

Conformational Flexibility of GPCRs: A Structural Key to Functional Selectivity and Therapeutic Targeting

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Date of Submission: 15-10-2025

Date of Acceptance: 25-10-2025

ABSTRACT:

G protein-coupled receptors (GPCRs) form the largest super family of membrane proteins that transduce cellular responses to hormones, neurotransmitters, and sensory stimuli. They govern physiological processes, including those in the cardiovascular, neurological, and endocrine systems, and are a target for nearly 35% of FDA-approved drugs. GPCRs have been characterized historically as simple on-off switches. Still, we have come to realize that GPCRs can exist as dynamic ensembles of conformations that are flexible in character (i.e. conformational flexibility). GPCR conformational flexibility may enable functional selectivity or biased signaling, where a ligand stabilizes unique receptor conformations that activate the G protein or β -arrestins to different degrees, which could enable safer and efficacious therapeutics. GPCR conformations are governed by conserved structural motifs - DRY, NPxxY, W6.48 toggle switch. Advances in cryo-electron microscopy (cryo-EM), nuclear magnetic resonance (NMR), double electron-electron resonance (DEER), single-molecule fluorescence, molecular dynamics, and machine learning are beginning to illustrate the intricate conformational landscapes of GPCRs. These advancements will influence structure-based drug discovery through revealing structures for biased ligands and allosteric modulators that stabilize the favorable therapeutic states of the receptor.

Keywords: G protein-coupled receptors (GPCRs), Conformational flexibility, Biased signaling, Allosteric modulation, Drug discovery.

800 encoded by the human genome. They identify a range of ligands, including ions, small messenger molecules like hormones and neurotransmitters, and sensory stimuli (light and odorants). They regulate some of the most important physiological

systems, including cardiovascular regulation, metabolism, neuronal signaling, and immune response (Cary et al., 2023). Due to their significant role in human physiology, approximately 35 percent of all FDA-approved drugs target GPCRs (Congreve et al., 2020).

In general, GPCRs were traditionally considered using a two-state model whereby receptors shift between inactive and active states. Agonist binding identified the active state, while antagonist binding prevented activation, and inverse agonist binding promoted the inactive state (Hilger, 2021). However, this framework of binary states did not sufficiently account for partial agonism and biased signaling that had been observed experimentally.

Progress in structural biology methods like X-ray crystallography, cryo-electron microscopy (cryo-EM), and NMR spectroscopy has demonstrated that GPCRs display dynamic ensembles of conformations, not static on/off states (Dalton et al., 2015; Casiraghi et al., 2019). The ligands that interact with GPCRs preferentially stabilize certain receptor states, which can account for potential phenomena such as partial agonism and signaling bias. The β 2-adrenergic receptor (β 2AR) studies demonstrate how the presence of several conformations results in significantly different functional outcomes (Congreve et al., 2020).

I. INTRODUCTION:

G protein-coupled receptors (GPCRs) constitute the largest and most diverse family of receptors found on the cell surface, with more than

The nature of the conformational diversity is one of the contributing factors to functional selectivity or biased agonism, which occurs when ligands preferentially drive distinct intracellular signaling pathways, such as G protein, β -arrestin signaling, or other pathways (Wingler & Lefkowitz, 2020). The biased μ -opioid receptor

agonist TRV130 (Oliceridine), for example, activates G protein receptor signaling preferentially, which provides analgesia that is less likely to cause respiratory depression (Maharana et al., 2022).

At the molecular level, conserved "micro-switches" are integral to the receptor architecture that allow for conformational flexibility. These micro-switches can include the DRY motif (TM3), the NPxxY motif (TM7), the PIF motif, and the toggle switch (Trp6.48). The conformational alterations in these micro-switches result in transitions between distinct signaling states. For example, the outward movement of TM6 and the inward movement of TM7 indicate G-protein activation, but arrestin-biased signaling indicates a different type of rearrangement of TM7 and helix 8 (Dalton et al., 2015; Buyanov & Popov, 2024).

Lately, new experimental and computational techniques have begun to uncover dynamics for GPCRs. Cryo-EM provides structures for multiple signaling complexes; NMR or DEER spectroscopy generates conformational distributions (Casiraghi et al., 2019; Elgeti and Hubbell, 2021); and molecular dynamics or machine learning techniques illuminate activation pathways and expose hidden allosteric sites (Aranda-García et al., 2025).

The synthetic development of biased ligands and allosteric modulators facilitates a targeted pathway therapeutic approach, with increased signaling efficacy and decreased side effects (Casadó-Anguera & Casadó, 2022). This movement is being extended across various receptor families, including opioid, angiotensin, dopamine, chemokine, and GLP-1.

In summary, GPCRs are not considered simply as binary molecular switching systems; they are structurally dynamic, with signaling outcomes driven by structural plasticity. Combining structural, computational, and pharmacological considerations to maximize receptor bias and flexibility to enhance therapeutic efficacy.

II. STRUCTURAL BASIS OF GPCR CONFORMATIONAL FLEXIBILITY

G protein-coupled receptors (GPCRs) possess a well-conserved seven-transmembrane (7TM) helical architecture, but a distinct feature of GPCRs is their conformational flexibility, which permits ligand-specific signaling. GPCR architectural flexibility enables them to convert several external signals into specific internal

responses through G proteins, β -arrestins, and other effectors. To understand this flexibility, it is necessary to consider their prototypical architecture, conserved microswitch motifs, conformational states, and data generated from experimental and computational studies.

2.1 Canonical Architecture:

GPCRs have a seven α -helices (TM1-TM7) transmembrane domain and are joined together by extracellular loops (ECLs) and intracellular loops (ICLs). The extracellular portions (the N-terminus and ECLs) form the ligand-binding pocket, and the intracellular portion is involved with G proteins and β -arrestins (Cary et al., 2023). These proteins all follow a conserved scaffold, but GPCRs are organized into subfamilies that are structurally different: Class A (rhodopsin-like), Class B1 (secretin-like peptide receptors (e.g., GLP-1R), Class C (metabotropic glutamate receptors, which form dimers), and Class F (Frizzled/Smoothed receptors).¹ Activation typically incorporates conserved sequences that are structural motifs that act as molecular switches coupling ligand binding to intracellular signaling for all classes of GPCRs.

2.2 Conserved Microswitch Motifs:

GPCR activation is coordinated by several conserved motifs:

- DRY motif (TM3): Acts as an "ionic lock" stabilizing the inactive conformation that releases upon activation to permit G protein coupling (Dalton et al., 2015).
- Toggle switch (Trp6.48, TM6): Rotational movement of this amino acid causes outward movement of TM6, which is a hallmark of the activation process (Martí-Solano et al., 2016).
- PIF motif: Couples conformational switches across helices 3, 5, and 6.
- NPxxY motif (TM7): Interacts with helix 8 to stabilize both the active state and the receptor-arrestin-bound state (Sente et al., 2018).
- Sodium ion pocket: Present in several Class A GPCRs, it modulates ligand affinity as well as intra- and inter-subunit conformational equilibria (Agasid et al., 2021).
- These motifs work together as allosteric nodes, coupling the binding of orthosteric ligands with conformational changes.

Conserved micro switch motifs that relay ligand-binding information via coordinated helical rearrangements orchestrate GPCR activation. Table

1 summarizes major structural motifs and their functional significance in controlling receptor

conformational changes.

Table 1. Key Structural Motifs and Their Roles in GPCR Activation

S.No	Microswitch Structural Motif	Location (TM helix)	Structural Role in Activation	Representative Evidence / Study
1	DRY motif (Asp-Arg-Tyr)	TM3	Forms the “ionic lock” (R3.50–E6.30), stabilizing the inactive state; disruption triggers G-protein coupling.	Dalton et al. (2015); Hilger (2021)
2	Toggle switch (Trp 6.48)	TM6	Rotates upon agonist binding, initiating TM6 outward movement that defines the active state.	Martí-Solano et al. (2016)
3	PIF motif (Pro 5.50–Ile 3.40–Phe 6.44)	TM3-TM5- TM6	Couples ligand-binding pocket rearrangements to cytoplasmic opening.	Dalton et al. (2015)
4	NPxxY motif (Asn 7.49- Pro 7.50-Tyr 7.53)	TM7	Coordinates helix 8 and stabilizes active or arrestin-biased conformations.	Sente et al. (2018); Garcia-Nafria & Tate (2021)
5	Sodium ion binding pocket	TM2-3-7 core	Acts as an allosteric modulator that stabilizes inactive states and tunes ligand efficacy.	Agasid et al. (2021)
6	Hydrophobic lock / CWxP motif	TM6	Provides structural coupling between ligand binding and TM6 displacement.	Casiraghi et al. (2019)
7	Phosphorylation barcode	C-terminal tail / ICL3	Differential phosphorylation patterns regulate arrestin subtype engagement and biased signaling.	Latorraca et al. (2020)

III. FUNCTIONAL SELECTIVITY AND BIASED SIGNALING

The concept of functional selectivity, sometimes called biased signaling, has revolutionized GPCR pharmacology by demonstrating that different ligands interacting with the same receptor can preferentially activate different intracellular pathways. For many years, ligands were classified according to their intrinsic efficacy (full, partial agonists, antagonists, and inverse agonists) under the assumption that the receptor generated a single signalling output (Wingler & Lefkowitz, 2020). Biased signalling dismantles this simplistic view, and in fact, two completely opposing ligands acting on the same GPCR can produce completely opposing physiological outcomes. The difference is not due to higher/lower binding affinity of the ligand, but

rather the two ligands stabilize different receptor conformations that can then preferentially couple to different G proteins, β -arrestin, and other effectors. GPCRs therefore act as allosteric signal-processors where the ligand effect (signal transduction) is realised through conformational states (dynamic range) (Goricanec et al., 2016).

3.1 Molecular Basis of Biased Signaling

Functional selectivity occurs due to ligand-induced conformational ensembles. G protein-biased ligands select receptor conformations with outward TM6 displacement and an open cytoplasmic pocket to favor coupling to G proteins, while arrestin-biased ligands stabilize variations in conformations around TM7, helix 8, and ICL3 that favor engagement with β -arrestin (Maharana et al, 2022; Hilger, 2021). Some

ligands displayed "mixed bias"; they may activate both pathways.

A separate layer of regulation comes from the hypothesis of a phosphorylation barcode, which says that ligand-specific phosphorylation of the receptor in intracellular domains will drive distinct arrestin conformations and signaling outcomes (Latorraca et al, 2020). Allosteric modulators modulate bias through action at non-orthosteric binding sites: positive allosteric modulators (PAMs) enhance G protein signaling (e.g., in

mGluRs), while negative allosteric modulators (NAMs) push receptors into

inactive states or other signaling states (Casadó- Anguera & Casadó, 2022).

The use of GPCR conformational bias and allosteric modulation to devise ligands with enhanced selectivity and safety promises to reveal future insight. Table 2 highlights examples of biased and allosteric ligands, which demonstrate how conformational flexibility leads to therapeutic variation.

Table 2. Representative Examples of Biased or Allosteric GPCR Ligands and Therapeutic Implications

S.No	Receptor / Target	Ligand (Type)	Signaling Bias / Modulation	Therapeutic Outcome / Status	Reference
1	μ-Opioid receptor (MOR)	Oliceridine (TRV130) (G-protein-biased agonist)	Favors G-protein coupling; reduces β-arrestin recruitment.	Analgesia with reduced respiratory depression; FDA-approved 2020.	Maharana et al. (2022)
2	Angiotensin II Type 1 receptor (AT1R)	TRV027 (β-arrestin-biased agonist)	Promotes arrestin-mediated cardioprotective pathways.	Investigated in heart-failure trials; mixed clinical outcomes.	Sente et al. (2018)
3	β2-Adrenergic receptor (β2AR)	Carvedilol (biased β-blocker)	Induces arrestin-biased signaling while antagonizing G-protein activity.	Cardioprotection beyond classical β-blockade.	Schafer et al. (2016)
4	GLP-1 receptor (GLP-1R)	Exendin-P5 / Semaglutide analogues (biased agonists)	Bias toward cAMP pathway with minimal β-arrestin recruitment.	Enhanced insulin secretion; improved tolerability in diabetes.	Zhao et al. (2022)
5	Muscarinic M1 receptor	BQCA (Positive allosteric modulator)	Potentiates acetylcholine binding; pathway-selective enhancement.	Cognitive improvement in Alzheimer's models; preclinical.	Casadó-Anguera & Casadó (2022)
6	mGlu5 receptor	ADX47273 (Positive allosteric modulator)	Strengthens glutamate efficacy without direct activation.	Potential for schizophrenia and anxiety disorders.	Casadó-Anguera & Casadó (2022)
7	Chemokine receptor CCR5	Maraviroc (Negative allosteric modulator/antagonist)	Stabilizes inactive conformation, blocking HIV entry.	Approved for HIV therapy; model for allosteric inhibition.	Van Baelen et al. (2022)

3.2 Structural Insights

Structural determinants of biased signaling have been found through Cryo-EM and crystallography. In the μ-opioid receptor (MOR) variant, Oliceridine (TRV130) stabilized conformations biased towards G protein coupling, but blocked arrestin (Maharana et al., 2022). The angiotensin II type 1 receptor (AT1R) biased

ligands interact with arrestin binding but also limited G protein activation (Sente et al., 2018). The β2- adrenergic receptor (β2AR) biased ligands are shown to sample distinct conformations that correlate with the amount of pathway bias seen after G protein activation followed by arrestin recruitment (Garcia- Nafría & Tate, 2021). Using complementary NMR and single-molecule FRET

studies with biased ligands, the equilibria of the receptor are shown to tip towards certain conformational states.

3.3 Case Studies

At the MOR, G protein-biased ligands were developed, such as Oliceridine, to achieve analgesia without respiratory depression, although clinical responses were mixed for this class of compounds. At the AT1R, TRV027 provided proof-of-concept for arrestin-biased cardioprotective signaling, although no clinical studies progressed beyond early-phase studies (Sente et al., 2018). Within the family of β -blockers, drugs such as carvedilol have arrestin bias, suggesting that cardioprotective effects are accomplished through mechanisms unrelated to β -blockade (Schafer et al., 2016). Biased signaling has been reported for chemokine receptors, e.g., CCR5, using allosteric modulators aimed at proteins that mediate inflammation and cancers (Van Baelen et al., 2022), and there are GLP-1R biased agonists in the pipeline to mediate insulin release while not causing bias to desensitization (Zhao et al., 2022).

3.4 Therapeutic Relevance

Biased ligands are an important step to precision pharmacology, separating therapeutic efficacy from side effects. The major applications are MOR-biased agonists for safer analgesia, AT1R and β AR ligands for heart failure, GLP-1R agonists in diabetes and obesity, dopamine receptor ligands for neuropsychiatric disorders, and biased chemokine receptor modulators for inflammatory and oncologic diseases. There are some issues with quantifying bias across biological systems and translating cell-based findings into clinical outcomes (Hilger, 2021).

Functional selectivity stems directly from the conformational flexibility characteristic of GPCRs. Various ligands stabilize specific receptor conformations that bias signaling through G proteins, β -arrestins, or other effectors. Collectively, the information provided by structural, spectroscopic, and pharmacological techniques provides a strong basis for deriving biased agonism for therapeutics developed for pathway-selective GPCR therapy, which is considered to be an exciting new frontier of modern GPCR drug development.

Characterizing the conformational flexibility of GPCRs necessitates the use of methods that involve both static and dynamic states

being monitored. GPCRs are ensembles of interconverting conformers, and ligand, modulators, and intracellular partners will bias the equilibrium towards functional states. A combination of structural, spectroscopic, computational, and AI-driven methodologies has been implemented to describe this transition.

IV. STRUCTURAL DYNAMICS

4.1 Structural Biology Approaches

X-ray Crystallography: The initial structural studies of GPCRs, namely rhodopsin (2000) and β_2 -adrenergic receptor (β_2 AR, 2007), elucidated the canonical seven-transmembrane fold in the inactive and active states (Congreve et al., 2020). Crystallization generally stabilizes the receptor in only a limited number of conformations, allowing only a static view of protein structures, not the dynamic equilibrium that typically elucidates the mechanism of action.

Cryo-Electron Microscopy (Cryo-EM): Cryo-EM is a very robust method to visualize GPCR-G protein and GPCR-arrestin complexes in the absence of crystallization. Cryo-EM is able to visualize a receptor in multiple activation states in the presence of a binding partner. For example, a cryo-EM structure of μ -opioid receptor-G protein complexes reflects how biased ligand might alter receptor conformations to enable specific signaling (Yang et al. 2021).

Stabilization Methods: Engineering methods using nanobodies and fusion proteins can stabilize the transient structural states of GPCRs to facilitate the determination of structure. Nanobodies stabilize the receptor in an active or inactive state or can stabilize the receptor in an intermediate conformation resembling G-proteins/arrestin interaction (Laeremans et al., 2022).

4.2 Spectroscopy and Biophysical Techniques

Nuclear Magnetic Resonance (NMR): NMR can be used to observe conformational equilibria, and ligand-induced transitions from inactive to intermediate and active states. 19 F-NMR labeling is especially responsive to local structural changes of the receptor (Frei et al., 2020).

Double Electron-Electron Resonance (DEER): DEER detects distance measurements between spin-labeled sites and characterizes distributions of active-like and inactive conformations (Elgeti & Hubbell, 2021).

FRET/BRET Assays: FRET and BRET can both detect conformational change and signaling interactions within live cells. FRET sensors can detect movement of helices (Kauk & Hoffmann, 2018), and BRET biosensors can measure G protein activation and recruitment of β -arrestin with real-time detection approaches (Wright et al., 2024).

Single-Molecule FRET (smFRET): With the development of smFRET, it has become possible to monitor individual receptor molecules, which demonstrated that agonists speed transition between states, while biased ligands appeared to stabilize a unique intermediate (Vafabakhsh et al., 2015; Bostock et al., 2019).

4.3 Computational Approaches

Molecular Dynamics (MD) Simulations: MD is an atomic-level study of receptor activation, ligand efficacy, and the interactions between lipids and proteins that is, without a doubt, an indispensable technique (Alhadeff et al., 2018; Prasanna et al., 2016). One of the enhanced sampling techniques, Metadynamics, can reveal the rare transitions that are currently beyond the reach of experimental observation.

Coarse-Grained and Hybrid Models: By simplifying the modeling approach, the researchers can study GPCR dimerization, oligomerization, and membrane effects faster and for longer timescales (Gusach et al., 2020).

4.4 Artificial Intelligence and Machine Learning

AI and ML enhance both experimental and simulation data. Receptor active and inactive conformations could be predicted using a modified AlphaFold2 algorithm (Sala et al., 2023). Neural networks trained on MD trajectories predict receptor states and identify hidden allosteric sites (Buyanov & Popov, 2024; Aranda-García et al., 2025). ML in deep mutational scanning predicts what would happen functionally to the receptor, based on receptor variants (Jones et al., 2020).

4.5 Integrative Approaches and Summary

Hybrid strategies (e.g., cryo-EM plus MD and NMR verified by DEER) merge both static and dynamic information. Live-cell FRET studies also enable assessment of the structure-function relationship. Collectively, all these approaches provide a multi-dimensional understanding of GPCR activity. Crystallography and cryo-EM contribute to an atomic understanding;

spectroscopy and fluorescence provide information on real-time dynamics; and computation-supported AI will allow us to visualize conformational landscapes. This integrated approach enhances our ability to map GPCR flexibility precisely and will facilitate and inform the rational design of pathway-selective, biased therapeutics.

V. THERAPEUTIC IMPLICATIONS AND DRUG DISCOVERY

G protein-coupled receptors (GPCRs) are the most extensively targeted proteins in pharmacology, with nearly 35% of FDA-approved drugs acting through them.² Traditional drug discovery focused on orthosteric ligands—agonists, antagonists, or inverse agonists—designed to switch receptors “on” or “off.” The recognition of GPCR conformational flexibility and functional selectivity has transformed this paradigm toward designing biased ligands, allosteric modulators, and multi-target drugs that precisely stabilize desired receptor states.

5.1 From Classical to Modern GPCR Pharmacology

Traditional models considered ligand efficacy to be a matter of stabilization of the active state of the target receptor, which has led to drugs such as β -blockers and opioids. More sophisticated models have changed the view of GPCRs as a variety of conformations, with ligands biasing signaling toward certain intracellular pathways (Hilger, 2021). Functional selectivity provides the possibility to have ligands that have therapeutic efficacy, while at the same time, down-regulating the chance of deleterious side effects (Wingler & Lefkowitz, 2020). Allosteric modulators can also interact with an alternative binding site, thus allowing control of a modulator that is more specific (Cary et al., 2023).

5.2 Biased Ligands in Therapy

μ -Opioid Receptor (MOR): Traditional opioids act on both G protein and β -arrestin pathways, produce analgesia, but often cause significant adverse effects. The biased agonist Oliceridine (TRV130) activates for analgesia G protein signaling while less β -arrestin pathway is engaged, and less respiratory depression, but clinical data were mixed (Maharana et al. 2022).

AT1 Receptor: TRV027 resulted in biased activation promoting β -arrestin-mediated cardioprotective signaling while reducing vasoconstrictive G protein activation. Clinical trials

in acute heart failure were less than definitive, but confirmed the concept of biased signaling (Sente et al. 2018).

β -Adrenergic Receptors: Carvedilol (a β -blocker) was later shown to have bias in the β -arrestin signaling pathway, and produced independent cardioprotective effects specifically beyond β -antagonism (Schafer et al., 2016).

GLP-1 Receptor: Biased agonists produce an insulinotropic signal and minimize desensitization and nausea seen with GLP-1 agonists and thus help individuals manage diabetes (Zhao et al. 2022).

5.3 Allosteric Modulators

Allosteric modulators interact with sites distant from the orthosteric pocket and modulate signaling without competing with an endogenous ligand at the orthosteric binding site. By definition, PAMs, to indicate positive allosteric modulation, generally enhance receptor function, and NAMs generally reduce receptor function. SAMs, indicated as negative allosteric modulation, suppress the modulation of the allosteric site and therefore suppress the receptor intrinsic activity. Allosteric regulators have many benefits, including improved selectivity, saturable effects, and possible bias in signaling (Casadó-Anguera & Casadó, 2022). Examples of potential therapeutics are mGluR PAMs for the treatment of neurodegenerative disorders, or muscarinic receptor NAMs for the treatment of Parkinson's disease.

5.4 Polypharmacology and GPCR Complexes

GPCRs can be either homo- or heterodimers, influencing the signaling profiles. GABAB receptors are heterodimers, as are dopamine-adenosine receptor complexes that modulate neuropsychiatric circuits (Gusach et al., 2020). Therapeutics that selectively target these receptors assemblies may produce more efficacious polypharmacological effects.

5.5 Orphan GPCRs and Future Targets

More than 140 orphan GPCRs have no known ligands, indicating a promising therapeutic target for neurodegeneration, oncology, and immunity. GPR52 (schizophrenia, Huntington's disease) and GPR40 (metabolic disease) are two examples of orphan GPCRs. There are now significant advances underway to identify ligands for orphan GPCRs through artificial intelligence modeling, large-scale mutational studies, and new

avenues in cell biology (Aranda-García et al., 2025).

5.6 Challenges

It is still challenging for biased signaling to be brought into the clinic. Not only may bias change between the cellular context in which it is studied or how it has been assayed, but also many biased ligands will exhibit suboptimal pharmacokinetics. Integrated approaches with cryo-EM, molecular dynamics, and AI-based prediction approaches have now started informing structure-based design of biased ligands, bitopic modulators, and personalized pharmacology (Buyanov & Popov, 2024; Jones et al., 2020).

Modern GPCR drug discovery considers the conformational diversity of GPCRs in order to design ligands that modulate biased signaling exquisitely, as embodied by the biased agonists and allosteric modulators. While biased agonism and allosteric modulation suggest improved efficacy and decreased toxicity, they represent a transition from one-dimensional pharmacology to conformationally dynamic, structure-based therapeutic

VI. FUTURE PERSPECTIVES

The recognition of GPCR conformational flexibility as the basis of functional selectivity has altered the landscape of modern pharmacology. Nevertheless, many hurdles and possibilities remain to fully translate structural and mechanistic insights into usable therapies in the clinic.

6.1 Merging Structural Biology with Pharmacology

Recent advances in technology, specifically cryo-EM, NMR, and spectroscopic methods, have afforded novel insights into the many conformations of GPCRs and the diverse signaling complexes GPCRs can adopt. However, static structures alone cannot mythologize the dynamic equilibria that underlie biased signaling. The future combines structural biology-atomic snapshots, live-cell sensors to monitor signaling in real time, and computational modeling of energy landscapes and allosteric pathways, in a single framework. An integrative framework would afford the rational design of ligands to modulate receptor states and connect molecular mechanisms to pathway physiology (Yang et al., 2021; Hilger, 2021).

6.2 Expanding Therapeutic Space through Biased Signaling

Biased signaling presents an unprecedented opportunity to treat therapeutic efficacy separately from undesired side effects. Rationally developed biased ligands can selectively activate beneficial pathways while suppressing pathways leading to unwanted outcomes. Potential high-impact uses of biased signaling include:

- **Neurological disorders:** Inappropriate dopamine receptor–biased ligands that reduced the unwanted motor side effects of the antipsychotic drugs.
- **Metabolic diseases:** GLP-1 and GIP receptor agonists, which can increase insulin secretion without unwanted gastrointestinal side effects.
- **Immunology and oncology:** biased chemokine receptor modulators that can reduce unwanted inflammation and even reduce tumor metastasis.

Until structural biology studies consistently produce high-resolution structures, the field of drug discovery is expected to switch away from an empirical trial-and-error approach to that of engineered bias: purposely designing ligands to achieve predetermined conformational and functional results (Casadó-Anguera & Casadó, 2022).

6.3 Allosteric and Bitopic Ligands

Receptor domains outside the orthosteric site will increasingly be dabbled during drug discovery. Allosteric modulators affect receptor functionality by stabilizing distinct receptor conformations, and bitopic ligands will target both the orthosteric and allosteric sites. These newer hybrid molecules will allow pathway- or tissue-selective pharmacological profiles that provide better safety and predictability of response. Continuous improvement of cryogenic electron microscopy (cryo-EM) and molecular dynamics allows for continued mechanisms of allostery to identify new allosteric pockets and cooperative modulation mechanisms of action (Gusach et al., 2020).

6.4 Artificial Intelligence and Personalized Pharmacology

Artificial intelligence (AI) is proving to be an increasingly significant aspect of GPCR studies. Machine learning models developed on structural and pharmacological datasets are capable of predicting bias at the ligand level and classifying

conformational states (Buyanov & Popov, 2024; Aranda-García et al., 2025). Integrating pharmacogenomic data will help facilitate personalized GPCR pharmacology by providing patient-specific receptor variants for use in selecting and prescribing medications (Jones et al., 2020). In the near term, AI-powered modeling may contribute to the rational design of bias-tuned therapeutics for orphan GPCRs or GPCRs with more difficult-to-characterize functions.

6.5 Remaining Challenges

Translating biased signaling into clinical relevance is an ongoing challenge. Cellular bias often does not translate to in vivo efficacy, as has been shown with Oliceridine and TRV027 (Maharana et al., 2022; Sente et al., 2018). Factors at the system-level, like receptor density, the expression of effectors, and tissue context, make it hard to interpret. Additionally, many functionally relevant states are short-lived and escape detection with existing methods.

Conformational landscapes of receptors will drive the design of bias-driven, allosterically tuned, and patient-personalized medications. GPCRs have progressed from binary switches to allosteric machines that present conformational diversity central to next-generation drug discovery.

VII. CONCLUSION

G protein–coupled receptors (GPCRs) are multifunctional signaling molecules that convert external stimuli into specific cellular responses. While they were traditionally thought of as being in a simple two-state model, GPCRs should now be considered to consist of dynamic conformations, which are steered in bias by ligand binding and binding partners to produce unique signaling responses. This conformational flexibility is the structural basis of functional selectivity, meaning that even though a ligand can bias beneficial pathways, the effects of side effects may be lessened.

Biased ligands and allosteric modulators provide specific examples of functional selectivity (e.g., Oliceridine (μ -opioid receptor), TRV027 (angiotensin II receptor), biased GLP-1R agonist (diabetes)). Advances in cryo-EM, spectroscopy, molecular dynamics, and AI have improved our understanding of the receptor family by allowing individual receptor states and allosteric networks to be visualized and predicted.

Yet, converting cellular bias to clinical utility is difficult due to system-level properties

that include receptor density, effector expression, and tissue context. Ultimately, mitigating these limiting properties will require standardized quantification, systems pharmacology, and personalized approaches.

We now think of GPCRs as allosteric machinery that is dynamic rather than a static switch. Their inherent conformational plasticity is responsible for their therapeutic utility, allowing us to create the next generation of precision drugs that will improve efficacy, safety, and specificity of drugs. Ultimately, this will launch GPCR pharmacology into a new era of rational drug discovery.

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