coupling, are thought to underlie ligand efficacy and promote transducer selectivity. Yet, how efficacy is encoded in the ligand-receptor interactions and how many receptor states exist in living cells remain poorly understood. To address this, we combined genetic code expansion with bioorthogonal labeling to engineer a panel of single-color GPCR activation biosensors for the M_2 muscarinic acetylcholine receptor (M_2R). These biosensors enable real-time monitoring of agonist-induced conformational dynamics across the receptor's extracellular surface in intact cells. Using this approach, we found that different agonists stabilize equilibria of at least four distinct active states of G protein–bound M_2R, each with different capacities to activate G proteins. The formation of these M_2R–G protein complexes follows trajectories involving both shared and ligand-specific conformational changes, which appear to dictate G protein selectivity. Together, these findings demonstrate that GPCR activation proceeds via efficacy-driven conformational trajectories rather than a concerted switch, providing molecular insight into GPCR–transducer allostery. Selectively exploiting these distinct activation pathways may open new avenues for the rational design of GPCR-targeting drugs with improved signaling specificity and therapeutic profiles.

Unconventional GPCR Signaling in the Hedgehog Pathway and Beyond

Benjamin Myers, Huntsman Cancer Institute, University of Utah

The Hedgehog pathway controls tissue and organ development across animal evolution and drives several widespread skin and brain cancers. The primary regulator of Hedgehog pathway at the cell surface is an atypical class F GPCR known as Smoothened (SMO). For decades, little was known about how SMO becomes activated or signals intracellularly, severely limiting our understanding of Hedgehog pathway function in physiology and disease, or to effectively control this pathway to therapeutic ends. A central challenge is that SMO defies the canonical signaling paradigms used by most other GPCRs. We and our collaborators have begun to unravel the unusual mechanisms underlying SMO activation and intracellular signal transduction, using an interdisciplinary approach that combines structural biology, biochemistry, cell biology, and *in vivo* development/cancer models. This work has revealed: (1) critical and unexpected roles for membrane sterols as endogenous SMO ligands, and (2) an unprecedented, G protein-independent mechanism for controlling protein kinase A (PKA) enzymatic activity in which SMO directly binds PKA's catalytic subunit and physically blocks its enzymatic activity. These findings help resolve longstanding mysteries in developmental and cancer signaling, point toward mechanism-inspired therapeutic strategies for cancers driven by ectopic Hedgehog pathway activity, and uncover new lipid- and kinase-dependent signaling principles that may extend throughout the GPCR superfamily.

Exploring non-canonical signaling mechanisms across diverse GPCRs

Ruth Huttenhain, Stanford University

G protein-coupled receptors (GPCRs) are essential mediators of cellular signaling, detecting a wide range of external stimuli and translating them into specific cellular responses. According to the prevailing signaling paradigm, the ~1,000 human GPCRs exert their biological effects primarily by engaging two canonical effectors: heterotrimeric G proteins and β-Arrestins (ARRBs). Yet this model does not fully account for the remarkable diversity of GPCR-mediated physiological outcomes. Emerging evidence indicates that GPCRs also engage additional, non-canonical signaling pathways via interactions with proteins beyond G proteins or ARRBs, often through intrinsically disordered regions (IDRs) in GPCR cytoplasmic domains. These non-canonical interactions can be initiated by selective phosphorylation of the receptors' active conformations by GPCR kinases (GRKs), ensuring that the resulting signaling events are dependent on receptor activation. To understand the prevalence of non-canonical GPCR signaling, we developed a high-throughput proximity labeling (PL) proteomics platform creating time-resolved maps of GPCR interactions. By profiling interaction networks of biologically diverse GPCRs after activation in HEK293A wildtype, GRK-, and ARRB-knockout cells, we identify both known and novel interactions of the activated receptors and their dependence on GRKs and/or ARRBs. Integrating proximity-labeling proteomics data with AlphaFold-based structural prediction and biochemistry, our study promises to reveal previously unrecognized, non-canonical signaling mechanisms across GPCR families, providing new insights into GPCR function and extending the current signaling paradigm.

Conformational coupling between extracellular and transmembrane domains modulates holo-adhesion GPCR function

Demet Araç, University of Chicago

For years, the adhesion GPCR field has been focused on the tethered agonist (TA)-mediated model of aGPCR activation. This mechanism proposes that autoproteolysis within the conserved GPCR Autoproteolysis-Inducing (GAIN) domain allows for the exposure of a TA peptide, which then activates the receptor. While compelling and widely studied, this model does not explain all observed phenomena, and several findings challenge its universality. Could a complementary mechanism address issues of the TA-dependent mechanism while offering a more flexible and reversible form of regulation? We hypothesized that the extracellular region (ECR)-mediated mechanism, involving direct conformational coupling between the ECR and TM of aGPCRs, could answer these challenges. We resolved the cryo-EM model of the Latrophilin-3 holoreceptor, revealing critical features of its organization. We found that the GAIN domain adopts a parallel orientation relative to the 7TM region and displays constrained movement, limited to a small range. This configuration suggests a possible interaction between the ECR and 7TM that can be altered by ligand-binding and mechanical force, supporting a direct coupling mechanism. Importantly, the TA peptide is tightly packed within the core of the GAIN domain, is not exposed to the solvent, and does not interact with the TM. This structural stability contrasts with the previous assumptions of extensive conformational flexibility of the ECR and is consistent with an ECR-mediated mechanism of signal transduction. To complement our structural data, we used single-molecule FRET to measure real-time conformational changes between the ECR and 7TM regions and confirmed the validity of our cryo-EM results. We also focused on full length ECR structures from several adhesion GPCRs and correlated the changes in their conformations to downstream signaling. Intriguingly, we noticed that the ECRs of adhesion GPCRs are NOT like beads-on-a-string but have compact “closed” conformations where the far N-terminal domain is indeed nearby the transmembrane domain and often interacts with the GAIN domain. Our observations suggest a closed-to-open conformational change correlates with receptor activation, and alteration of conformational populations may lead to disease.

Unnatural Amino Acid Labeling and smFRET Reveal Intermediate States in Glucagon Receptor Signaling

Shi Feng, Stanford University

G protein–coupled receptors (GPCRs) constitute the largest family of membrane proteins in the human genome, mediating diverse physiological processes including vision, olfaction, taste, endocrine regulation, cardiac function, immune responses, and neurotransmission. Among them, the glucagon receptor (GCGR) family, a major subset of family B GPCRs, plays a central role in metabolic regulation and glucose homeostasis, which exerts significant cardiostimulant effects, such as increasing heart rate and contractility. Despite their physiological importance, the mechanistic features that distinguish family B GPCRs, including GCGR, from family A GPCRs remain incompletely understood. Here, we established an unnatural amino acid (UAA) incorporation strategy to enable site-specific labeling of GCGR at transmembrane helices 4 and 6 without perturbing its native structure or function. Using single-molecule fluorescence resonance energy transfer (smFRET), we directly monitored GCGR conformational dynamics in the presence of agonists and G proteins, revealing an intermediate state that represents an unprecedented signaling event triggered by nucleotide binding to G proteins. These findings provide new insights into the activation and signaling mechanisms of GCGR and more broadly advance our understanding of family B GPCR function.

Single G Protein Turnover Defines DRD2's Coupling Efficiency

Desve Ambaw, The University of Queensland

The dopamine D2 receptor (DRD2), a Gi/o-coupled GPCR, has two splice variants, DRD2 long and short, expressed in post- and presynaptic neurons, respectively. DRD2S lacks a 29-amino-acid third intracellular loop insert, a region involved in G protein engagement. Functional differences between isoforms remain unresolved, with conflicting reports attributing greater coupling efficiency to either variant. To address this, we investigated G protein activation (GoA/β17) and cAMP inhibition downstream of both isoforms in CHO cells using lentiviral and Flp-In stable cell lines, which yielded differential expression. Signalling Emax scaled with receptor density, independent of isoform identity (no EC50 change). To further test the relationship between cell-surface receptor number and signalling output, we modulated DRD2 long expression in stable CHO cells using different promoters, as well as functionally depleting receptor number using long-residence-time antagonists. In all cases, Emax dropped with reducing receptor number, while EC50 remained unchanged, suggesting a 1:1 stoichiometry between active receptor and G protein. This contradicts accepted receptor reserve theory, which predicts preserved Emax when the EC_{50} lies left of agonist affinity (Furchgott, 1966). This stoichiometric coupling is supported by prior studies, including biochemical co-purification of pituitary DRD2 with Gi/o (Senogles et al., 1987) and [^{35}S]GTPγS binding kinetics in DRD2-CHO membranes (Quirk et al., 2007). The finding suggests that cellular output may be more dependent on receptor availability than ligand concentration (which is high at neuronal synapses), potentially explaining narrow therapeutic windows observed with DRD2-targeting pharmaceuticals. For example, clinical data for DRD2-targeting antipsychotics show efficacy at 60–75% occupancy, while >78% is linked to extrapyramidal symptoms (Nordström, 1993; Kapur, 2001). To further investigate this mechanism, we are applying single-particle tracking of DRD2 and GoA, which will provide insights into the single-turnover behaviour of DRD2 at the level of individual receptor–G protein interactions.

Exploring Intracellular GPCRs and Their Role in Pathway Crosstalk

Nikoleta Tsvetanova, Duke University

We investigate fundamental questions about the process of cell signaling and ask how it contributes to physiological and pathological states. Our main focus is on the function and regulation of G protein-coupled receptors (GPCRs), and we apply cell biology and genomic approaches to dissect the molecular strategies that allow cells to achieve functionally diverse responses to GPCR activation. Over the past years, we have made significant breakthroughs by showing that one strategy relies on compartmentalization of GPCR signaling. Specifically, we have demonstrated that 1) receptors can be activated not only on the plasma membrane but also on intracellular compartments, 2) intracellular- and ***not*** plasma membrane-delimited signaling drives various receptor-dependent outputs, including selective transcriptional and translational responses, and 3) the site of receptor activation can dictate how cells “interpret” different ligands, including medically relevant drugs. I will be presenting our ongoing work on the mechanisms and consequences of compartmentalized receptor signaling.

Age-dependent GPCR expression dynamics guides precision medicine for fetal, pediatric, and geriatric populations

Katarina Nemec, St. Jude Children's Research Hospital

G protein-coupled receptors (GPCRs) represent one of the largest and most versatile families of cell surface receptors, orchestrating a wide range of biological functions throughout the human lifespan. However, GPCR expression and that of their interacting partners varies significantly across developmental stages and aging, profoundly impacting the timing of physiological roles and responses to therapeutics. We analyzed time-resolved gene expression datasets to uncover the dynamic expression patterns of GPCRs and their interacting partners during human development and aging. Our analysis identified developmental switches in GPCR expression between prenatal and postnatal stages, revealing their critical roles in physiological and pathophysiological processes. By integrating transcriptomics with pharmacological data, we demonstrate how age-related developmental changes modify GPCR signaling and therapeutic efficacy of essential drugs. This integrated approach enables several key advances: (1) identification of developmental switches in GPCR expression; (2) discovery of mechanisms underlying age-related system bias in GPCR signaling; and (3) determination of optimal time points for drug effectiveness to support age-specific precision dosing. Combining target gene expression trends with pharmacological metrics allows for tailored therapeutic recommendations across pregnant women and neonatal, pediatric, and geriatric populations. Furthermore, exploring the relationship between dynamic GPCR expression and drug side-effect profiles enhances our understanding of secondary pharmacology, supporting the development of safer therapeutics. This research advances personalized medicine by improving the efficacy and safety of pharmacologically most relevant protein families to tailor treatments to different life stages, ultimately enabling more precise and effective therapeutic interventions across the human lifespan.

A SPS method for GPCR drug screening

Xiangyu Liu, School of Pharmaceutical Sciences, Tsinghua University

G protein-coupled receptors (GPCRs) regulate nearly all physiological processes and represent the most successful class of FDA-approved drug targets. Consequently, drug screening against GPCRs has garnered intense interest from both industry and academia. However, high-throughput screening methods based on conventional GPCR signaling assays have several limitations, including high costs, technical complexity, and a short window for signal detection. To address these challenges, we developed a novel approach for GPCR agonist screening by coupling target GPCR activation to downstream yeast growth readouts. By employing a growth-controlling reporter protein and a protein-fragment complementation strategy, we achieved accelerated yeast growth upon agonist stimulation of the target GPCR. We term this method Survival Pressure Selection (SPS), which has been successfully applied to identify novel agonists for several GPCRs. Furthermore, by replacing the reporter with a toxic protein, we inverted the system: antagonist treatment promotes faster yeast growth (while agonists slow growth). This reverse configuration is named reverse Survival Pressure Selection (rSPS) and enables the screening of novel antagonists for target GPCRs. I will also present an atypical GPCR signaling phenomenon—sustained G protein signaling mediated by a GPCR–G protein–β-arrestin megacomplex—stabilized by an allosteric agonist identified through the SPS screening platform.

Activation of the relaxin receptor by chemically divergent agonists

Andrew Kruse, Harvard Medical School

The RXFP1 relaxin receptor is a key mediator of physiological adaptation of the cardiovascular system to pregnancy, and its vasodilatory and anti-fibrotic effects have made it an attractive target for development of drugs to treat severe cardiac and pulmonary diseases. A number of RXFP1 agonists are currently in Phase 2 clinical trials, including both small molecule drugs and engineered peptides. We sought to understand the molecular basis for activation of RXFP1 by these diverse ligands using cryo electron microscopy, hydrogen deuterium exchange mass spectrometry, and other methods. Collectively, our data show that the small molecule drug candidate AZD5462 activates RXFP1 quite differently from peptide agonists, stabilizing a distinct conformation by interacting with the membrane-embedded surface of transmembrane helix 7. In contrast, the native hormone triggers a series of conformational rearrangements beginning with the receptor's ectodomain, culminating in selective activation of G protein relative to arrestin. Together, these data show how chemically divergent agonists can induce distinct conformations and signaling outcomes in a clinically relevant GPCR.

Assembly required – how cells build functional ion channel complexes

Tino Pleiner, Stanford University

Nearly half of all ~5,000 human membrane proteins need to assemble into defined oligomeric complexes to function. How do thousands of newly synthesized membrane proteins navigate the crowded ER membrane environment and avoid promiscuous interactions, aggregation or premature degradation to find their correct binding partners? How are hydrophilic subunit interfaces protected within the hydrophobic lipid bilayer prior to assembly? We propose that membrane protein assembly does not occur through chance encounters of randomly diffusing subunits; rather, it is highly regulated and requires assembly factors that collaborate with membrane insertion machinery. To begin exploring the diversity of factors and mechanisms controlling membrane protein assembly at the ER, we are using the large family of *voltage-gated ion channels (VGICs)* as important model complexes. VGICs fulfill many essential functions; for example, they mediate excitation-contraction coupling in heart and muscle cells, and trigger hormone and neurotransmitter release in secretory and neuronal cells. Mutations that impair VGIC biogenesis or function cause severe cardiological, neuropsychiatric, and neurodevelopmental diseases. A better understanding of ion channel assembly and quality control pathways could therefore reveal critically needed therapeutic targets to modulate their cellular levels. In my talk, I will present our latest findings showing that the assembly of heterotrimeric voltage-gated calcium channels is highly regulated and requires the abundant and highly conserved ER membrane protein complex (EMC) as an assembly factor at the ER membrane. The EMC is well known for its function in inserting membrane proteins into the lipid bilayer (1-4), but had long been speculated to have additional roles in membrane protein biogenesis. Combining cell-based calcium channel reporter assays, inhibitory anti-EMC nanobodies, and reconstruction of early calcium channel assembly events in an *in vitro* translation and ER membrane insertion system, we could provide first direct evidence for a chaperone function of the EMC. We found that the EMC engages nascent calcium channels co-translationally as part of an unprecedented large ribosome-multipass translocon supercomplex to prevent pre-mature channel degradation. Our work highlights a sophisticated network of biogenesis factors that collaborate dynamically to ensure membrane protein folding and assembly intermediates are protected from otherwise wasteful premature degradation or toxic aggregation.

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Unravelling non-canonical interactions at GPCR intrinsically disordered regions

Mia Greeson, Stanford University

Under the classic GPCR signaling paradigm, the functional outputs of GPCR activation are primarily dictated by two canonical factors: G proteins and β-arrestins (ARRBs). However, this simplified model does not fully explain GPCR signaling specificity. Emerging evidence highlights GPCR kinase (GRK)-mediated phosphorylation of intrinsically disordered regions (IDRs) of GPCRs as a potential driver of non-canonical GRK-dependent, but ARRB-independent protein interactions, potentially fine-tuning signaling outputs and contributing to the functional diversity of GPCRs. Yet, the lack of systematic approaches to study non-canonical GPCR interactions has hindered their exploration, leaving the fundamental mechanisms and overall prevalence of non-canonical signaling poorly defined. We developed a comprehensive experimental pipeline integrating APEX-based proximity labeling and quantitative proteomics with multi-layered validation strategies to map and characterize non-canonical GPCR interactions. Thirty-five GPCRs were prioritized using AlphaFold-guided predictions of conditional folding in their C-terminal tails, which we hypothesize may represent key interfaces for non-canonical interactions with GPCR IDRs. We established a genomic landing pad system across HEK293a WT, ΔGRK2/3/5/6, and ΔARRB1/2 cell lines, allowing us to rapidly generate >100 stable GPCR-APEX cell lines. Together with our optimized proximity labeling protocol in 12-well plate format and biotin enrichment in an automated 96-well plate format, our pipeline allows for higher throughput GPCR-APEX to resolve both the location and protein interaction networks of GPCRs over time, as well as the GRK- and ARRB-dependence of interactors. Applying this framework, we have identified non-canonical GRK-dependent, ARRB-independent interactors at activated GPCRs, as well as a putative IDR-mediated non-canonical interactor at an orphan GPCR. For both cases, AlphaFold-based predictions support high-confidence receptor-effector interactions. By redefining our understanding of GPCR-effector interactions, this work has the potential to uncover new mechanisms of GPCR signaling and inspire novel therapeutic strategies.

Multimodal β-arrestin functions through membrane nanodomain formation

Asuka Inoue, Kyoto University

β-arrestins are multifunctional scaffolding proteins that regulate GPCR desensitization, endocytosis, and signaling through spatiotemporally controlled mechanisms. Building upon well-studied models distinguishing plasma membrane and endosomal β-arrestin functions, we uncover an additional layer of spatial organization within the plasma membrane, where β-arrestins dynamically partition into distinct membrane compartments. Using live-cell single-molecule imaging, NanoBiT biosensor tools, and biochemical assays, we found that β-arrestins localize to specific membrane structures with unique roles. In clathrin-coated pits (CCPs), β-arrestins concentrate at the center, suggesting they may enter CCPs independently and prior to ligand-activated GPCRs. In caveolae, β-arrestin is required for the confinement of active GPCRs. Moreover, β-arrestin actively remodels the local lipid environment by promoting the formation of PIP_2-enriched nanodomains through its non-canonical C-edge lipid-binding site. We further found that GRKs display subtype- and ligand-dependent heterogeneous distributions across the plasma membrane, which may underlie spatial differences in receptor phosphorylation and β-arrestin engagement. Together, these findings suggest that β-arrestin function is orchestrated through multiple layers of spatial regulation involving membrane compartmentalization, lipid remodeling, and GRK distribution. In this workshop, I will discuss how these spatial mechanisms may refine current models of spatiotemporal GPCR signaling and open new directions for understanding GPCR signal regulation.

Application of genetically encoded dopamine and 2nd messenger sensors challenge paradigms of dopamine receptor signaling in the striatum

Ulrik Gether, University of Copenhagen

The striatum constitutes an essential subcortical forebrain nucleus that receives major input from midbrain dopaminergic neurons and plays a key role in movement, motivation, reward-related behavior and learning. The main neuronal type in the striatum is the medium spiny neuron (MSN), which is further classified into dopamine D1-receptor-expressing MSNs (D1R-MSNs) and D2-receptor-expressing MSNs (D2R-MSNs). The Gαs/olf-coupled D1Rs are generally considered to have lower affinity for dopamine (DA), responding in the micromolar range, whereas the Gαi/o/z-coupled D2Rs exhibit higher affinity, responding in the nanomolar range. Accordingly, it has been proposed that D1R-MSNs primarily respond to phasic DA release, while D2R-MSNs are more sensitive to tonic DA levels. In the context of natural rewards or cocaine administration, despite a global increase in extracellular striatal dopamine, only a very small fraction -referred to as an ensemble - of striatal neurons (primarily D1R-MSNs) become active at the level of c-Fos transcription. This ensemble is crucial for learning and memory, encoding both adaptive and maladaptive behaviors, as in the case of substance use disorders (SUDs). Here, we employed the genetically encoded protein kinase A sensor ExRai-AKAR2 and the genetically encoded cAMP sensor G-Flamp1 to investigate D1R-MSN signaling in striatal primary cultures and acute brain slices using live epifluorescence imaging or two-photon microscopy. This approach enabled the assessment of dose-response relationships in individual cells and revealed striking differences in cAMP and PKA responsiveness to dopamine: some D1R-MSNs responded to nanomolar dopamine concentrations, while others required micromolar concentrations. The response profile of individual neurons often remained unchanged after 24 hours, although a subset shifted their sensitivity, becoming either more or less responsive to dopamine. Notably, overexpression of D1R in D1R-MSNs increased the fraction of hyperresponsive neurons, suggesting that differential receptor expression levels may, at least in part, contribute to the observed phenotype. To explore the relationship between dopamine sensitivity and transcriptional activity *in vivo*, we performed iDISCO brain clearing with c-Fos immunostaining following cocaine or saline administration in mice. We mapped c-Fos-enriched regions and aligned them with the Allen Brain Cell Atlas to examine patterns of gene expression. Additionally, we analyzed single-cell RNA sequencing datasets from rodent and human striatum to assess D1R expression in relation to c-Fos induction at the single-cell level. Thus, beyond revealing a previously unappreciated and surprising heterogeneity of dopamine signaling within MSNs, our findings challenge the classical assumption of low-affinity D1Rs and high-affinity D2Rs. Moreover, they provide new insight into the proposed roles of these receptors in dopamine-mediated memory processes by suggesting that D1R hypersensitivity may be a contributing factor in ensemble selection.

The role of protein dynamics in G protein coupled receptor signaling

Brian Kobilka, Stanford University

G protein coupled receptors (GPCRs) conduct the majority of transmembrane responses to hormones and neurotransmitters and mediate the senses of sight, smell and taste. Thanks to advances in protein engineering, crystallography and cryo-electron microscopy (cryoEM) over the past 18 years there are now more than 500 deposited high-resolution structures of GPCRs including inactive states, active states and GPCR-G protein complexes. These structures have provided important insights into common mechanisms of G protein activation for Family A and Family B GPCRs. However, they don't fully explain the complex behavior of many GPCRs that signal through more than one G protein isoform, and through G protein independent pathways. Moreover, we still do not fully understand the mechanism of G protein coupling specificity. This complex functional behavior provides evidence for the existence of multiple functionally distinct conformational states that may be too transient or unstable to be captured by crystallography or cryoEM. We have used fluorescence, EPR and NMR spectroscopy to study the dynamic properties of several GPCRs. I will discuss a few examples of what these studies have taught us about the role of protein dynamics in GPCR signaling.

Poster Abstracts

P-1 Conformation, dynamics, and signaling of an intermediate GPCR-G complex

Libin Ye, University of South Florida

Unraveling the signaling roles of intermediate complexes is pivotal for G protein-coupled receptor (GPCR) drug development. Despite hundreds of GPCR-Gαβγ structures, these snapshots primarily capture the complex structure in a fully activated conformational state. Consequently, the functions of intermediate GPCR-G protein complexes remain elusive. Guided by a conformational landscape visualized via ^{19}F quantitative NMR and molecular dynamics (MD) simulations, we determined the structure of an intermediate GPCR mini-G_s complex at 2.6 Å using cryo-EM by blocking its transition to the fully activated complex. Furthermore, we present direct evidence that the complex at this intermediate state initiates a rate-limiting nucleotide exchange before transitioning to the fully activated complex. In this state, BODIPY-GDP/GTP-based nucleotide exchange assays further indicated the α-helical domain of the Gα is partially open, allowing it to grasp a nucleotide at a non-canonical binding site, distinct from the canonical nucleotide-binding site. Our ongoing research also indicated that the intermediate and fully activated states regulate downstream signaling differently, contributing to both signaling efficacy and biases. These advances bridge a significant gap in our understanding of the complexity of GPCR signaling.

P2 Fine-tuning the Biased Agonism of G Protein-Coupled Receptors

Guodong He, Tsinghua University

G protein-coupled receptors (GPCRs) are critical drug targets, with approximately 34% of currently marketed drugs exerting their effects by modulating GPCR activity. Since the 1990s, the paradigm of GPCR signal transduction has been well established, recognizing that GPCRs initiate intracellular signaling through two key transducers: G proteins and β-arrestins. These transducers typically compete with each other, triggering distinct signaling cascades that can lead to the "on-target" side effects of some GPCR-targeted drugs. Biased agonism has emerged as a promising strategy to address this challenge. To explore the mechanisms underlying biased signal transduction, we identified (R)-141, the first exclusively G protein-biased agonist for the μ-opioid receptor (μOR). (R)-141 robustly activates G protein signaling of μOR while exhibiting negligible β-arrestin recruitment. By resolving the structure of the μOR-Gi-141 complex, we identified a key motif on transmembrane helix 7 (TM7) that acts as a signaling-selective conduit, propagating conformational changes from the orthosteric pocket to the intracellular surface upon receptor activation. Mutations in this motif were shown to modulate the signaling profile of μOR in a predictable manner. Notably, sequence alignment of Class A GPCRs revealed that this motif becomes stepwise conserved along TM7, suggesting its role as a funnel that integrates agonist-specific perturbations into stereotyped transducer coupling. These findings provide novel structural insights into the molecular determinants of GPCR signaling bias and identify a conserved motif for modulating GPCR signaling. This structural information establishes a framework for developing therapeutics with improved signaling bias and reduced side effects.

P-3 Deep mutational pharmacology reveals basis of opioid efficacy

Matthew K. Howard, UCSF

Understanding the residue-level interactions that drive molecular recognition and allostery in G Protein-Coupled Receptors (GPCRs) remains a central challenge in modern protein science. Emerging methods like Deep Mutational Scanning (DMS) are poised to enable efficient experimental profiling of GPCR function in a deep, holistic manner. Already, DMS has been used to map how individual stimuli interact with a receptor, shedding light on recognition patterns and activation mechanisms. A key gap in our understanding, however, is how we can leverage this information to understand how different chemotypes achieve differing efficacy at the same receptor. Here, we use the μ-opioid Receptor (μOR) as a model system and generate over 1 million measurements of mutational effects on efficacy, potency, and internalization across diverse opioid ligands. Our analyses uncover critical receptor positions dictating ligand recognition and allosteric networks governing ligand efficacy and potency. We reveal nuances of how each residue is differentially important to receptor activation by chemically diverse opioid scaffolds and derivatives. Additionally, we further resolve trafficking motifs in the intracellular regions that regulate receptor internalization and recycling – providing a more holistic view of how receptor variation can influence drug response. By integrating these data, we gain insight into fundamental principles of ligand-receptor interactions and how they percolate down to cellular responses. This technology provides a novel and quantitative lens that will aid in understanding receptor biology and accelerate drug development for GPCRs and beyond.

P-4 G protein Selectivity in Dopamine Receptors is Determined Before GDP Release

Ravinder Abrol, California State University, Northridge

Dopamine receptors are G protein-coupled receptors (GPCRs) that trigger dopaminergic signaling cascades with important implications for neurophysiology and associated diseases. The dopamine receptor subtypes D1R and D5R selectively couple to Gs/olf proteins while subtypes D2R through D4R are selective for Gi/o proteins. Although significant progress is being made in the structural biology of cognate receptor-G protein complexes establishing the key role of the α5 helical domain of Gα in receptor coupling, the molecular mechanisms underlying G protein selectivity are still not clear. Almost all complex structures have captured G protein in a nucleotide-free state (normally very short-lived in a cell), whereas biophysical studies are beginning to hint that the selectivity is enabled during the encounter of the receptor with the GDP-bound G protein. To provide a mechanistic insight into G protein selectivity, structural models of dopamine D1R and D2R were built in complex with their cognate G proteins (Gs and Gi, respectively) as well as non-cognate G proteins (Gi and Gs, respectively), where for each G protein two different conformations were used: GDP-bound or nucleotide-free. These eight complex structures were relaxed in a lipid bilayer environment through 2-microsecond-long molecular dynamics (MD) simulations to generate conformational ensembles for each complex. A thermodynamic analysis of these ensembles provided free energies of G protein binding to the receptors that were consistent with known cognate vs non-cognate partners of the D1R and D2R receptors but only for the GDP-bound states of the G proteins, suggesting that Gs vs Gi selectivity is conferred before GDP release during GDP-bound G protein's interactions with the receptors. Measurements of receptor pre-association with G protein in cells supported the D1R receptor's preference for Gs relative to D2R. To further probe the structural mechanisms underlying this selectivity and the role of G protein's α5 helical domain, receptor complexes with chimeric G proteins were constructed, where the last 18 residues on the α5 helix were switched between Gs and Gi (Gi18s and Gs18i). Thermodynamic analysis of MD-relaxed chimeric complexes revealed a complete switch in G protein binding selectivity for both D1R and D2R receptors, but only for the GDP-bound G proteins. Measurements of receptor pre-association with G protein in cells also supported the D1R receptor's preference for the Gi18s chimera relative to D2R. These structural modeling and biochemical studies have shown that receptor binding to GDP-bound G protein determines the observed G protein selectivity of the dopamine receptors and additional molecular events may be needed for a productive coupling to enable a successful GDP/GTP exchange.

P-5 Dissecting cannabinoid receptor 2 signalling in HER2+ breast cancer

Andrea Vinaga, Queen Mary University of London

HER2 overexpression leads to an aggressive form of breast cancer. While HER2-targeting agents have improved patient survival, relapse is common and reduces their therapeutic benefit. A subpopulation of highly plastic cancer cells displaying stem-like properties, cancer stem cells, is thought to play a role in tumour initiation, progression and metastasis. Increasing evidence indicates that cancer stem cells can resist HER2-targeting therapies and lead to clinical relapse by recreating tumour heterogeneity, indicating the need for more treatments targeting the cancer stem cell compartment. While cannabinoid receptor 2 (CB_2) is normally expressed by cells of the immune system, HER2 can increase its expression in cancer cells. Additionally, CB_2^{Hi} HER2+ breast tumours which have metastasised to the lymph nodes have a worse prognosis than CB_2^{Lo} tumours, underlining the potential of using CB_2 as a biomarker for poor prognosis. Preliminary data from this project indicate that HER2+ breast cancer cells expressing CB_2 have a cancer stem cell-like phenotype. Using CB_2-targeting therapies in conjunction with traditional treatments could reduce metastasis and recurrence rates by specifically targeting the cancer stem cell compartment, thereby improving patient survival. Mechanistically, CB_2 has been shown to bind to HER2. However, little is known about which signalling pathways are activated downstream of CB_2 in cancer cells. This project uses phosphoproteomics to investigate signalling downstream of CB_2 in HER2+ cancer cells, identifying druggable signalling nodes which may represent novel targets, or for which current anticancer drugs could be repurposed, for the treatment of CB_2^{Hi} HER2+ breast cancer. The effects of CB_2-induced cancer stem cell phenotypic changes on tumour behaviour, and the potential of reversing these using cannabinoids, are studied in 2D and 3D models in vitro.

P-6 Exploiting Gαq structural dynamics for new uveal melanoma therapies

Huixia Wang, UCSF

Uveal melanoma is a devastating cancer with a 10-year mortality of 50%. Despite dramatic successes in therapeutic development for other melanoma subtypes, immune checkpoint blockade and targeted therapies have seen limited success for uveal melanoma. We aim to develop targeted therapies that directly inhibit the oncogenic driver of uveal melanoma, the Gαq/11 oncoproteins. Recent studies suggest that oncogenic Gαq/11 mutants engage a unique spectrum of signaling effectors, potentially due to unique conformations of the constitutively active oncogenic Gαq/11 proteins. Our preliminary biophysical studies provide support for a distinct conformation of oncogenic Gαq/11 mutants. These observations open the door to the discovery of a “wild-type sparing” inhibitor which selectively targets only the oncogenic Gαq/11 proteins, thereby providing an effective therapeutic target for this central node in uveal melanoma oncogenic signaling. Here we combine deep biophysical interrogation of mutant Gαq proteins with innovative drug discovery approaches. First, we determine the unique conformations and dynamics of oncogenic Gαq mutants using a combination of structural techniques (nuclear magnetic resonance, electron microscopy). Second, we optimize conformation- and mutant-specific covalent inhibitors that target the Gαq protein–protein interface with downstream effector proteins then evaluate changes in PLCβ signaling via a variety of in vitro assays, including BLI and fluorescence polarization. We will iteratively refine these inhibitors for cellular reactivity and mutant specificity, with the ultimate goal of testing them in vivo. These studies are poised to set the foundation for the discovery of new precision medicine therapies for this neglected “orphan” cancer.

P-7 A long-acting μ-opioid receptor inverse agonist for durable opioid reversal and withdrawal suppression

Peng Huang, Stanford

The opioid overdose epidemic remains a critical global health crisis. Naloxone, the current emergency antidote, rapidly reverses μ-opioid receptor (μOR) activation but is limited by its short duration of action and its tendency to precipitate withdrawal symptoms. Here, we report the discovery of HG15, a potent and long-acting μOR inverse agonist identified from a DNA-encoded library. Unlike the morphinan scaffold of naloxone, HG15 features a distinct chemical feature that confers slow receptor dissociation and prolonged efficacy in reversing morphine-induced antinociception *in vivo*. HG15 robustly inhibits DAMGO-stimulated μOR signaling, reducing G protein activation, cAMP accumulation, and β-arrestin recruitment in cell-based assays. A high-resolution cryo-electron microscopy (cryo-EM) structure of the μOR-HG15 complex reveals that HG15 stabilizes the receptor in an inactive conformation via a cyclopropyl group engaging the conserved microswitch residues $W293^{6.48}$ and $Y326^{7.43}$. The cyclohexane moiety of HG15 occupies a previously uncharacterized sub-pocket formed by TM2, TM3, and ECL1, where residues $Q124^{2.60}$ and $N127^{2.63}$ modulate μOR constitutive activity. Site-directed mutagenesis confirms that HG15's inverse agonism is conferred by its cyclohexane moiety engaging residues $Q124^{2.60}$ and $N127^{2.63}$ within the sub-pocket. In mouse models, HG15 effectively reverses morphine-induced analgesia in both pretreatment and co-administration paradigms, without precipitating withdrawal symptoms. Collectively, these findings establish HG15 as a structurally distinct, long-acting μOR inverse agonist with potential as a next-generation therapeutic for opioid overdose reversal and withdrawal management.

P-8 Infrared Spectroscopy Reveals Sodium Ion-Mediated Allosteric Modulation in the kappa Opioid Receptor (KOR)

Kota Katayama, Nagoya Institute of Technology

Opioid analgesics such as morphine activate opioid receptors (ORs), a subclass of G protein-coupled receptors (GPCRs), to produce analgesic effects. However, their use is limited due to serious side effects, including dependence and tolerance. This has led to increasing interest in structure-based drug development to reduce these adverse effects. Recent studies have shown that sodium ions bind to an allosteric site within GPCRs, distinct from the orthosteric site targeted by endogenous ligands and drugs. This allosteric binding can modulate receptor activity. While such sodium-dependent modulation has been observed in ORs, the detailed mechanism of sodium binding and its functional impact remain unclear. Targeting this sodium-binding pocket offers a new approach to opioid drug design beyond conventional orthosteric strategies. In this study, we used infrared (IR) spectroscopy, a method we have refined for monitoring GPCR structural changes via vibrational spectra[1–4], to examine sodium-induced changes in the κ-opioid receptor (KOR), a pharmacologically important OR subtype. When sodium ions were added to KOR pre-bound with the agonist nalfurafine, we observed a down-shift in the amide-I band. This shift reflects strengthened hydrogen bonding in local α-helices and suggests a conformational change toward the inactive state. Previously, we showed that the amide-I band shifts upward upon agonist binding (activation) and downward upon antagonist binding (inactivation) in the absence of sodium ions[5]. The present findings support the idea that sodium promotes receptor inactivation. We further tested other agonists and antagonists with different chemical structures. Although sodium consistently caused a down-shift in the amide-I band, the extent of the shift varied by ligand. This indicates that sodium acts as a general negative allosteric modulator of KOR, with ligand-dependent modulation strength. We will also present IR spectra of amino acid side chains and molecular dynamics simulations. Together, these findings reveal the sodium ion entry pathway and provide mechanistic insights into sodium-mediated allosteric control of GPCRs.

References:
[1]Katayama et al., J. Phys. Chem. Lett. (2019); [2]Katayama et al., Commun. Biol. (2021); [3]Suzuki et al., RSC. Adv. (2021); [4]Sugiura et al., J. Am. Chem. Soc. (2025); [5]Suno-Ikeda et al., Nat. Commun., under revision.

P-9 Structural insights into spectral tuning and retinal exchange in cone visual pigments

Sayaka Ohashi, Nagoya Institute of Technology

Primates' color vision relies on red, green, and blue cone photoreceptors, which bind the common 11-cis-retinal chromophore but differ in absorption maxima. In catarrhine primates, including humans, red and green pigments diverged through gene duplication and differ at just three key amino acid positions, enabling fine spectral discrimination for trichromatic vision. Unlike rhodopsin, which mediates dim-light vision, cone pigments have faster activation and regeneration, supporting daylight vision, though the structural basis remains unclear. Here, we present cryo-EM structures of red- and green-sensitive cone pigments from a non-human primate, combined with vibrational spectroscopy and quantum-chemical modeling. The red-to-green spectral shift is primarily caused by a threonine residue, whose -OH dipole alters the chromophore electrostatics. Minor steric and electrostatic effects contribute less. Differences between green cone pigment and rhodopsin involve chromophore distortion and counterion relocation. We also identify a lateral membrane opening in the inactive state, absent in rhodopsin, which likely serves as a retinal entry site. This gating mechanism links retinal photoisomerization to its entry and exit pathways, explaining the rapid regeneration of cone pigments. These findings offer molecular insights into spectral tuning and activation-coupled retinal exchange, shedding light on daylight color vision and phototransduction.

P-10 Neurocardiac interactions in the human heart: The role of neurons in improving cardiomyocytes GPCRs responses

Cristina Molina, University Medical Center Hamburg-Eppendorf

Over the past decades significant progress has been made in understanding heart function, particularly in the context of excitation-contraction coupling and cardiac electrophysiology. These insights have provided a clear picture of the major pathophysiological mechanisms underlying cardiac diseases, including neurohormonal regulation and the role of GPCRs in heart function. Nevertheless, understanding cell-cell interactions and how neurogenic mechanisms may influence cardiomyocytes remodeling or vice versa has been a challenging experimental problem. There is a profound gap of knowledge regarding the characterization of the neurons in the heart, in our understanding of this crosstalk and in the impact of neuronal GPCRs function and targeting with commonly used cardiovascular therapies. We explore neurocardiac interactions via GPCRs using, for the first time, living primary human cardiac myocytes and neurons.

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P-11 Design of Small Non-Peptidic Ligands That Alter Heteromerization between Cannabinoid CB1 and Serotonin 5HT2A Receptors

Marc Ciruela-Jardí, Universitat Autònoma de Barcelona

Cannabinoid agonists have proven their analgesic potential, which could make them useful for treating migraine, rheumatoid arthritis or cancer-related pain. However, their use has been hampered by side effects like memory impairment. Recent findings linked the cognitive side-effects of cannabinoid agonists such as delta-9-tetrahydrocannabinol (THC) to complexes formed between cannabinoid CB1R and serotonin 5-HT2A receptor (5-HT2AR). The expression of CB1R-5-HT2AR heteromers was demonstrated in native tissues and in behavioural studies using mice lacking 5-HT2AR (Vinals et al. 2015). Notably, synthetic peptides capable to disrupt CB1R-5HT2AR heteromerization, led to a selective abrogation of memory impairment caused by exposure to THC in vivo (Gallo et al. 2021). Using these synthetic peptides as templates, a ligand-based virtual screening exercise identified compounds that mimic the pharmacophoric features of the synthetic peptides. These nonpeptidic small molecules prevent CB1R-5HT2AR heteromerization in bimolecular fluorescence complementation assays and the heteromerization-dependent allosteric modulations in cell signalling experiments (Matsoukas et al. 2025). These results provide proof-of-principle for the design of optimized ligand-based disruptors or glues for GPCR heteromers. Modelling binding sites located at the receptor-membrane interface remains challenging. Most molecular docking algorithms assume an aqueous environment, which limits their accuracy in such contexts. To enable more effective structure-based virtual screening, a better representation of the membrane is necessary. To address this, we adapted a virtual screening protocol that accounts for the lipophilic nature of the membrane by explicitly incorporating a lipid membrane portion into the docking scoring process, thereby improving the design of optimized ligands. This study offers a new perspective on cannabinoid analgesia by exploiting the allosteric effect of GPCR heteromerization.

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P-12 Identification of Proteins Controlling mu Opioid Receptor Trafficking in Cultured Cells Using a Novel Chemical Biology Platform

Aleksandra Dagunts, Oregon Health & Science University

A critical homeostatic mechanism for regulating G protein-coupled receptor (GPCR) activity is agonist-induced GPCR endocytosis and trafficking to the lysosome for proteolytic downregulation. The mu opioid receptor (MOR) is a notable example of this type of cellular regulation where prolonged exposure to high efficacy opioid drugs causes MOR to traffic to the lysosome. Here, we used functional genomics to identify cellular proteins which control MOR downregulation. We found that the central regulator of MOR post-endocytic trafficking is the Retromer complex, which rescues MOR from opioid-induced downregulation by promoting MOR recycling from endosomes to the plasma membrane. Critically, the ability of MOR to access the Retromer recycling pathway is dependent on its non-canonical bi-leucine recycling motif, and we show this novel pathway for Retromer-based recycling protects opioid receptors from pharmacological tolerance. Additionally, we demonstrate that other membrane proteins besides MOR can access Retromer-based recycling through similar bi-leucine motifs. Overall, this study reveals a novel mechanism by which MOR is regulated following chronic exposure to opioid drugs.

P-13 Unveiling the molecular mechanism of odorant recognition by the human OR52 family

Chulwon Choi, Seoul National University

Human odorant receptors (ORs), which constitute nearly half of all G protein-coupled receptors (GPCRs), are essential for odor detection and olfactory signaling. However, structural studies of ORs have been limited by their low surface expression in heterologous systems and their intrinsic instability. To overcome these challenges, we employed a consensus strategy and determined the cryo-EM structure of octanoate-bound consensus OR52 ($OR52_{cs}$), representing the human OR52 family. The structure, combined with mutagenesis data, revealed that a conserved Arg residue at the extracellular tip of transmembrane helix 6 plays a critical role in recognizing the carboxyl group of odorants. To further explore how this structure can enhance our understanding of odorant binding in native ORs, we engineered $OR52_{cs}$ to mimic the odorant pocket of the native human OR52 family subtype, which recognizes the major human sweat odorant. Structural study of this chimeric receptor bound to carboxylate sweat odorant, along with docking simulations and mutagenesis studies, provides the molecular insights into sweat odor recognition by this human OR.

P-14 Molecular basis of CXC chemokine receptor 3 ligand multispecificity

Alexandre Bouyssou, Genentech

The C-X-C motif chemokine receptor 3 (CXCR3) is a key player in immune cell function. It drives T helper 1 (Th1) cell infiltration in autoimmune and chronic inflammation and also promotes tumor proliferation and metastasis. However, the mechanisms by which the endogenous ligands CXCL9, CXCL10 and CXCL11 differentially recognize and activate CXCR3 are not fully understood. Here, we present cryo-EM structures of all three chemokine-CXCR3-Gi complexes. We also provide live-cell binding, systematic mutagenesis, molecular dynamics and chimeric-chemokine analysis. Structures reveal a membrane-distal receptor N-terminus engagement (CRS0.5) as a key determinant of ligand recognition. By systematically comparing the pharmacological and interaction profiles of CXCL9, CXCL10, and CXCL11, we rationalize their varying efficacies and potencies and demonstrate the critical role of the membrane-distal CXCR3 N-terminus in ligand binding and signaling. Using chimeric chemokines and molecular dynamics, we reveal key sequence interactions that shape distinct signaling signatures. Together, these insights enable us to propose a multimodal, sequence-encoded binding and activation model that explains CXCR3 multispecificity and signaling versatility, while providing structural handles for selective modulation of CXCR3 function. These mechanistic insights provide a blueprint for rational design of biased agonists and antagonists to selectively tune immune or tumor responses via CXCR3 therapies.

P-15 Structural Insights into Allosteric Activation and Inactivation Mechanisms of FFA2

Mai Kugawa, The University of Tokyo

The Free Fatty Acid Receptor 2 (FFA2), a G protein-coupled receptor, selectively recognizes short-chain fatty acids (SCFAs). The activation of FFA2 plays a crucial role in modulating immune and metabolic functions, making potent FFA2 ligands viable therapeutic options for treating metabolic and immune-mediated diseases such as ulcerative colitis, diabetes, and obesity. Among these, GLPG0974, a selective FFA2 antagonist, has advanced to Phase II clinical trials for ulcerative colitis treatment, but the precise mechanism by which this structurally unique ligand acts as an antagonist was previously unclear. Similarly, 4-CMTB, serving as both an agonist and a positive allosteric modulator (ago-PAM), has shown potential in allergic asthma treatment, but its mechanism for activation has yet to be clarified. In this study, we determined the cryo-electron microscopy (cryo-EM) structures of FFA2 in (1) its active state, bound to 4-CMTB and TUG-1375 (a synthetic orthosteric agonist), and (2) its inactive state, bound to GLPG0974 (Kugawa et al., Nature Communications, 2025). These structures revealed the unique receptor activation and inactivation mechanisms employed by these ligands. First, GLPG0974 functions as an “allosteric” antagonist by binding next to the orthosteric ligand-binding pocket, thus modifying the pocket’s conformation by pushing Y90 towards it. Conversely, 4-CMTB acts as an ago-PAM by binding to the surface of transmembrane helices 6 and 7, thereby stabilizing the receptor in its active conformation. 4-CMTB forms a hydrogen bond with N230, which disrupts the N230-D269 interaction that stabilizes the inactive state of FFA2. This disruption facilitates receptor activation by promoting the stabilization of the active conformation. These findings significantly advance our understanding of FFA2's allosteric modulation mechanisms, demonstrating the complex relationship between ligand binding and receptor activity. They also pave the way for rational drug design, leveraging receptor modulation for therapeutic development and broadening the scope of precision treatments for metabolic and immune disorders.

P-16 Structural Basis of Opioid Receptor Activation by Hallucinogens

Tao Che, Washington University School of Medicine

Several hallucinogens, including psychedelics and dissociatives, offer medical benefits, such as anesthetic, analgesic, and antidepressant activities. These substances primarily act as agonists at the serotonin GPCRs or antagonists at the ion channels. However, the mechanisms behind their analgesic and antidepressant effects, as well as their potential for abuse, are not well understood. Our research demonstrates that these hallucinogens exhibit polypharmacological profiles, interacting with multiple targets, including opioid receptors, although this interaction is often underappreciated due to their weaker binding affinity. Here we present the cryo-EM structures of opioid receptors bound to both natural and synthetic hallucinogens. These high-resolution structures confirm that different hallucinogens act as orthosteric agonists at opioid receptors and reveal key motifs responsible for their recognition. Interestingly, hallucinogens with varying scaffolds adopt different binding poses between KOR and MOR due to distinct local environments. Through MD simulations and SAR analyses, we have identified specific interactions within opioid receptors that could guide the development of next-generation antidepressants with reduced abuse liability. Our findings emphasize the importance of considering opioid receptors to fully understand the therapeutic potential of hallucinogens in clinical settings.

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P-17 Look Ma, No Ligand! Structural Basis for the High Constitutive Activity of the 5-HT1B Receptor

Elspeth Allen, California State University Northridge

GPCRs exhibit different degrees of constitutive activity, i.e., activation in the absence of a ligand. Despite the clinical and pharmacological significance of this topic, the mechanisms resulting in some GPCRs' constitutive signaling at levels as high as 50% of their maximum value – while others exhibit little or no constitutive activity – are not well understood. Here, molecular dynamics simulations are employed to interrogate the high constitutive activity of the serotonin 5-HT1B receptor and a mechanism is proposed to account for the significantly higher level of constitutive activity 5-HT1B exhibits compared to the closely related β2 adrenergic receptor. 5-HT1B preferentially couples to Gi/o proteins and was selected due to robust documentation of its high level of constitutive activity (e.g., ~42% of its maximum level, as measured by G protein recruitment). It is therapeutically targeted with agonists for the treatment of migraines, so a mechanistic understanding of its constitutive activity can provide novel avenues for therapeutic applications as well. MD simulations were performed on the 5-HT1B receptor using apo models of the two functional states: an inactive conformation (template: PDB ID 5V54) and an active conformation (template: PDB ID 6G79). Additionally, a holo model of the inactive conformation, bound to the inverse agonist methiothepin, was used as a control to see if the inverse agonist can prevent the inactive apo model from constitutively converting to the active state. Simulations of each of the three models embedded in explicit lipid bilayers were run with four 10 ms replicas for a total simulation time of 40 μs/model. Clustering of MD snapshots revealed 12 distinct conformational states for the inactive apo model, 4 distinct states for the active apo model, and only 1 state for the inactive holo model. So, the largest conformational dynamics was displayed by the inactive apo model and lowest for the inverse agonist bound inactive model, consistent with the hypothesis about the inverse agonist bound receptor model. The TM3-TM6 ionic lock (3.50-6.30) distance analysis of the MD snapshots showed a trimodal distribution for the inactive apo and active apo models, seemingly sampling active-like, intermediate-like, and inactive-like ionic lock distances. The ionic lock in the inverse agonist bound inactive model simulation remained locked in the inactive-like state. The localization of the inverse agonist in the 5HT1B binding pocket suggests that it shuts down constitutive activity by preventing rotation of the toggle switch at W6.48 and, thereby, stabilizing packing of the $P^{5.50}I^{3.40}F^{6.44}$ motif. This was substantiated by a locked W6.48 residue and smaller fluctuations of the $P^{5.50}$, $I^{3.40}$, and $F^{6.44}$ residues in the inverse agonist bound inactive model compared to apo forms of the inactive and active models. The closely related β2 adrenergic receptor differs from 5-HT1B in its relatively low levels of constitutive activity. Structure and sequence comparison of the inactive conformations of 5-HT1B and β2 receptors reveal the presence of variations at key locations around the $W^{6.48}$ residue and the PIF motif. These differences can cause increased constitutive activity of 5-HT1B (compared to b2) through decreased stability of the PIF motif packing and a dynamic $W^{6.48}$ rotamer. One example is the $S207^{5.46}$ – $T118^{3.37}$ hydrogen bond in b2, right above the PIF motif, which is a weak nonpolar contact $A216^{5.46}$ ··· $T134^{3.37}$ in 5-HT1B. This weak link between TM3 and TM5 is exploited by the apo inactive model of 5-HT1B, enabling it to become active. However, the inverse agonist is able to prevent activation of the

5-HT1B inactive model by strengthening the interaction between TM3 and TM5, thereby offsetting the absence of TM3-TM5 hydrogen bond in 5-HT1B ($A216^{5.46}$ ··· $T134^{3.37}$), which is present in b2 ($S207^{5.46}$ – $T118^{3.37}$). So, the long MD simulations are beginning to provide mechanistic insight into the highly constitutively active 5-HT1B receptor.

P-18 Discovery of Allosteric Modulators of the Ghrelin Receptor

Yuki Shiimura, Kurume University

Ghrelin is a stomach-derived peptide hormone that regulates growth hormone release, appetite, and energy balance through the ghrelin receptor, a class A GPCR. Owing to these functions, the receptor represents a promising therapeutic target for conditions such as cancer cachexia and eating disorders. Positive allosteric modulators (PAMs) of GPCRs enhance receptor activity only in the presence of an orthosteric ligand, typically the endogenous hormone *in vivo*, thereby preserving physiological rhythms of hormone secretion. This unique feature makes PAMs attractive as next-generation therapeutics, but also difficult to identify by conventional activity-based screening, since they lack intrinsic agonist activity. To overcome this challenge, we employed DNA-encoded library (DEL) screening, which evaluates direct compound binding independently of receptor signaling. Using purified ghrelin receptor–Gq protein complexes, we identified 20 candidate compounds with binding activity. Subsequent Ca2+ mobilization assays revealed that several of these compounds functioned as PAMs of the ghrelin receptor. We are currently characterizing the binding sites of these PAMs and assessing their ability to potentiate ghrelin-induced growth hormone secretion. These findings highlight DEL screening as a powerful strategy for discovering novel GPCR allosteric modulators and may open new avenues for drug discovery targeting the ghrelin receptor.

P-19 Regulation of Melanocortin 4 Receptor in GT1-7 hypothalamic cells by Setmelanotide

Maha Hammad, Kuwait University

Background: Melanocortin 4 receptor (MC4R) plays a key role in regulating appetite and energy homeostasis. MC4R has been a promising target for the treatment of obesity for some time. However, animal studies reported serious cardiovascular side effects with the many tested MC4R ligands delaying its use as a therapeutic option. Setmelanotide, a selective MC4R agonist, was approved in 2020 for obesity treatment in patients with leptin-melanocortin pathway mutations. We hypothesize that Setmelanotide exhibits tissue-specific signaling distinct from the physiological agonist α- melanocyte-stimulating hormone (α- MSH). Objective: To compare MC4R-mediated signaling pathways and functional outcomes induced by α-MSH and Setmelanotide, using hypothalamic GT1-7 cells. Methods: HEK293-MC4R and GT1-7 cells were treated with α-MSH or Setmelanotide. cAMP production, ERK1/2 phosphorylation, glucose uptake, and insulin signaling markers were assessed to evaluate pathway activation and metabolic effects. Results: In HEK293-MC4R cells, Setmelanotide induced stronger cAMP signaling but weaker ERK1/2 activation than α-MSH, indicating functional selectivity. In GT1-7 cells, both ligands activated ERK1/2 similarly; however, only Setmelanotide increased glucose uptake. Conclusion: Setmelanotide exhibits ligand- and tissue-dependent MC4R signaling, highlighting its therapeutic potential and the importance of selective pathway targeting in anti-obesity drug development.

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P-20 Decoding the logic of neuromodulation: Functional diversity among G protein coupled receptors co-expressed in hippocampal neurons

Jenny He, UCSF

Neurons communicate not only through fast synaptic transmission, but also through neuromodulation by a vast array of GPCRs that can have prolonged effects on neural circuits. Recent transcriptomic analyses suggest that individual neurons express dozens of such receptors, indicating the potential for processing a huge bandwidth of chemical information. However, given that these GPCRs converge on only a few transduction pathways, how is specificity achieved? To address this, we compared receptors for two classes of neuromodulators, catecholamines and neuropeptides, which have stark differences in presynaptic release and ligand availability. We found 4 Gs-coupled receptors for these ligands co-expressed in primary hippocampal neurons (B1-AR, D5R, VIPR1, and CRHR1). These receptors show similarities in their abilities to produce global cAMP and PKA activity in the neuron, but begin to diverge at downstream signaling pathways. While all receptors are able to produce a transcriptional response to a brief ligand exposure, VIPR1 is uniquely able to produce a strong response to sustained ligand application. The ability for VIPR1 to produce sustained transcription may come from its resistance to desensitization in response to repetitive ligand application, in contrast to D5R and B1-AR which do desensitize. These differences in transcription suggest that these receptors are differentially tuned to the duration of ligand exposure. VIPR1 is primed to respond to sustained elevations of neuropeptide, while B1-AR and D5R are primed to respond to transient release events. These results unveil a distinct temporal code to the signaling specificity of neuropeptide and catecholamine receptors, which adds another dimension to the chemical language of neuromodulation.

P-21 *In silico* discovery of nanobody antagonists to the G protein coupled receptor MRGPRX2 using AlphaFold-Multimer

Jeffrey S. Smith, Harvard Medical School

Antibodies comprise a growing share of approved therapeutics, however generation of preclinical antibody candidates is often both time intensive and costly. We sought to develop an alternative approach to antibody fragment discovery to a GPCR using *in silico* antibody screening methods and bypass certain experimental steps such as immunization or library display. VHH antibodies, also known as nanobodies, are single-domain antibodies naturally derived from llamas and related species. They can be humanized and have recently received FDA approval. The smaller size and biochemical stability of nanobodies offers certain advantages, such as increased tissue penetration relative to conventional antibody biologics as well as modular architecture for multi-specific binder development. We computationally screened a library of nanobodies against the GPCR MRGPRX2, a promising target for the treatment of itch and chronic spontaneous urticaria. Based on our *in silico* confidence scores, we selected ten nanobody candidates to purify and evaluate biochemically. Our results show that 3/10 purified and tested nanobodies exhibited mid-nanomolar affinity for MRGPRX2. Binding affinity was tested in both the ROSA mast cell line at endogenous receptor levels and in HEK293T cells overexpressing MRGRPX2. We validated that these nanobodies are functional antagonists through various approaches. Pretreating mast cells with the three nanobodies resulted in a significant reduction in degranulation ($p<0.05$, one-way ANOVA) as well as a rightward shift in EC50 and reduced Emax in G protein activation relative to control, non-binding nanobodies ($p<0.05$, two-way ANOVA). Structural predictions suggest that the nanobodies block the MRGPRX2 orthosteric binding pocket, which was supported by mutagenesis studies. Our results demonstrate a computational antibody discovery pipeline for a GPCR that can effectively bypass experimental library screening.

P-22 A Positive Allosteric Modulator for the Mu-Opioid Receptor Provides Analgesic Relief

Teja Peddada, Stanford University

The safe and effective management of acute and chronic pain remains a major unmet medical challenge. Opioid drugs are limited by adverse effects and risk of misuse, highlighting the need for safer pain therapeutics. Positive allosteric modulation of the mu-opioid receptor (MOR), a G protein–coupled receptor, may offer a strategy to enhance analgesia while reducing toxicity by fine-tuning receptor activity. To identify new MOR positive allosteric modulators (PAMs), we performed a DNA-encoded library (DEL) screen of over 30 billion small molecules.From this screen, we identified **HG13** as a MOR PAM and used radioligand binding and GTP turnover assays to guide structure–activity relationship optimization, yielding the improved analog **AP58**. Radioligand binding assays showed that AP58 decreased naloxone binding (IC_{50} ≈ 30 μM), consistent with a shift in receptor equilibrium away from the inactive state. Functionally, AP58 enhanced agonist-stimulated GTP turnover without intrinsic agonist (ago-PAM) activity. In vivo, AP58 potentiated the analgesic effects of subtherapeutic morphine doses in antinociceptive assays and appeared to enhance endogenous opioid signaling in a chronic constrictive injury model of neuropathic pain. Importantly, while AP58 exacerbated morphine-induced respiratory depression, it exhibited no respiratory depression or reinforcement on its own and did not increase morphine preference in conditioned place preference assays. To elucidate its mechanism of modulation, we determined a 3.2 Å cryo-electron microscopy structure of an AP58 analogue bound to the MOR-Gi1 complex with met-enkephalin, an endogenous peptide. The structure revealed that HG13 binds to a previously uncharacterized allosteric pocket located between transmembrane helices 3, 4, and 5 and the intracellular loop 2 (ICL2). Next steps include using biophysical tools to study the effect of AP58 on ICL2 dynamics to characterize its mechanism further. Together, these findings demonstrate the power of large-scale DEL screening combined with structural biology to discover novel and chemically diverse allosteric modulators of clinically relevant GPCRs. AP58 defines a new chemical scaffold and binding site for MOR allosteric modulation, representing a promising lead compound for developing safer, more effective opioid analgesics.

P-23 Biophysical Characterization of Intracellular Loop 2 and Other Understudied Regions of the μ-opioid Receptor

Nicholas Sims, Stanford University

Chronic pain significantly reduces quality of life, and opioids remain the most effective treatment option. However, their use is limited by severe side effects, including addiction and respiratory depression, largely mediated through the μ-opioid receptor (μOR), a G protein-coupled receptor (GPCR). While canonical μOR activation is often associated with the outward movement of transmembrane helix 6 (TM6), recent findings (Zhao et al., 2024, Nature) show that full agonists like morphine can activate the receptor without substantial TM6 displacement, suggesting alternative activation pathways involving other receptor regions. To investigate this, we examined the conformational dynamics of two understudied regions: Intracellular Loop 2 (ICL2) and Helix 8. Our single-molecule FRET (smFRET) studies reveal ligand-specific changes in these regions. Notably, the number of distinct ICL2 conformational states decreases with increasing agonist efficacy, supporting a transition from disordered to helical conformations, consistent with findings in other Class A GPCRs. Ongoing studies using double electron-electron resonance (DEER) and continuous-wave electron paramagnetic resonance (CW-EPR) spectroscopy aim to complement smFRET results and provide high-resolution insight into μOR dynamics. These findings identify ICL2 and Helix 8 as critical modulators of μOR activation and highlight their potential as novel targets for structure-guided drug design. Targeting these receptor regions could enable the development of next-generation opioids with reduced side effects, offering strategies to address the ongoing opioid crisis.

P-24 Alpha-helical domain displacement of $G\alpha_s$ triggers GTPase-independent $G\alpha_s$-ERK signaling

Yonghoon Kwon, Gwangju Institute of Science and Technology

G protein-coupled receptors (GPCRs) constitute the largest superfamily of membrane proteins, regulating pivotal physiological processes and contributing to various diseases. Among the major GPCR signaling pathways, extracellular signal-regulated kinase (ERK) serves as a master regulator of cell proliferation and survival. However, the precise mechanisms and major transducers of GPCR-mediated ERK activation remain unresolved despite extensive investigation. Given that the alpha-helical domain (AHD) of $G\alpha_s$ protein undergoes large conformational displacement upon coupling with active GPCRs, we investigated the role of this displacement in controlling GPCR-mediated ERK activation. Our results demonstrate that the GDP-released open conformation of $G\alpha_s$ and its AHD is critical for ERK1/2 activation. The AHD directly binds to cRaf through the Ras like domain (RLD) of $G\alpha_s$ binding interface, indicating that cRaf interaction occurs exclusively in the open conformation. Furthermore, we investigated disease-associated $G\alpha_s$ mutants to determine their roles in ERK activation, providing mechanistic insights into their potential pathological functions. These findings highlight a noncanonical role of $G\alpha_s$ in controlling GPCR signaling that does not require its conventional GTPase activity, paving the road for discovering previously unrecognized $G\alpha_s$ dependent signaling pathways mediated by protein-protein interactions.

P-25 Inhibition of the μ-opioid receptor by orphan GPR88

Berta Carrasco-Martinez, Universitat Autònoma Barcelona

From the approximately 800 members of the G protein-coupled receptor (GPCR) family, more than 100 remain orphans (oGPCRs). There is evidence indicating that some oGPCRs may carry out a physiological role independently from endogenous ligands, such as forming heteromers with other GPCRs and changing their functional and pharmacological properties via allosteric interactions [1]. One of these oGPCRs is GPR88. Recently, some findings suggest that GPR88 may be acting as an inhibitor of opioid activity, by interacting with opioid receptors and allosterically changing their functionality [2,3]. Opioids are analgesic drugs which have become a standard treatment for pain. However, these compounds also display fatal addictive potential, which has led to the current worldwide opioid crisis caused by their misuse. μ-opioid receptor (μOR) is a GPCR targeted by opioids, and its activation is responsible for the analgesic properties as well as the dependence-related side-effects of these compounds. Thus, the GPR88μOR heteromer is of potential therapeutic interest, since GPR88 counteracts the effects of μOR agonists. Here, we have focused on the characterization of the interaction between GPR88 and μOR, to explore at the molecular level the allosteric mechanism that GPR88 exerts on μOR activity. Using a peptide-interfering approach combined with biophysical and biochemical techniques, we identified that GPR88 and μOR interact via transmembrane helix (TM) 6, acting as a protomer-protomer interface. A combination of molecular dynamic (MD) simulations and site-directed mutagenesis have permitted to propose that the negative regulatory role of GPR88 on μOR is due to the side chain of $Q298^{6.49}$ of GPR88. Understanding this architecture of the GPR88-μOR heteromer is of great value, due to its potential to combat neurologic disorders.

P-26 Cryo-EM structure of a single-chain β1-adrenoceptor – AmpC β-lactamase fusion protein in complex with cyanopindolol

Roger M. Benoit, PSI Center for Life Sciences

The insertion of fusion proteins has enabled the crystallization of a wide range of G protein-coupled receptors. By searching the Protein Data Bank using a BioJava script, we identified AmpC β-lactamase as a relatively large protein (39 kDa) that is geometrically suited for insertion into intracellular loop 3 (ICL3) of class A GPCRs via extended helix fusions. We genetically inserted AmpC β-lactamase into ICL3 of ultra-thermostable β1-adrenoceptor (β1AR). The resulting fusion protein was monodisperse and able to bind cyanopindolol. The protein particles comprised sufficient mass and discernable structural features to elucidate the cryo-EM structure in complex with cyanopindolol without any protein binding partners, to a resolution of 4.2 Å. The overall receptor architecture in the inactive conformation is clearly resolved. 3D variability analysis revealed significant flexibility between the two protein domains and within the GPCR helices, offering insights into conformational dynamics. The map contains clear density for the cyanopindolol. The fusion protein geometry fits many class A GPCRs, presenting a strategy for structure elucidation of diverse GPCR–ligand complexes. The approach furthermore holds potential for structure elucidation of GPCRs in the absence of ligands. We also discuss related protein engineering strategies, including molecular biomimetics and *de novo* protein and binder design.

P-27 Structural Insights into the Biased Signaling Mechanism of the Human Delta Opioid Receptor

Ryoji Suno, Kansai Medical University

The delta-opioid receptor (DOR) is a member of the G protein-coupled receptor (GPCR) family and regulates cellular responses via transducers such as G proteins and arrestins. (-) KNT-127 is a DOR agonist that is a G protein-biased DOR agonist (-)-KNT-127, and it is expected to be used as a pharmaceutical agent because it has the potential to reduce arrestin-mediated side effects such as convulsions while maintaining its G protein-mediated analgesic and antidepressant effects. Here, we used cryo-electron microscopy (cryo-EM) to elucidate the dynamics of the structure of the human DOR-G_i signaling complex bound to (-) KNT-127 or SNC80 (a balanced agonist) and identified the ligand binding mode in the active state. Using cell-based mutagenesis experiments, we found that three amino acid interaction networks ($W274^{6.48}$, $V281^{6.55}$, and $Y308^{7.42}$; Ballesteros-Weinstein numbering is indicated by superscript) are important for the biased signal transduction selectivity. $V281^{6.55}$ and $Y308^{7.42}$ are important for suppressing arrestin signaling activity, suggesting that they affect the conformational change of TM6 and 7 upon activation. Our findings open the way to enhance the therapeutic efficacy of DOR-mediated therapy while reducing undesirable side effects by targeting specific residues within the ligand-binding pocket of DOR.

P-28 Structural insights into ligand recognition and G protein preferences in histamine H_1 and H_4 receptors

Yuma Matsuzaki, The University of Tokyo

Histamine regulates diverse physiological processes by activating four receptor subtypes, each with a specific G protein preference. Among these, the histamine H_4 receptor (H_4R) influences chemotaxis and interferon production through G_i protein coupling, highlighting its therapeutic relevance. However, the molecular basis of H_4R signaling and of G protein preference among histamine receptors remains unclear. In this study, we determined the cryo-electron microscopy structure of the H_4R–G_i complex, revealing unique features of histamine binding and receptor activation. We also resolved the structures of the histamine H_1 receptor (H_1R) bound to the non-canonical G proteins G_i and G_s. Together with functional and computational analyses, intracellular loop 2 was identified as a critical determinant of G protein preference in H_1R and H_4R. Collectively, these findings provide a structural framework for understanding distinct ligand-recognition and activation mechanisms and shed light on G protein preference across histamine receptor subtypes.

P-29 Structure, function, and physiology of vasopressin–oxytocin crosstalk

Ayami Fukunaga, The University of Tokyo

Vasopressin (VP) and oxytocin (OT) are neuropeptides that are crucial for regulating various social behaviors. VP and OT have been understood to activate their canonical G protein–coupled receptors (GPCRs), the vasopressin V1 receptor (V1R) and the oxytocin receptor (OTR), respectively. Recent studies, however, have shown that VP can also activate OTR and OT can also activate V1R. This crosstalk has been proposed to influence social behaviors, but most previous evidence was obtained under pharmacological conditions, and its physiological relevance has remained uncertain. To address this question, we carried out a combined molecular, cellular, and physiological study. We determined the cryo-EM structure of VP-bound V1R, thereby revealing the molecular basis of VP recognition by the V1R. Structural comparison with the previously reported OT-bound OTR structure identified both common and distinct features of ligand recognition. Leveraging these insights, we engineered a V1R mutant with reduced affinity for OT (VP-selective V1R) and an OTR mutant with reduced affinity for VP (OT-selective OTR). We then generated OT-selective OTR knock-in mice and examined their social behaviors. These experiments demonstrated that VP-mediated activation of OTR contributes to the regulation of social behaviors under physiological conditions. Our findings provide the first direct evidence that the VP–OTR axis has a physiologically relevant role in the regulation of social behaviors.

P-30 A combined structural and computational approach reveals the odorant-binding mechanism of human OR10G7

Minseok Kim, Seoul National University

Odorant receptors (ORs) are the largest subfamily of class A G protein-coupled receptors (GPCRs), yet the structural basis of their ligand recognition remains poorly understood. ORs are also ectopically expressed in non-olfactory tissues; notably, OR10G7 is expressed in undifferentiated keratinocytes of patients with atopic dermatitis. To elucidate the odorant binding mechanism of OR10G7, we employed the consensus strategy, which has been previously applied in structural studies of ORs. We determined the cryo-EM structure of an odorant-bound consensus OR10 and constructed an odorant-bound OR10G7 model using template-based modeling and docking. The predicted binding pose of the odorant was validated by site-directed mutagenesis, cAMP assays, and molecular dynamics simulations. Comparative analyses revealed that subtle changes in residues forming the odorant binding pocket reshape the odorant-binding cavity, leading to the altered odorant preference of OR10G7. These results provide structural insights into the odorant recognition mechanisms of OR10G7. Furthermore, our study demonstrates that combining computational and structural approaches enables the investigation of molecular interactions between odorants and native ORs, whose structural studies are often hindered by low surface expression.

P-31 Probing allosteric activation with rigidity transmission allostery (RTA) theory, NMR, and simulations

Adnan Sljoka, RIKEN, Japan

Rigidity Theory Allostery predictions algorithms provide a theoretical and mechanical interpretation of allosteric signaling in protein structures. Rigidity Transmission Allostery (RTA) methods are designed to predict if perturbation of rigidity at one site of the protein can propagate across a network and in turn cause a change in rigidity at a second distant site, resulting in allosteric transmission. Here we focus on the application of RTA on allosterically driven functional dynamics in G Protein Coupled Receptors (GPCRs). We show how ligands trigger allosteric changes that propagate to functionally important regions; the role of beta-gamma subunit in G proteins as critical facilitators of allostery within the ternary complex with adenosine A2AR; the role of differentiated allosteric pathways in GPCR promiscuity and selectivity mechanisms in A2AR in the presence of different G protein subtypes. We combine 19F-NMR and RTA to delineate allostery and distinct key functional activation and precoupled intermediate states in Adenosine A2A GPCR complexed with heterotrimeric G protein. NMR showed that binding of G protein stabilizes a precoupled activation intermediate state and two distinct active states which facilitate nucleotide exchange by full or partial agonists. RTA showed beta-gamma subunit in G proteins is critical in facilitating allostery within the ternary complex. We present results on several GPCR structures and recent work which investigates the selectivity and efficacy in A2AR in the presence of different G protein subtypes. Using a two-dimensional RTA allostery map, we quantify how local rigidification at the orthosteric pocket or conserved microswitches propagates across the A2AR–G protein complex. This 2D analysis highlights a tryptophan-enriched dynamic and adaptive allosteric pathway linking the orthosteric site to the nucleotide pocket. 19F-NMR using 5-fluorotryptophan reporters track these couplings and validate the predicted allosteric pathways.

P-32 Native Mass Spectrometry Reveals the Interplay Between the Disordered Loop and G Proteins in GPCR Activation

Hsin-yung Yen, Academia Sinica, Taiwan

G protein-coupled receptors (GPCRs) regulate diverse physiological processes through selective coupling to G proteins, yet the mechanisms that fine-tune this process remain incompletely understood. Using native mass spectrometry (nMS) and hydrogen–deuterium exchange (HDX)-MS, we previously showed that ligand binding to the β1-adrenergic receptor (β1AR) modulates receptor–G protein coupling and enhances the dynamics of intracellular loop 3 (ICL3). In the present study, we reveal a key function of ICL3 in the early recruitment of G proteins by capturing pre-equilibrium states of β1AR–mini-Gs complexes. ICL3, an intrinsically disordered region located between transmembrane helices (TM) 5 and 6, exerts agonist-specific effects that contribute to pharmacological diversity. To dissect its structural contributions, we performed truncations and topological modifications, identifying the N-terminal segment of ICL3 connected to TM5 as a critical determinant of complex formation. Furthermore, by partially regressing mini-Gs toward the wild-type G protein, we probed the steric interplay between G protein helices and ICL3, showing that the loop alleviates steric hindrance to facilitate receptor–G protein engagement. Together, these findings establish ICL3 as a dynamic structural element that integrates pharmacological input with structural transitions, thereby fine-tuning the early steps of GPCR signaling.

P-33 Structural Insights Into Angiotensin Receptor Biased Signaling

Jinghan Liu, Harvard Medical School

The angiotensin II type 1 receptor (AT1R) is a G protein–coupled receptor (GPCR) that regulates cardiovascular and renal homeostasis in response to angiotensin II (AngII). Overstimulation of AT1R causes hypertension, making it a major therapeutic target. Around 5% of U.S. adults currently take angiotensin receptor blockers (ARBs) to manage blood pressure. AT1R signals through two distinct intracellular pathways, mediated by proteins of the Gq and β-arrestin families. Gq coupling drives vasoconstriction and raises blood pressure, while β-arrestin signaling enhances cardioprotection and contractility. For patients with both hypertension and heart failure, current therapies remain suboptimal: ARBs lower blood pressure but can compromise cardiac performance. An ideal drug would lower vascular resistance while preserving or even boosting cardiac performance. β-arrestin–biased agonists uniquely offer this profile by blocking detrimental G protein signaling while activating cardioprotective arrestin pathways, but currently no drug-like candidates exist. Despite decades of biochemical studies and recent structural breakthroughs in GPCR signaling, the molecular basis by which AT1R discriminates between signaling partners remains incompletely understood, and efforts to develop biased small molecules remain underexplored. Herein, I employed a miniG fusion approach and determined two cryo-EM structures of the AT1R–G protein complex bound either to AngII or to a G protein-biased peptide agonist, TRV055, representing the first structural view of a G protein–biased agonist at this receptor. These structures provide a reference state for comparative studies. Through structural comparisons, mutagenesis and paired G protein/β-arrestin BRET assays, residues N294 and N298 were identified as important for G protein biased signaling. Together, these results highlight structural features that contribute to biased signaling at AT1R and provide a framework for further studies aimed at understanding and exploiting AT1R biased agonism.

P-34 Discovery of protein-peptide interactions using a large, target-agnostic yeast display library

Joe Hurley, Harvard Medical School

Protein–peptide interactions underlie key biological processes and are commonly utilized in biomedical research and therapeutic discovery. It is often desirable to identify peptide sequence properties that confer high-affinity binding to a target protein. However, common approaches to such characterization are typically low throughput and sample only regions of sequence space near an initial hit. To overcome these challenges, we built a yeast surface displayed library representing ~6.1×10^9 unique peptides. We then performed screens against diverse protein targets, including two antibodies, an E3 ubiquitin ligase, and a membrane-bound bacterial enzyme. In each case, we observed motifs that appear to drive peptide binding, and we identified multiple novel, high-affinity clones. These results highlight the library's utility as a robust and versatile tool for discovering peptide ligands and for characterizing protein–peptide binding interactions more generally. We are now extending this platform to characterize the ligand-binding properties of a panel of G protein coupled receptors (GPCRs). This work aims to identify peptides with unusual pharmacology or sequences highly divergent from known ligands and could serve as a starting point for deorphanization of receptors for which there are no known ligands.

P-35 Metadynamics Simulations and Site-Directed Mutagenesis Determines the Binding of Cannabidiol to the Adenosine A_{2A} Receptor

Aleix Quintana García, Universitat Autònoma de Barcelona

Cannabidiol (CBD), the second most abundant active compound found in the Cannabis sativa plant, is nonaddictive and of increasing interest due to its potential therapeutic utilities. The actions of CBD are mediated by cannabinoid receptors and other targets, both G protein-coupled receptors (GPCRs) and non-GPCR proteins. It has recently been shown that CBD is not an orthosteric ligand of the adenosine A_{2A} receptor ($A_{2A}R$), but rather a negative allosteric modulator. Because of the multitude of non-conserved allosteric pockets in GPCRs, the binding mode of CBD to $A_{2A}R$ remains unknown. To fill this knowledge gap, and due to the therapeutic relevance of CBD and $A_{2A}R$, we have used metadynamics simulations and site-directed mutagenesis to identify the binding mode of CBD. These theoretical and experimental results show that the allosteric binding site of CBD is along the ligand binding pathway of adenosine to $A_{2A}R$, near the orthosteric binding site.

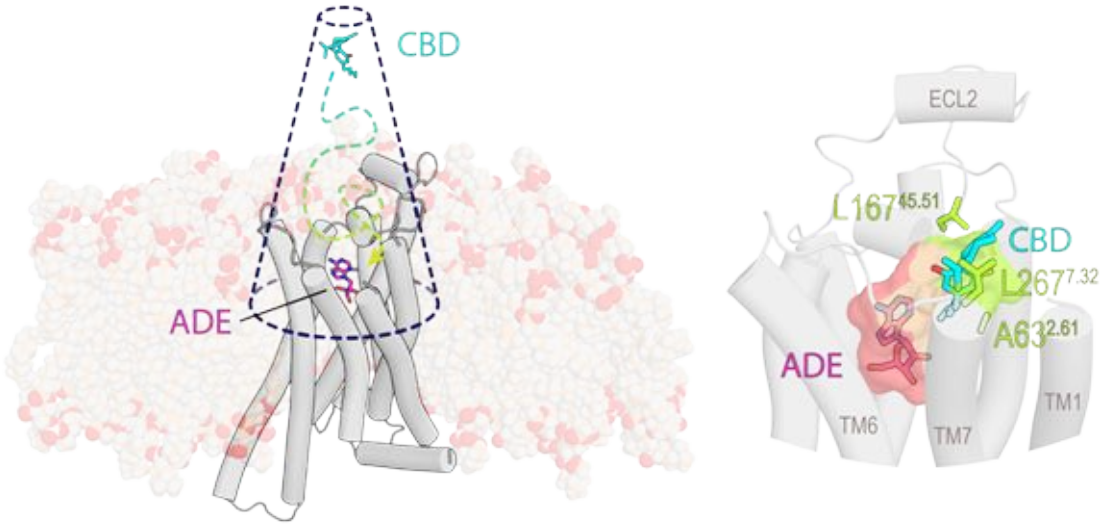

Figure 1. (Left) Schematic of the ligand-binding metadynamics approach employed for sampling the binding of CBD to $A_{2A}R$. CBD (cyan sticks) starts from an unbound state and diffuses within a confined region of the simulation box (dashed lines) to bind to $A_{2A}R$ (gray cartoon) embedded in a lipid bilayer membrane with bound agonist ADE (magenta sticks). (Right) Binding mode of CBD within the ADE and $A_{2A}R$ complex. The allosteric binding site and mutated residues (lemon sticks) differ from the orthosteric binding site (coral surface).

P-36 RXFP2 Mechanism of Activation

Xunan Wang, Harvard Medical School

Relaxin-family peptide receptor 2 (RXFP2) is a class A GPCR important for regulating male reproductive physiology. Its primary role is to regulate testicular descent during prenatal development. RXFP2 has a very unique structure: in addition to the seven-transmembrane domain conserved across all GPCRs, it has a large ectodomain composed of leucine-rich repeats (LRRs), and a low-density lipoprotein class A (LDLa) module tethered to the LRRs through a flexible linker. Out of all the GPCRs, the LDLa module is only present in RXFP1 and RXFP2, and it is critical for the activation of those two receptors. Multiple cryo-EM structures of RXFP1 have been elucidated by our lab, but densities of the LDLa module have never been seen, suggesting its dynamic nature. The mechanism of activation of RXFP2 by its cognate peptide hormone agonist insulin-like peptide hormone 3 (INSL3) and the role of LDLa module in promoting RXFP1 and RXFP2 activation are not yet understood in the literature. Hence, this study aimed to elucidate the mechanism of RXFP2 activation by INSL3 through structure elucidation by cryo-EM and complemented by single-molecule Förster resonance energy transfer (smFRET). Cryo-EM structures were elucidated for the inactive state RXFP2, and active state RXFP2 bound to INSL3 and the heterotrimeric G protein complex. Similar to observations in the RXFP1 cryo-EM structures elucidated so far, LDLa module density was not present. INSL3-bound RXFP2 structure revealed INSL3 B chain binding to the LRRs of RXFP2 and A chain engaging the linker tethering to the LDLa module. Preliminary smFRET data from the sample of receptor only detected two states of the LDLa module, a high FRET state and a low FRET state, representing a transmembrane domain-bound state and an unbound state. When the hormone was preincubated with the receptor, the population distribution shifted to more molecules in the high FRET state, indicating the LDLa module-bound state is stabilized by the hormone. Our data suggest INSL3 activates RXFP2 through recruiting the linker tethered to the LDLa module to position LDLa module in close proximity to the transmembrane domain for receptor activation.

P-37 Exploring Activation Dynamics and Conformational Landscapes of GPCRs using Molecular Dynamics Simulations

Shun Yokoi, University of Tsukuba

G protein-coupled receptors (GPCRs) are central players in cellular signaling and are among the most important drug targets. While static structures have provided invaluable insights, they cannot fully capture how receptors move or how these motions shape signaling outcomes. Understanding such dynamic processes is essential for bridging structural snapshots with functional outcomes and for advancing GPCR-targeted drug discovery. To address this, we employ large-scale molecular dynamics (MD) simulations, integrated with structural and functional data from our collaborators. In our recent work on the orexin 2 receptor (OX2R), we traced activation pathways and identified intermediate conformations that bridge inactive and active states. These findings offer a quantitative framework for understanding receptor activation and highlight possible atomic-level mechanisms of signal regulation. We have also investigated biased signaling in the κ-opioid receptor (KOR) and other GPCRs. By combining MD simulations with cryo-EM structures and mutagenesis experiments, we examined how specific ligand–receptor interactions and critical residues can shift the balance between G protein– and β-arrestin–mediated pathways. These insights provide a foundation for the rational design of biased ligands that preserve therapeutic efficacy while minimizing side effects. Overall, our aim is to integrate structural biology, computational physics, and medicinal chemistry to deepen our understanding of GPCR function. At the GPCR Workshop 2025, I will present ongoing studies on OX2R and KOR, focusing on conformational landscapes and biased signaling as potential routes toward dynamics-based drug discovery.

P-38 Discovery of GPCR agonist antibodies with unique activation mechanisms using the FAST platform

Monica Schwartz, Abalone Bio

Antibodies are highly specific, avoiding off-target activities that challenge GPCR drug discovery. However, their pharmacological actions on GPCRs have been predominantly inhibitory. Abalone Bio's Functional Antibody Selection Technology (FAST) platform combines synthetic biology and machine learning (ML) to discover and design functionally active antibody drugs—such as agonists, positive or negative modulators, or antagonists. FAST goes beyond mere binding by simultaneously measuring the function of 100 million antibodies against GPCRs directly, allowing us to find rare antibodies that are hard to discover through traditional screening methods with limited throughput. Furthermore, the platform does not depend on structural information, which is limited for dynamic receptors like GPCRs, bypassing the challenge of predicting allosteric interactions from static structures. FAST couples GPCR function to cell growth in a quantitative and tunable manner, enabling sensitive identification of functional antibodies with a wide range of activity. These discovery campaigns produce library-scale sequence-function datasets that uniquely power generative protein language models to design novel active antibody sequences. Using FAST, Abalone has identified multiple agonists against four GPCRs. FAST-discovered CB2 agonist antibodies have demonstrated activity both in vitro and in vivo. We constructed a sequence-to-functional activity map by fine-tuning a protein large language model on data generated as part of our discovery campaigns. We used the learned activity map to efficiently guide a generative model in sampling predicted active sequences from novel sequence space near the seed, then experimentally tested them. Our generative approach not only enriched for active novel sequences but also created sequences with greater activity than the original sequence. To our knowledge, these are the first designed GPCR antibody sequences that are functionally activating. CB2 is predominantly expressed on immune cells such as macrophages, microglia, and Kupffer cells. Activating CB2 effectively suppresses the activation of these cell types, leading to reductions in inflammation and fibrosis. While several small molecule CB2 agonists have been evaluated in clinical trials, their development has been hindered by off-target effects on the CB1 receptor. AB120 and AB150 effectively reduce mouse and human macrophage activation in vitro and reduce fibrosis in a therapeutic model of F4 stage liver fibrosis. This model recapitulates advanced liver cirrhosis and is a high bar for potential therapeutics. Thus, success of AB120 and AB150 in this challenging model generates enthusiasm for the ongoing preclinical development of these candidates.

P-39 Developing a Toolbox to Resolve Isoform-Specific Signaling of the Dopamine D2 Receptor

David Reiner-Link, Stanford University

Alternative splicing of G protein–coupled receptor (GPCR) genes expands the functional repertoire of receptor signaling, yet isoform-specific mechanisms remain underexplored. The dopamine D2 receptor (D2), the most widely drug-targeted GPCR, exists as two isoforms with distinct expression across tissues, neuronal subtypes, and subcellular compartments. The short isoform (D2S) is enriched presynaptically, whereas both isoforms contribute postsynaptic signaling, dominated by D2L. We systematically compared isoform signaling using G protein activation assays. D2L consistently exhibited higher efficacy than D2S across six Gi/o subtypes (GoA, GoB, Gi1, Gi2, Gi3, Gz), while D2S showed faster activation kinetics at GoB, Gz, and Gi1. Isoform-specific differences were most pronounced for GoA, GoB, and Gz, which dominate Gi/o expression in the brain. To probe the molecular basis of these differences, we developed isoform-selective proximity labeling sensors for proteomic mapping of signaling complexes, and engineered isoform-specific induced pluripotent stem cells (iPSCs) to establish advanced neuronal models of isoform signaling. Together, this toolbox establishes a first platform for resolving GPCR isoform-specific signaling at spatiotemporal resolution, bridging molecular pharmacology with isoform-centric drug discovery.

P-40 Positive allosteric modulation of CCK1R through the third intracellular loop

Morrhyssey Benz, University of Minnesota

The cholecystokinin 1 receptor (CCK1R) has emerged as a promising therapeutic target for treating obesity. When CCK1R is activated, it triggers downstream events that inhibit gastric emptying, functioning to maintain the feeling of satiation. A major pitfall of the development of GPCR drugs is the occurrence of unintended side-effects. This is due, in part, to off-target binding due to sequence homology at the ligand binding site. Allosteric modulators can regulate GPCR signaling by selectively targeting a site that is distant from the active site. Recently, we have shown that the third intracellular loop (ICL3) of GPCRs is autoregulatory. When targeted, ICL3 can be biased into an open or closed conformation, which alters G protein coupling. Importantly, the ICL3 is highly sequence divergent among receptor subtype isoforms, making it a promising candidate for selective modulation. The goal of this study was to identify and characterize single domain antibody fragments that selectively target the ICL3 of CCK1R and positively modulate signaling. Prospective nanobodies (Nb) were isolated from a yeast surface display library containing ~108 unique sequences. The naive library was subjected to magnetic and fluorescence activated cell sorting. Clones that demonstrated selective binding to a CCK1R ICL3 construct were isolated and 19 unique Nbs were identified. In order to verify Nb binding to the full length CCK1R, Nb sequences were inserted into an intramolecular FRET SPASM sensor. If the Nb demonstrated binding to the CCK1R ICL3, FRET between the donor and acceptor within the sensor would occur. To determine if the Nb modulates signaling, IP1 accumulation was measured downstream of CCK1R-Nb SPASM sensors. Positive modulation of CCK1R signaling through the Nb would cause increased levels of intracellular IP1 relative to a CCK1R control. We discovered two unique Nb sequences that act as positive allosteric modulators of CCK1R. Furthermore, when the two Nbs were inserted in a FRET sensor containing the CCK2R sequence, the other isoform in the CCK receptor sub-family, IP1 levels were not significantly different relative to the control. Our data demonstrate that Nbs targeting the ICL3 of CCK1R can selectively modulate receptor activity. The findings from this study reveal general Nb features that can be applied for the identification of novel positive allosteric modulators for other GPCRs of interest.

P-41 Exploring Endosomal GPCR Signaling by Endogenous G proteins

Andrew Dates, UCSF

For many GPCRs, the subcellular location of signaling plays a central role in shaping ultimate cellular responses. Endomembrane signaling is best characterized on endosomes, the first intracellular compartment encountered by GPCRs after activation. However, the fundamental mechanisms that regulate G protein activity on endosomes remain poorly understood, particularly under conditions where G proteins are expressed at native levels to preserve the natural organization of signaling components. Here, we employ a suite of biosensors to monitor the subcellular activity of G proteins at endogenous levels. These sensors recognize distinct surfaces of Gα subunits to enable real-time tracking of signaling events in living cells. By applying these tools across the G_s, $G_{i/o}$, and $G_{q/11}$ families and using multiple GPCRs as inputs, we aim to uncover the basic principles that govern G protein activation on endosomes. Upon receptor stimulation, all G protein subfamilies exhibit elevated activity on endosomes, following a characteristic kinetic delay relative to plasma membrane signaling. We found that ligand concentration and receptor expression levels influence the kinetic profile of signaling at the plasma membrane, but not on endosomes. We also observed differences in subcellular G protein activation across G protein subtypes and receptor inputs. The kinetics of both plasma membrane and endosomal activation appear to differ between G protein subfamilies. Across receptors, the magnitude of endosomal G protein activity varies, suggesting that cells might be able to use endosomal signaling signatures to distinguish inputs that converge on the same G protein pathway. Together, these preliminary studies provide initial insights into the subcellular signaling dynamics of endogenous G proteins, informing the basic rules by which GPCRs encode signals across compartments.

P-42 Receptor-miniGs interactions reveal the kinetics of partial agonism

Michael Ritt, University of Minnesota

Partial agonism describes a state of receptor activation less than the maximum achieved from a full agonist. Using FRET-based biosensor measurements in combination with ordinary differential equation modelling, we identified two key kinetic features that determine agonist efficacy: the concentration-dependent association rate of the G protein to the agonist-bound receptor, and the catalytic rate of G protein activation by the receptor. While both rate constants scale proportionally with reported molecular efficacy metrics, we find that the concentration-dependent association rate is the governing parameter for a given cellular signaling system. Our model explains discrepancies between expected ligand efficacy and efficacy values derived from receptor overexpression systems. Furthermore, we find that our model for agonist efficacy is applicable to multiple distinct GPCR signaling systems.

P-43 Quantifying β2AR TM6 dynamics at single-molecule resolution

Arnab Modak, St. Jude Children's Research Hospital

GPCRs, the largest class of integral membrane protein drug targets, regulate cellular homeostasis by eliciting versatile intracellular signaling outcomes in response to ligand binding at extracellular orthosteric and/or allosteric sites. Despite extensive cellular, structural, and biophysical characterization, the order and timing of the physical events underpinning ligand efficacy remain incompletely understood. Previously, our collaborative team has employed single-molecule FRET (smFRET) imaging to quantify the rate and extent of β2AR (a prototypical class A GPCR)-mediated stimulatory G protein (Gs) coupling by tracking the ~15Å outward deflection of its transmembrane helix 6 (TM6) element in the nucleotide-free state. At that time, it was not possible to directly examine the conformational landscape of β2AR in the absence of G protein. Hence, the molecular events in β2AR that render it capable of productively engaging G protein when agonized remain obscure. To address this knowledge gap, we have advanced technological developments in microscope design, self-healing organic fluorophores and site-specific labeling of β2AR that now enable us to directly visualize TM6 movements at sufficient spatial and temporal resolution to directly reveal the sequence of transitions between inactivated and fully activated states when agonized. Using conformation-specific nanobodies and allosteric modulators, we assign the distinct FRET states observed to specific conformations and quantify the relationship between our findings and the energy landscape underpinning the activation that has been derived from ensemble investigations.

P-44 Conformational dynamics governing G protein recognition and activation by Neurotensin Receptor 1

Kazuhiro Kobayashi, The University of Tokyo

G protein–coupled receptors (GPCRs) constitute the largest membrane protein family in humans, with approximately 800 members regulating nearly every aspect of physiology, making them critical targets for basic research and drug discovery. GPCR signaling occurs through heterotrimeric G proteins, classified into four subtypes, whose selective activation leads to distinct cellular outcomes. Here we present cryo-EM structures of human neurotensin receptor 1 (NTSR1) complexed with Go and Gq heterotrimers, revealing how the receptor's intracellular surface dynamically rearranges to optimally accommodate each G protein subtype. Moreover, time-resolved cryo-EM analyses of NTSR1–Gi complexes enable visualization of G protein dissociation processes upon GDP or GTP binding. Structural characterization of over 20 intermediates, complemented by mutational, biophysical, and computational analyses, identifies distinct aspects of Gi dissociation: GDP/GTP-induced Gi release from both canonical and unique non-canonical active states; dynamic remodeling of the receptor's intracellular surface during Gi dissociation, suggesting that the receptor dynamically changes its intracellular surface both to recognize different G protein subtypes and to facilitate G protein activation; a Gi dissociation pathway significantly distinct from that of Gs; and orchestrated sequential conformational rearrangements in the Gα subunit's switch I–III regions during separation from Gβγ. These findings provide a comprehensive view of GPCR–G protein signaling dynamics, deepening our mechanistic understanding and providing a robust foundation for the development of signal-targeted GPCR therapeutics.

P-45 UniSens – One Assay for the Future of GPCR Drug Discovery

Alexei Sirbu, ISAR

The modern GPCR drug discovery pipeline integrates decades-old methods, from classical ligand-binding to advanced biosensor-based functional assays. Over time, researchers have developed numerous second messenger and recruitment assays to study GPCR activity in cells. While comparing outputs across assays to gauge functional selectivity is now routine, such practices offer limited predictive value for drug performance in patients. A major limitation of signaling assays is their focus on downstream events composed of multiple protein–protein interactions, which makes results highly sensitive to experimental setup and cellular context. A more promising approach is to probe drug action directly at the receptor. GPCRs occupy a dynamic conformational landscape with multiple states of varying activity, and ligands that modulate this equilibrium, even subtly, are potential therapeutics. Although the mechanisms remain incompletely understood, receptor-level interrogation promises a more realistic and reproducible metric for drug discovery. ISAR Bioscience, in collaboration with six international SMEs and academic labs and supported by the European Union*, is advancing the UniSens technology platform to meet evolving discovery needs. UniSens employs a panel of novel engineered GPCR activity sensors that monitor multiple receptor epitopes, generating distinct functional fingerprints (patent pending). Early results with the prototype panel suggest sufficient resolution to distinguish basal, agonist-, and antagonist-bound states of the β2 adrenergic receptor. In the next two project years, we will expand the sensor panel and fingerprint diverse GPCRs with broad ligand libraries. Integrating these textured data with AI-assisted workflows, our goal is to overcome current challenges while enabling transformative approaches in pharmaceutical research. Our vision is a cost-effective, community-vetted assay for smart, sustainable drug discovery worldwide.

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P-46 Lighting up signalling of native GPCRs to facilitate discovery efforts for dementia

Karen Gregory, Monash University

Dementia is characterised by impaired cognition (memory and thinking symptoms) and comprises multiple distinct underlying neurodegenerative disorders, of which Alzheimer's disease is the most common. Moreover, commonly people living with dementia also experience symptoms of depression and/or anxiety. Current medicines have limited efficacy and are associated with side effects, which impact quality of life. There is an urgent need to discover new and safer medicines to treat the memory, mood, and thinking symptoms that are top priorities for people living with dementia. G protein-coupled receptors (GPCRs) are highly druggable targets, which mediate the actions of diverse neurotransmitters and have critical roles in maintaining higher order brain functions. Neuroscience drug discovery is associated with high attrition rates, which may be partially attributable to a reliance on preclinical model systems with poor translational relevance. In addition, early-stage drug discovery efforts largely depend on canonical measures of GPCR activity for hit and lead identification. We aim to address this challenge through optimising biosensor-based assays in translationally relevant model systems amenable for medium throughput formats. Fluorescent and/or resonance energy transfer biosensors were introduced into different cell types using mRNA-mediated transfection. A range of different cell types have been tested, including recombinant cells transfected with human GPCRs of interest, primary neuronal cultures and induced pluripotent stem cells differentiated into neurons. We performed high-content imaging to simultaneously visualise and quantify expression of fluorescent biosensors, cell viability, and cell number over time. Our preliminary data show successful introduction of intracellular signalling biosensors with the optimal time and conditions differing across biosensor and cell type. We will continue to optimise and validate these new assay formats by benchmarking against endogenous ligands and synthetic small molecules, as well as clinical candidates and existing medications for the symptoms experienced by people living with dementia. We envisage that biosensor assays in translationally relevant cellular models could be incorporated early within discovery pipelines to improve triage or selection of promising new leads for neuroscience and beyond.

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P-47 Cross-Species Benchmarking of SSTR2 Ligands in Fresh-Frozen Brain Sections

Shil Patel, Ratio Therapeutics

Somatostatin receptor subtype 2 (SSTR2) is a G protein-coupled receptor (GPCR) that regulates hormone secretion in neuroendocrine tissues and is over-expressed in neuroendocrine tumors (NETs) and multiple other cancers including small cell lung cancer (SCLC), hepatocellular carcinoma (HCC), and cancers of the breast, prostate, and colon. Approval of the β-emitting agonist [^{177}Lu]DOTATATE (Lutathera®) for gastroenteropancreatic neuroendocrine tumors (GEPNETs) has validated SSTR2-targeted radioligand therapy and sparked efforts to develop next-generation ligands with enhanced tumor uptake, retention, and dosimetry profiles. Progress toward these goals is accelerated by experimental platforms that preserve native receptor conformation and provide quantitatively comparable binding data across species. We present a fresh-frozen tissue-section assay that uses the high-affinity antagonist [^{177}Lu]-DOTA-LM3 as a tool radioligand, enabling cross-species assessment of SSTR2-targeted ligands and informing translation of next-generation compounds from rodents to non-human primates to humans. [^{177}Lu]-DOTA-LM3 demonstrated specific binding to a single population of high-affinity sites in mammalian brain sections where sub-regional autoradiographic signal intensity correlated strongly with known SSTR2 expression. In parallel, SSTR2 immunohistochemistry (IHC) on adjacent fresh-frozen sections aligned with the autoradiograms, providing further support for radiolabeled DOTA-LM3 as an in vitro tool compound in this setting. Importantly, regional differences in binding affinity were not observed across mouse (m), rat (r), cynomolgus monkey (NHP) or human (Hu) brain sections. These data were confirmed in competition assays where the apparent binding affinity (K_i) for unlabeled DOTA-LM3 matched the saturation binding assay-derived binding affinity (K_D) for the radiolabeled compound. Competition curve affinities (K_i) for reference SSTR2-selective ligands (DOTATATE, Somatostatin-28, Octreotate, Somatostatin-14, DOTA-JR11, and PRL-2915) aligned with published values and were consistent across species. This fresh-frozen tissue-section assay enables cross-species quantification of SSTR2 ligand affinity. [^{177}Lu]-DOTA-LM3 serves as a tool radioligand, enabling evaluation of emerging SSTR2 RLT candidates and facilitating translation from rodents to non-human primates to humans. Although the reference ligands evaluated here showed concordant affinities, next-generation molecules may diverge; this assay offers a platform to detect such differences early and guide appropriate translation.

P-48 Mechanisms of aberrant thyrotropin receptor signaling in Graves' disease

Karthik Srinivasan, UCSF

Autoantibodies that alter GPCR signaling are implicated in many diseases. Graves' disease (GD) is a classic example where autoantibodies constitutively activate the thyrotropin receptor (TSHR), causing hyperthyroidism. Our lab has described the structural basis by which a pathogenic GD autoantibody mimics the endogenous TSH hormone to activate TSHR and proposed a model for receptor activation. However, this model does not fully explain clinical heterogeneity among GD patients, who exhibit varying degrees of hyperthyroidism, ophthalmopathies, and dermopathies. To uncover the molecular underpinnings of this variance, we are characterizing GD patient-derived autoantibodies. Using TSHR as an antigen bait, we have isolated single B-cells and obtained sequences of several monoclonal antibodies. Functional assays reveal that these antibodies display a spectrum of binding affinities and potencies in activating TSHR-mediated Gs signaling. In parallel, we are employing deep mutational scanning (DMS) to map receptor residues critical for autoantibody-mediated activation. Together, these complementary approaches aim to unravel the landscape of TSHR-autoantibody interactions and illuminate mechanisms of aberrant thyroid signaling, providing a foundation for developing new therapeutic strategies for GD.

P-49 The far extracellular CUB domain of the adhesion GPCR ADGRG6/GPR126 is a key regulator of receptor signaling

Sumit J. Bandekar, University of Chicago

Adhesion G protein-coupled receptors (aGPCRs) transduce extracellular adhesion events into cytoplasmic signaling pathways. ADGRG6/GPR126 is an aGPCR critical for axon myelination, heart development, and ear development; ADGRG6 is also associated with developmental diseases and cancers. ADGRG6 has a large, alternatively spliced, five-domain extracellular region (ECR) that samples different conformations and is essential for receptor function in vivo. However, the mechanistic details of how the ECR regulates signaling are unclear. Herein, we studied the conformational dynamics of the conserved CUB domain, which is located at the distal N-terminus of the ADGRG6 ECR and is deleted in an alternatively spliced isoform (ΔCUB). We show that the ΔCUB isoform has decreased signaling and is insensitive to inclusion of an activating splice insertion (+ss). Molecular dynamics simulations suggest that the CUB domain is involved in interdomain contacts to maintain a compact ECR conformation. A cancer-associated CUB domain mutant, C94Y, drastically perturbs the ECR conformation and results in elevated signaling, whereas another CUB mutant located near a conserved Ca^{2+}-binding site, Y96A, decreases signaling. Our results suggest an ECR-mediated mechanism for ADGRG6 regulation in which the CUB domain instructs conformational changes within the ECR to regulate receptor signaling.

P-50 Unveiling dynamic molecular events during GPCR–G protein engagement

Jiemin Shen, Stanford University

High-resolution structures of nucleotide-free GPCR–G protein complexes have provided insights into the mechanism of G protein activation. However, these static models capture only stable conformations and do not reflect the transient intermediates that occur in the cellular environment, where GDP and GTP are abundant. Recent cryo-EM studies have revealed a non-canonical GPCR–G protein engagement mode rotated ~45° relative to the canonical state, raising the question of whether such alternative conformation represents an intermediate along the activation pathway. Here, we employed single-molecule Förster resonance energy transfer (smFRET) microscopy to monitor real-time conformational changes between site-specifically labeled receptors and G proteins reconstituted in lipid nanodiscs. By strategically placing fluorophores at multiple positions on both partners, we were able to map a coherent FRET-state landscape that reveals domain-wise rotational motions of the G protein relative to the receptor in the presence of GDP. We resolved three distinct FRET states consistent with the canonical, non-canonical, and a pre-engagement state with some further rotation. These states form a sequential transition pathway, with the non-canonical state as a kinetically important intermediate. Kinetic analyses show that the relative populations and transition rates between the canonical and non-canonical states correlate with ligand efficacy, G protein subtype preference (Gs vs. Gi) for β_2AR, and its higher signaling efficacy through Gs. Our findings define the sequence of molecular events during GPCR–G protein engagement and reveal transient intermediates that underline how GPCRs select and activate distinct G protein subtypes.

P-51 Elucidation of the Pharmacological Association between GPCRs and Phosphoinositides using Mass Spectrometry

Guan-Ting Lian, Academia Sinica, Taiwan

G protein-coupled receptors (GPCRs) convert ligand binding into intracellular signaling via conformational changes that recruit effectors such as G proteins and arrestins. Lipids, such as cholesterol and phospholipids, critically modulate receptor signaling, underscoring the importance of the membrane environment. However, given the heterogeneous compositions of membrane lipids, it remains essential to quantitatively assess the molecular factors that govern lipid interactions to advance our understanding of lipid-associated functions. Herein, we employed native mass spectrometry (native MS) to resolve the interactions between GPCRs and diverse phospholipids with high definition. By integrating a sequential binding model, we quantified lipid affinities as a function of binding stoichiometry. Our results revealed that both the acyl chain composition and phosphorylation pattern of phosphoinositides critically modulate their binding affinities. Intriguingly, several receptors, including the β-adrenergic receptor 1 (β_1AR), the Adenosine A2A receptor (A2AR), and the Cannabinoid receptor 1 (CB1), exhibit distinct lipid interaction profiles. Notably, these differences emerge when receptors are stabilized by ligands with diverse pharmacological effects, underscoring the strong link between receptor structural configuration and lipid interactions. To map lipid-binding contacts, we established a native top-down MS (nTDMS) approach. Through gas-phase fragmentation, we localized specific lipid contacts at the junction between transmembrane (TM) domain seven and helix 8, implicating this region as pivotal in regulating receptor activity. Collectively, our findings highlight the utility of native MS in elucidating membrane protein–lipid interactions and providing detailed mechanistic insight into their regulation.

P-52 Structure-guided allosteric modulation of the delta opioid receptor

Jesse I. Mobbs, Monash Institute of Pharmaceutical Sciences, Australia

Opioid analgesics remain essential for pain management but are associated with significant adverse effects, including respiratory depression, tolerance, and dependence. The δ-opioid receptor (δOR) represents a promising therapeutic target for developing safer opioid analgesics with reduced adverse effects compared to conventional μ-opioid receptor-targeting drugs. Positive allosteric modulators (PAMs) offer advantages over direct agonists by enhancing endogenous opioid signaling while preserving natural spatiotemporal activation patterns, potentially avoiding tolerance and dependence issues. Here, we present high-resolution cryo-EM structures of δOR complexed with the peptide agonist DADLE and the PAM MIPS3614, revealing a novel lipid-facing allosteric binding site formed by transmembrane helices 2, 3, and 4. MIPS3614 stabilizes the active receptor conformation through a critical hydrogen bond with residue N131$^{3.35}$ in the conserved sodium binding site, a key regulatory region controlling GPCR activation. Comprehensive mutagenesis, molecular dynamics simulations, and structure-activity relationships validate this proposed mechanism. Structure-guided optimization yielded MIPS3983 with enhanced binding affinity and retained cooperativity. Our findings establish the first molecular framework for δOR allosteric modulation and provide a structural foundation for the rational design of safer opioid therapeutics.

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