https://doi.org/10.1038/s44386-025-00011-8

# Intracellular GPCR modulators enable precision pharmacology



Brian E. Krumm[1] & Bryan L. Roth[1,2] ✉

G-protein-coupled receptors (GPCRs) have proven to be the most successful target class for drug discovery but their complicated signal transduction pathways cause difficulties for drug development. Recently, ligands have been identified that engage an intracellular binding site which promotes pathway biased signal in cooperation with orthosteric ligands. Here, we explore the topic of biased signaling and intracellular modulators to understand their application for precision pharmacology of Class A or Rhodopsin-Like GPCRs.

G-protein-coupled receptors (GPCRs) are a large and diverse family of cell surface receptors that play critical roles in transmitting extracellular signals to the interior of the cell. GPCRs represent one of the largest protein families in the human genome, with over 800 different GPCRs identified in humans, and are involved in regulating processes such as neurotransmission, hormone signaling, immune responses, and sensory perception. GPCRs are categorized into six classes based on sequence and function, namely Class A are rhodopsin-like receptors, Class B is the secretin family, Class C are the metabotropic glutamate receptors, Class D are the fungal mating pheromone receptors, Class E are cAMP receptors, and Class F are frizzled (FZD) and smoothened (SMO) receptors[1]. Similarly, vertebrate GPCRs have been grouped into what is referred to as GRAFS system of classification that groups GPCRs into five main families - **G**lutamate, **R**hodopsin, **A**dhesion, **F**rizzled/Taste2, and **S**ecretin[2]. The largest class of receptors is the Class A Rhodopson-Like receptors and will be the predominant focus of this perspective.

In part, due to their ubiquitous nature, GPCRs have been implicated in numerous diseases, including heart disease, cancer, neurological disorders, and infectious diseases, making them important therapeutic targets. Indeed, approximately 30% of all FDA approved medications target GPCRs[3]. In contrast to the current "one-size-fits-all" approach for disease treatment and prevention along with the underlying research and development of approved drugs, precision medicine would allow doctors and researchers to predict more accurately which treatment and potential prevention strategies for a particular disease will work in a subset group of individuals. While pharmacogenomics, the study of how genes affect an individual's response to a given drug, is a part of precision medicine and combines pharmacology and genomics to develop effective, safe medications and doses that are tailored to variations in a person's genes[4]. In this perspective, we will explore and discuss the topic of biased signaling and intracellular modulators to understand their application in precision medicine and the pharmacology of Class A or Rhodopsin-Like GPCRs but at times will discuss GPCRs of other classes to bring about a given topic.

## Canonical Class A GPCR Signaling

As transmembrane proteins, the intracellular portions of activated GPCRs couple to not only heterotrimeric G proteins but can also couple to regulatory and scaffolding proteins such as arrestins, PDZ-domain-containing scaffolds and non-PDZ scaffolds, that initiate or control distinct patterns of signaling[5]. GPCR canonical signaling involves the coupling of an activated GPCR to heterotrimeric G proteins that hydrolyze guanosine 5′-triphosphate (GTP) initiating downstream signaling with subsequent receptor phosphorylation by GRK(s) and the recruitment of β-arrestin(s) (discussed below) (Fig. 1)[6]. Heterotrimeric G proteins are comprised of three subunits: Gα, Gβ, and Gγ. When a GPCR is activated, G-protein Gα subunit dissociates from Gβγ subunit, allowing both subunits to perform their respective downstream signaling effects. Furthermore, G-protein Gα subunits are divided into four main subgroups: Gαs (GNAS/GNAL), Gαi/o (GNAI/GNAO), Gαq/11 (GNAQ/GNA11), and Gα12/13(GNA12/GNA13)[7]. Depending on the type of Gα subunit of the heterotrimeric G protein that a given GPCR couples, activation can lead to secondary messengers, such as inositol triphosphates (Gαq) production or cyclic adenosine monophosphate (cAMP) stimulation (Gαs) or inhibition (Gαi/o). G-protein Gβγ family consists of 5 Gβ and 12 Gγ isoform members. Upon release from G-protein Gα subunit, free Gβγ interacts and activates a diverse range of signaling regulators including kinases, lipases, GTPases, and ion channels. Gβγ family consists of 48 members, which show cell- and tissue-specific expressions, and recent reports show that cells employ the subtype diversity in Gβγ to achieve desired signaling outcomes[8,9].

Intracellular signaling via G proteins can be termed G protein-dependent signaling versus G protein-independent signaling (discussed below)[6]. G protein-dependent signaling can be modulated by accessory

[1]Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA. [2]National Institute of Mental Health Psychoactive Drug Screening Program (NIMH PDSP), School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
✉e-mail: bryan_roth@med.unc.edu

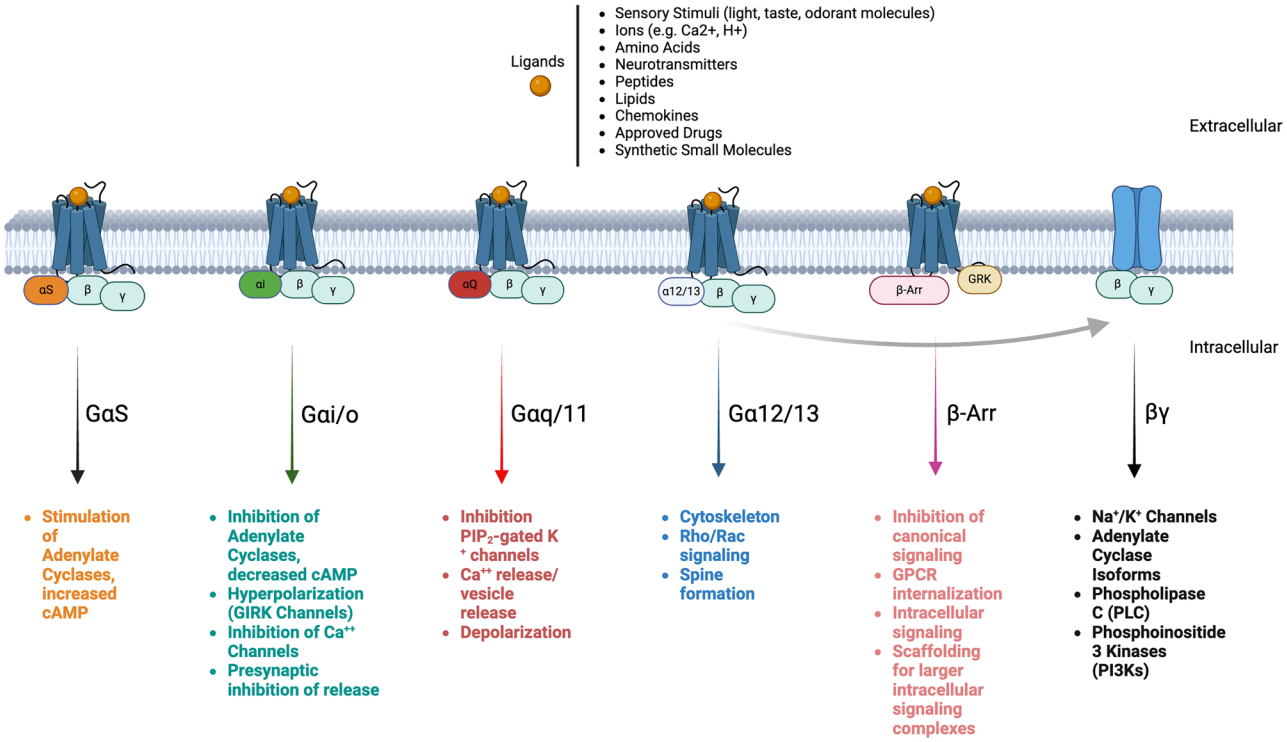


**Fig. 1 | Canonical Class A G Protein Signaling Pathways - Schematic diagram depicting the major Class A canonical GPCR signaling transduction pathways.** Upon GPCR stimulation with extracellular ligand, Gαs coupled receptors activate adenylyl cyclase (AC) resulting in an increase of cAMP; GαI coupled receptors inhibit AC, and the subunits activate ERK pathway; GαQ coupled receptors activate phospholipase C (PLC) to produce inositol trisphosphate (IP3) and diacylglycerol (DAG), which in turn increases intracellular calcium concentration ($Ca^{++}$); Gα12/13 coupled receptors activate small G protein RhoA GTPase. GPCR can also be phosphorylated by GRKs and in turn couple to β-arrestins resulting in desensitization and internalization. Upon liberation from heterotrimeric G protein complex, the βγ complex can in turn interact and regulate ion channels, PLC and PI3Ks. Figure was created with BioRender.com. Krumm, B. (2025) https://BioRender.com/x39b767.

proteins that alter and or enhance G protein activity. These accessory proteins include but not limited to activators of G protein signaling (AGS) and regulators of G protein signaling (RGS) proteins. Members of these families may contain GTPase-activating protein (GAP), guanine nucleotide exchange factor (GEF) or guanine nucleotide dissociation inhibitor (GDI) activities[10]. GEFs affects G protein activity by increasing the rate of GTP association with Gα subunits, whereas GDI-containing accessory proteins act to inhibit the dissociation of GDP from Gα subunits, inhibiting Gα protein-mediated signaling. RGS proteins that contain GAP activity accelerate GTPase activity of Gα subunits, which in turn decreases the amplitude and duration of Gα protein-mediated signaling[10–12].

Other intracellular effector proteins can bind to GPCRs and produce signal transduction pathways typically referred to as G protein-independent signaling as they proceed via transduction pathways different from G protein-dependent signaling pathways. The most widely recognized G protein-independent signaling involves β-arrestin dependent signaling pathway[13]. Upon the activation of GPCRs, a family of protein kinases called G-protein-coupled receptor kinases (GRKs) phosphorylate intracellular serine and threonine residues of GPCRs in what is referred to as phosphorylation barcoding[14]. Phosphorylated GPCRs then recruit β-arrestins, which often mediate the desensitization of GPCR signaling, internalization of GPCRs and therefore serve as negative feedback of G-protein-dependent GPCR signaling. It is also thought that variations of phosphorylation barcodes act as a molecular "code" that regulates receptor behavior, including desensitization, internalization, and signaling specificity which give rise to specific downstream signaling events[14–17]. Additionally, atypical chemokine receptors (ACKRs) bind with high affinity to chemokines but do not couple to any known G proteins, preferentially coupling to β-arrestins and thus signal via G protein-independent signaling pathways[18–20].

GPCRs can form functional complexes not only with signal transduction proteins such as heterotrimeric G proteins, β-arrestins, and GRKs but also adapter proteins and molecules to fine tune an intracellular message that is often contextually different depending on the orthosteric ligand and the cellular milieu. Although targeting the orthosteric site has been successful for many GPCRs[21–25], the conserved nature of the orthosteric site between GPCR subtypes renders the task of precisely design subtype-selective drugs difficult. Recently, a new sub-type of allosteric modulators has been identified along with their GPCR complex structures determined. These so called intracellular biased allosteric modulators (BAMs) selectively activate G protein or β-arrestin transduction pathways, allowing for the precise targeting of desired pathways while avoiding undesirable signaling events.

## GPCR Biased Signaling

Agonist binding to a given GPCR's orthosteric site promotes the stabilization of conformations that facilitate coupling to signal transducers which in turn elicit second messenger signaling, kinase cascade activation, and ion channel modulation, as discussed above. In some cases, such as with the binding of endogenous dopamine and partial agonist SKF-83953 to the dopamine D1R GPCR, the signaling cascade appears to be balanced between G protein signaling and β-arrestin signaling or recruitment, thus not favoring or biasing one signaling transduction event over another (Fig. 2)[26].

GPCR biased signaling also known as functional selectivity[27] refers to the phenomenon where a given ligand (such as a drug or endogenous molecule) binds to a given GPCR's orthosteric site and preferentially activates certain downstream signaling pathways. This bias occurs even though the ligand may trigger seemingly similar conformational changes in the receptor comparable to neutral or unbiased ligands. Ultimately, however,

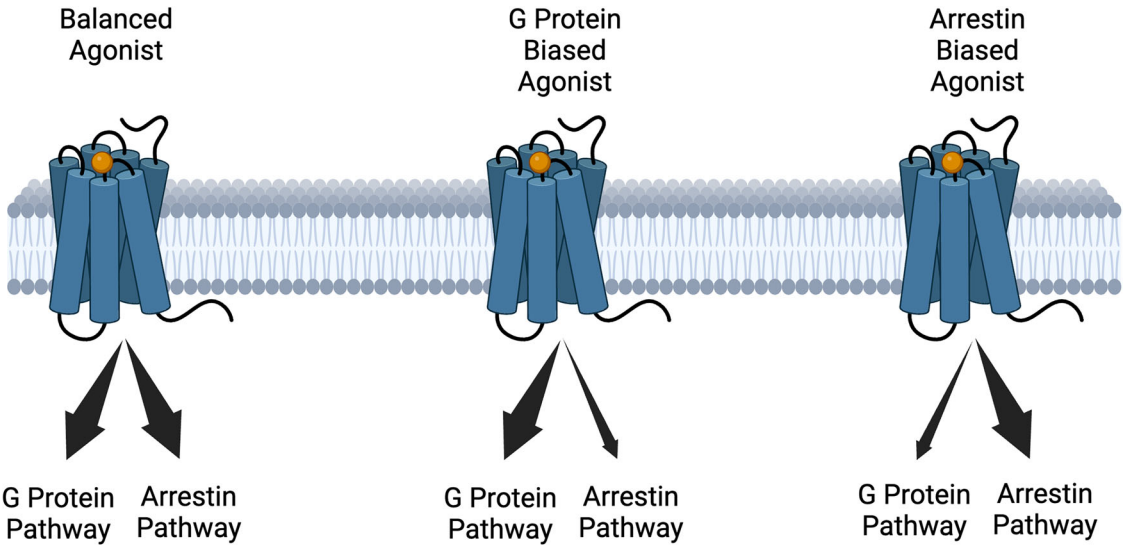


**Fig. 2 | Orthosteric Biased Agonism - Schematic diagram depicting GPCR biased signaling.** (Left) Balanced agonists activate both the G protein and β-arrestin signaling pathways. (Middle) G protein biased agonists preferentially activate G protein signaling pathway(s). Sustained G protein signaling can affect cellular responses through second messenger signaling. (Right) β-arrestin biased agonists preferentially activate β-arrestin signaling pathways leading to distinct physiological outcomes. Figure was created with BioRender.com. Krumm, B. (2025) https://BioRender.com/u39j412.

the ligand selectively activates one signaling pathway over another while minimizing or preventing activation of other pathways associated with the given GPCR[28,29]. The structural basis for ligand bias and G protein selectivity of multiple GPCRs has been revealed in combination with molecular dynamic and mutagenesis using known biased ligands that have been identified via functional assays. These include (but not limited to) β1AR[30] and β2AR[31], D2 dopamine receptor (D2R)[32], κ- and μ-opioid receptors[23,33–35], 5-HT1B, 5-HT2A[36], and 5-HT2B receptors[37]. Despite the abundance of data, there does not appear to be a conserved mechanism of ligand bias between orthosteric pockets of different GPCR families but some degree of conservation of ligand bias exist within the same family, as could be expected. It is also worth noting that there is a degree of conserved G protein selectivity within the conserved residues of the intracellular ICL2[36,38]. Additionally, in the GPCR intracellular, a study of β2AR phosphorylation patterns by individual GRKs, demonstrated that the β-arrestin-biased ligand (carvedilol) induced a phosphorylation pattern that was distinct from that of isoproterenol, an unbiased full agonist. This suggests that biased ligands can recruit distinct GRKs and produce a distinct phosphorylation pattern[14,28].

When the effects of a GPCR orthosteric agonist on different cellular pathways reveal differences in the activation of signaling pathways that are not due to observational or system bias[39], this can be attributed to ligand bias. Different intracellular signaling pathways have various sensitivities to agonists, such that ligand bias must be detected and quantified by comparing the activity of agonists within one assay to a selected standard agonist (in order to remove observational bias and system bias). The relative activity of each agonist in one assay can then be compared to its relative activity in other assays (compared to the same reference agonist in each assay) to yield a relative activity ratio that corrects for system bias and observational bias and can be used across assays to detect true ligand bias between signaling pathways[39].

Additional observational and system bias can be removed from the investigation by having comparable assay formats and conditions. For example, assays such as cAMP production (Glosensor)[40] and Presto-Tango[41] are amplified messenger assays. The GloSensor cAMP biosensor (Promega) uses a modified form of firefly luciferase containing a cAMP-binding motif. Upon cAMP binding, a conformational change leads to enzyme complementation followed by incubation with a luciferase substrate results in a luminescence readout[42]. In the Presto-Tango assay, the C-terminus of the GPCR of interest is fused with the C-terminal tail of the V2 vasopressin receptor followed by a Tobacco Etch Virus (TEV) protease cleavage site and a tTA transcription factor. Ligand stimulation of the GPCR of interest results in the recruitment of a β-arrestin - TEV fusion protein to the receptor which results in the cleavage of tTA from the receptor. The tTA can then translocate to the nucleus, in which it transcribes a stably expressing luciferase reporter gene followed by incubation with a luciferase substrate results in a luminescence readout[41]. However, other assays formats are available to measure GPCR activity including assays such as Pathhunter, calcium flux, Inositol trisphosphate (IP3) measurement to name a few[43].

For bias factor calculations, the mean efficacy (Emax) and potency (EC50) are obtained from at least three independent concentration-responses for all agonists in cAMP and β-arrestin recruitment assays. We prefer to use the bias factor calculation and formulas as recommended by Kenakin (2017)[44]. Briefly, Emax and EC50 values for each agonist are entered into the log(Emax/EC50) equation for both cAMP or Tango β-arrestin recruitment assays and then subtracted from the log(Emax/EC50) for the reference agonist, to obtain a Δlog(Emax/EC50). To obtain a ΔΔlog(Emax/EC50), the $\Delta\log(Emax/EC50)^{\beta\text{-arrestin}}$ is subtracted from $\Delta\log(Emax/EC50)^{cAMP}$. The inverse log of ΔΔlog(Emax/EC50) is the G protein bias factor. The β-arrestin bias factor is obtained by subtracting the $\Delta\log(Emax/EC50)^{cAMP}$ from $\Delta\log(Emax/EC50)^{\beta\text{-arrestin}}$ to calculate the ΔΔlog(Emax/EC50). The β-arrestin bias factor is the inverse of ΔΔlog(Emax/EC50). Compounds with values close to one represent unbiased agonists while compounds with large numerical values, typically >100, represent extremely biased agonists.

GPCR biased signaling holds significant therapeutic potential by allowing for the development of drugs that preferentially activate specific signaling pathways which could minimize unwanted side effects or enhance therapeutic activity[45]. This approach is particularly valuable in conditions where GPCRs mediate complex and diverse cellular responses, such as in pain management, cardiovascular diseases, and neurological disorders[21]. By selectively modulating G-protein versus β-arrestin pathways, biased agonists can reduce adverse effects like desensitization, tolerance, or off-target toxicity, which are common challenges in traditional GPCR-targeted therapies[21]. For example, biased agonists at the μ-opioid receptor (MOR), such as Oliceridine (TRV130), preferentially activate G-protein-mediated analgesia while minimizing β-arrestin-driven side effects like respiratory depression[46–48]. Conversely, carvedilol, a nonsubtype-selective ß-adrenergic receptor (ßAR) antagonist and an approved drug for the treatment of heart failure, was later discovered to be an arrestin biased signaling compound at the ß2AR[49]. However, other biased drug candidates have not fared as well

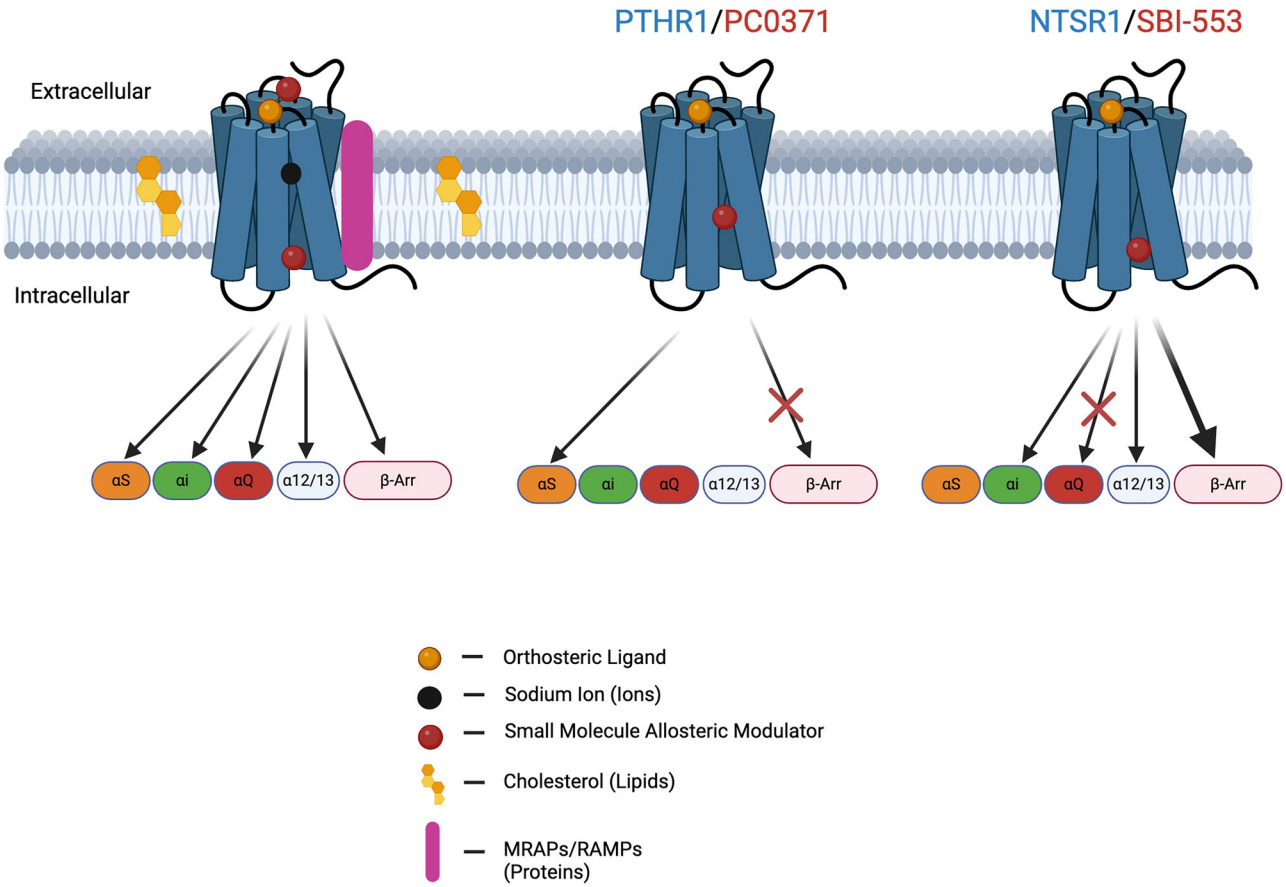


**Fig. 3 | GPCR Allosterism - Schematic diagram depicting GPCR allosterism and the effects of recent intracellular biased allosteric modulators (BAMs) on GPCR signaling.** (Left) Overview of GPCR allosterism in which a receptor's activity can be modulated by molecules such as ions, small molecules, cholesterol (lipids) and MRAPs/RAMPs. (Middle) Parathyroid Hormone Receptor 1 (PTHR1) in complex with intracellular biased allosteric modulator PC0371. In vitro assays revealed PC0371 blocked β-arrestin recruitment biasing PTHR1 towards G protein signaling. (Right) Neurotensin Receptor 1 (NTSR1) in complex with intracellular biased allosteric modulator SBI-553. In vitro assays revealed SBI-553 blocked Gαq signaling, potentiated β-arrestin recruitment biasing NTSR1 towards β-arrestin. Note - absent directional arrows indicate this pathway was not tested. Figure was created with BioRender.com. Krumm, B. (2025) https://BioRender.com/a15y277.

with biased ligands for the AT2-angiotensin receptor have been studied in Phase 2 trials humans but were not advanced to Phase 3[50,51]. It is worth noting that bias is context dependent and observed bias in one format or assay may not translate bias in an endogenous setting due to unforeseen variables outside the scope of this perspective.

## GPCR Allosterism

GPCR allosterism refers to the condition wherein an allosteric molecule interacts with a site distinct from the orthosteric GPCR binding site thereby modulating the activity of the orthosteric ligand (Fig. 3)[52]. Such allosterism can lead to a variety of outcomes, including changes in signaling pathways, shifts in ligand binding properties, or alterations in receptor desensitization and internalization[53–56]. Thus, "allosteric regulation" occurs when the binding of an effector at one site on the receptor influences the receptor's activity at another site. Thus, allosteric modulators can remotely regulate GPCRs and regulate their signal transduction pathways, offering novel strategies for the development of GPCR-targeted drugs[57,58].

Allosteric modulators can be divided into three principal categories depending on their pharmacological properties or effects on GPCR signaling[59]. Positive Allosteric Modulators (PAMs) are modulators that enhance the receptor's response to the orthosteric ligand. PAMs can increase the receptor's affinity for the orthosteric ligand or enhance the efficacy of signal transduction. Negative Allosteric Modulators (NAMs) are modulators that reduce the receptor's response to the orthosteric ligand, either by decreasing its binding affinity or by inhibiting the signaling cascade. Neutral Allosteric Modulators are modulators that do not have a positive or negative regulatory effect (neutral effect) on signal transduction of the receptor after binding to the allosteric site but can act as "silent" modulators that prevent other allosteric modulators (PAMs or NAMs) from binding and exerting their effects on the receptor. Additionally, some allosteric modulators exhibit intrinsic agonism (known as ago-PAMs) or inverse agonist profiles when tested in the absence of GPCR orthosteric ligand. Notably, an allosteric modulator's effects can depend on the orthosteric ligand, and the receptor signaling pathway and is therefore deemed to be probe-dependent[60].

Typically, three-independent dose-response assays for the known agonist and allosteric modulator of interest are obtained. As it is not usually known the effect the modulator will have on different transduction pathways, we typically employ BRET β-arrestin recruitment and G protein activation assays as a starting point[61–63]. Empirically it should be determined if pre-incubation with the allosteric modulator is necessary to achieve a significant response over the no allosteric modulator control. The dose-response curves are normalized to the 'no allosteric modulator' control and then fit to the Operational Model for Allosterism[64]. At this point, allosterism should be obvious when the dose-response curves are plotted on the same graph. An excellent review of the derivation of the Operational Model for Allosterism model parameters can be found in ref. 65.

## GPCR Allosteric Modulators

Many GPCRs have been shown to be modulated by ions such as sodium, magnesium, manganese, and zinc, of which the most studied of these ions being the effects of sodium ions on GPCR ligand binding[66]. It was first

suggested that sodium ions could decrease opioid receptor agonists binding while having relatively little effect on opioid receptor antagonists[67]. Subsequent studies suggested that the sodium ion effect was likely due to sodium-induced conformational changes of the receptor[68]. Mutagenesis studies further identified a highly conserved aspartic acid residue, $D^{2.50}$ (Ballesteros-Weinstein designation) in the second transmembrane helix of class A GPCRs as being key to the putative allosteric sodium site[69]. Subsequently, the structural basis for sodium ion allosterism of Class A GPCRs was shown in the high-resolution inactive state crystal structure of the delta opioid receptor[70]. The crystal structure revealed a highly coordinated sodium ion and water network in the transmembrane bundle in which the sodium ion allosteric site appears to collapse in active-state structures, suggesting that the allosteric site and sodium ions play key roles in constraining Class A GPCRs in an inactive state[66]. Furthermore, studies on D4 dopamine receptors indicated that the sodium site is essentially pre-formed in the inactive state[71].

The melanocortin receptor accessory protein (MRAP) family comprises two accessory proteins (MRAP1 and MRAP2) that generate distinct phenotypes by regulating different melanocortin receptors in vivo. As a single transmembrane protein, MRAP1 and MRAP2 both interact with all five melanocortin receptors (MC1R–MC5R) and modulate their cell surface expression and ligand-responsive properties[72]. Recent data also suggests that MRAP1 and MRAP2 can interact and alter the surface expression and pharmacological profile of other metabolic-related GPCRs[73].

Receptor Activity-Modifying Proteins (RAMPs) are a three-member family of single-pass transmembrane proteins that act as protein allosteric modulators for certain GPCRs, particularly the Class B calcitonin receptor-like receptor (CLR) and calcitonin receptor (CTR) and other receptor families of Class B GPCRs[74,75]. Although first identified in Class B GPCRs, recent data suggest that RAMP allosterism on GPCR activity can also be found in other GPCR classes[76]. MRAPS and RAMPs influence receptor conformation and signal transduction pathways at sites distinct from the orthosteric ligand-binding site, making family members of these accessory proteins GPCR allosteric regulators.

Recent Cryo-EM structures of GPCR:transducer complexes has revealed many GPCR with both bound lipids and cholesterol. Given that GPCRs are integral transmembrane proteins, it is not surprising that the lipid bilayer can have an influence on a given GPCR's behavior. Probably the best studied example of how a GPCR can be modulated by a lipid is through its interaction with cholesterol[77–79]. In the crystal structure of β2-AR, in which two cholesterol molecules were identified[80] a cholesterol consensus motif (CCM) was postulated for the β2-AR and for many other GPCRs. Although the extent to which the CCM site affects cholesterol binding, and the modulation of the receptor has not been completely explored. Given, increases in GPRC agonist affinity with increased membrane cholesterol content is not always observed for all GPCRs.

When one thinks of allosteric modulators it is typically in the context of small molecules that bind at a site different from the orthosteric site which affects the receptor's signal transduction. To date, there are numerous GPCRs with at least one identified small molecule allosteric modulators[81]. Structurally, allosteric modulators have been shown bind to multiple locations on GPCRs including the extracellular vestibule, transmembrane regions, and intracellular region[82–84].

Intracellular allosteric modulators that have recently been determined via Cryo-EM which may have actions similar to so-called molecular glues[85]. Molecular glues differ from other known allosteric modulators in that they cause apparent allosteric modulation by binding to both the GPCR and the transducer simultaneously, other known GPCR intracellular allosteric modulators bind only to the receptor[85]. For example, 2-PCCA is a synthetic ago-allosteric modulator of the orphan receptor GPR88. 2-PCCA binds to the exterior of the cytoplasmic ends of TM5 and TM6 but extends into the interior of the cytoplasmic cavity and interacts with the C-terminus of the Gi1 α5 helix and stabilizes the active state of the receptor[86].

An interesting subset of intracellular allosteric modulators are those that can either be a NAM or a PAM (or some combination thereof) depending on the signal transduction pathway. These compounds can function as biased allosteric modulators (BAMs) for a given pathway. Examples of intracellular biased allosteric modulators whose Cryo-EM structures have been determined recently include PCO371 in complex with the Class B GPCR parathyroid hormone type 1 receptor (PTH1R) and SBI-553 in complex with the Class A GPCR neurotensin receptor 1 (NTSR1)[55,87,88]. PCO371 binds within the intracellular cavity of PTH1R and directly interacts with the G protein Gαs subunit. The PCO371 binding mode rearranges the intracellular region of PTH1R towards the active conformation stabilizing the significantly outward-bent conformation of transmembrane helix 6, which in-turn biases PTH1R signaling towards the Gαs protein pathway with little to no recruitment of β-arrestins.

In- vitro assays of G protein activation and β-arrestin recruitment revealed SBI-553 has complex effects on NTSR1 signaling pathways[55,56,89]. It was discovered that SBI-553 can act as a NAM for Gq signaling, neutral for Gi signaling, and as an ago-PAM for β-arrestin recruitment. It was revealed in the Cryo-EM structure that SBI-553 interacts and rearranges the side-chain of $R167^{3.50}$, a member of the DRY motif[90] and a key residue for receptor activation and G protein interaction, thus competing for NTSR1 and blocking G protein interaction[55,88]. It is worth noting that in the Cryo-EM complex structure with the GoA Gα subunit, the interface is skewed allowing permissive binding of both SBI-553 and G protein in the NTSR1 intracellular cavity while having interactions with both NTSR1 and G protein. Thus, additional Cryo-EM structures of NTSR1 will be needed to fully understand the complex effects SBI-553 has on transduction pathways at the receptor:G protein interface.

## Discussion and Conclusion

Precision medicine, sometimes referred to as "personalized medicine" is an approach tailored towards disease prevention and treatment that takes into account differences in an individual's genetic makeup, environment, and lifestyle. Precision medicine is increasingly prevalent in areas of oncology, immunotherapy, pharmacogenomics, and rare diseases. For example, targeted cancer therapies for breast cancer patients with mutant HER2-positive tumors has significantly improved outcomes for these cancer patients[91]. More recently, the FDA has approved a bispecific antibody (talquetamab-tgvs) which targets both CD3 on T Cells and cancers cells that express the Class C GPCR GPRC5D for the treatment of multiple myeloma[92,93].

The growing understanding of GPCR structure and signaling mechanisms has provided opportunities for the design of safer and more effective drugs, offering potential breakthroughs in what we term 'precision pharmacology'. Orthosteric biased GPCR agonists opened the door for GPCR precision pharmacology by selectively activating specific signaling pathways and minimizing unwanted effects, these biased agonists have the potential to improve the treatment of diseases ranging from pain management to cardiovascular diseases, neurological conditions, and cancer. Given that GPCRs are highly diverse, with different subtypes that display subtype specificity with regards to orthosteric ligands *and* the complexity of GPCR signal transduction *along with* the need for context-dependent modulation of signal transduction, future research and drug development in orthosteric biased ligands will be challenging but hopefully rewarding.

The potential for pathway specific intracellular biased ligands for a given GPCR to treat and interrogate diseases and disease mechanisms, can be envisioned through the development of drug candidates and or molecular probes that target the intracellular cavities at the receptor:G protein interface. To illustrate this concept we propose two examples: (1) a single point mutation (W276C/A) in the endothelin receptor subtype B (ET(B)R) impairs Gq/11 signaling while having no effect on other signaling pathways which is associated with reduced congenital brain development leading to the intestinal disease, Hirschsprung's disease[94]; (2) mutations in G protein Gq/11 subunit at Q209 along with mutations at L129 in the Cysteinyl leukotriene receptor 2 (CYSLTR2) stimulate Gq/11 constitutive pathway activity and have been implicated in the oncogenic disease uveal melanoma (UM)[95,96]. A Gq NAM biased intracellular allosteric modulator specific for these receptors has the potential to ameliorate these conditions.

Consider the fact that owing to the conserved nature of the orthosteric binding sites between GPCR subtypes, developing biased orthosteric ligands between subtypes has been challenging[97,98]. One could also expect that this degree of GPCR subtype specificity could be extended to allosteric sites, making the design of selective allosteric modulators also challenging. GPCRs are conformationally flexible and undergo multiple conformational states, which can make it difficult to predict how allosteric modulators will behave across different receptor subtypes or cellular contexts. Thus, the interplay between the orthosteric binding site and potential allosteric sites can lead to complex signaling outcomes, requiring careful consideration of how these pathways are regulated in different physiological and pathological contexts[99]. Whereas orthosteric biased ligands and allosteric ligands are akin to "targeting signal transduction from afar" intracellular biased allosteric ligands (BAMs) and molecular glues are akin to targeting signal transduction at the direct site of interaction between the receptor and the transducer.

As well, the orthosteric site of GPCRs has evolved to be highly selective for a given GPCR's endogenous ligand, taking into account the potential number of endogenous ligands available. On the other hand, the GPCR intracellular cavity is less discriminative with many GPCRs being able to couple to different G protein classes and subtypes. Designing and screening intracellular biased allosteric ligands to investigate the diverse signaling pathways of GPCRs is an extremely complex and daunting task. Biased agonists such as PC0371 and SBI-553 were discovered and optimized via screening of large chemical libraries, and the exact mechanism governing their biased agonism and allosterism remained undetermined until their discovery by Cryo-EM complex structure determination[100,101]. Superposition of NTSR1 Cryo-EM structures in complex with Gq and GoA revealed a convincing overlap of receptor:G protein interface with key determinants of SBI-553's transducer selectivity (bias) which involved not only conserved residues of the G protein C-terminus but also the total receptor:G protein interface[55]. Despite these challenges, some very modest gains in selectivity of transducer pathways have recently been achieved using analogs of SBI-553 providing insight for the further development of SBI-553 analogs and potentially other intracellular biased allosteric modulators (BAMs)[102].

It is also worth considering the potential utility of precision intracellular biased allosteric modulators (BAMs) versus biased orthosteric ligands. As discussed above, orthosteric biased ligands selectively activate canonical G protein or β-arrestin pathways. Further testing of non-canonical G protein pathways[62] frequently reveals additional levels of real and potential bias[21]. Signaling pathway specific intracellular biased allosteric modulators such as SBI-553, provide potential models in which to precisely tune receptor-transducer interactions.

The discovery and rational development of intracellular biased allosteric modulators (BAMs) is an emerging concept for precision pharmacology which would have been impossible without the recent Cryo-EM structures of these compounds revealing their respective binding sites. However, further investigation into their mechanisms is needed especially given the apparent failure of PC0371 in clinical trials (https://clinicaltrials.gov/study/NCT04209179). Conceivably, BAMs have the potential to provide a powerful orthogonal approach for precision pharmacology. Current assays and large-scale docking to identify potential BAMs along with further structure guided design are already being used to advance the field, an approach not dissimilar to the identification of orthosteric ligands[103,104]. However, additional tools such as cell specific assays and pathway specific stable cell lines might be needed to interrogate the complexity of allosteric signaling and cell bias which has been discussed briefly previously.

## Data availability

No datasets were generated or analysed during the current study.

Received: 1 January 2025; Accepted: 20 March 2025;
Published online: 12 May 2025

## References

1. Foord, S. M. et al. International Union of Pharmacology. XLVI. G protein-coupled receptor list. *Pharm. Rev.* **57**, 279–288 (2005).
2. Fredriksson, R., Lagerstrom, M. C., Lundin, L. G. & Schioth, H. B. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol. Pharm.* **63**, 1256–1272 (2003).
3. Hauser, A. S. et al. Pharmacogenomics of GPCR Drug Targets. *Cell* **172**, 41–54.e19 (2018).
4. Collins, H., Calvo, S., Greenberg, K., Forman Neall, L. & Morrison, S. Information Needs in the Precision Medicine Era: How Genetics Home Reference Can Help. *Interact. J. Med Res.* **5**, e13 (2016).
5. Wootten, D., Christopoulos, A., Marti-Solano, M., Babu, M. M. & Sexton, P. M. Mechanisms of signalling and biased agonism in G protein-coupled receptors. *Nat. Rev. Mol. Cell Biol.* **19**, 638–653 (2018).
6. Pierce, K. L., Premont, R. T. & Lefkowitz, R. J. Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* **3**, 639–650 (2002).
7. Gilman, A. G. G proteins: transducers of receptor-generated signals. *Annu Rev. Biochem* **56**, 615–649 (1987).
8. Syrovatkina, V., Alegre, K. O., Dey, R. & Huang, X. Y. Regulation, Signaling, and Physiological Functions of G-Proteins. *J. Mol. Biol.* **428**, 3850–3868 (2016).
9. Senarath, K. et al. Regulation of G Protein betagamma Signaling. *Int Rev. Cell Mol. Biol.* **339**, 133–191 (2018).
10. Sato, M., Blumer, J. B., Simon, V. & Lanier, S. M. Accessory proteins for G proteins: partners in signaling. *Annu Rev. Pharm. Toxicol.* **46**, 151–187 (2006).
11. Sjogren, B., Blazer, L. L. & Neubig, R. R. Regulators of G protein signaling proteins as targets for drug discovery. *Prog. Mol. Biol. Transl. Sci.* **91**, 81–119 (2010).
12. Sjogren, B. & Neubig, R. R. Thinking outside of the "RGS box": new approaches to therapeutic targeting of regulators of G protein signaling. *Mol. Pharm.* **78**, 550–557 (2010).
13. Reiter, E., Ahn, S., Shukla, A. K. & Lefkowitz, R. J. Molecular mechanism of beta-arrestin-biased agonism at seven-transmembrane receptors. *Annu Rev. Pharm. Toxicol.* **52**, 179–197 (2012).
14. Nobles, K. N. et al. Distinct phosphorylation sites on the beta(2)-adrenergic receptor establish a barcode that encodes differential functions of beta-arrestin. *Sci. Signal* **4**, ra51 (2011).
15. Liggett, S. B. Phosphorylation barcoding as a mechanism of directing GPCR signaling. *Sci. Signal* **4**, pe36 (2011).
16. Baidya, M. et al. Key phosphorylation sites in GPCRs orchestrate the contribution of beta-Arrestin 1 in ERK1/2 activation. *EMBO Rep.* **21**, e49886 (2020).
17. Dwivedi-Agnihotri, H. et al. Distinct phosphorylation sites in a prototypical GPCR differently orchestrate beta-arrestin interaction, trafficking, and signaling. *Sci Adv* **6**. https://doi.org/10.1126/sciadv.abb8368 (2020).
18. Nibbs, R. J. & Graham, G. J. Immune regulation by atypical chemokine receptors. *Nat. Rev. Immunol.* **13**, 815–829 (2013).
19. Chen, Q. et al. ACKR3-arrestin2/3 complexes reveal molecular consequences of GRK-dependent barcoding. *bioRxiv*. https://doi.org/10.1101/2023.07.18.549504 (2023).
20. Ulvmar, M. H., Hub, E. & Rot, A. Atypical chemokine receptors. *Exp. Cell Res.* **317**, 556–568 (2011).
21. Che, T. & Roth, B. L. Molecular basis of opioid receptor signaling. *Cell* **186**, 5203–5219 (2023).
22. Zhang, D. et al. Structural insights into angiotensin receptor signaling modulation by balanced and biased agonists. *EMBO J.* **42**, e112940 (2023).

23. Faouzi, A. et al. Structure-based design of bitopic ligands for the micro-opioid receptor. *Nature* **613**, 767–774 (2023).
24. Kaplan, A. L. et al. Bespoke library docking for 5-HT(2A) receptor agonists with antidepressant activity. *Nature* **610**, 582–591 (2022).
25. Lyu, J., et al. AlphaFold2 structures guide prospective ligand discovery. *Science* **384**, eadn6354 (2024).
26. Lee, S. M. et al. SKF-83959 is not a highly-biased functionally selective D1 dopamine receptor ligand with activity at phospholipase C. *Neuropharmacology* **86**, 145–154 (2014).
27. Urban, J. D. et al. Functional selectivity and classical concepts of quantitative pharmacology. *J. Pharm. Exp. Ther.* **320**, 1–13 (2007).
28. Rankovic, Z., Brust, T. F. & Bohn, L. M. Biased agonism: An emerging paradigm in GPCR drug discovery. *Bioorg. Med Chem. Lett.* **26**, 241–250 (2016).
29. Kenakin, T. Biased Receptor Signaling in Drug Discovery. *Pharm. Rev.* **71**, 267–315 (2019).
30. Warne, T. et al. The structural basis for agonist and partial agonist action on a beta(1)-adrenergic receptor. *Nature* **469**, 241–244 (2011).
31. Masureel, M. et al. Structural insights into binding specificity, efficacy and bias of a beta(2)AR partial agonist. *Nat. Chem. Biol.* **14**, 1059–1066 (2018).
32. McCorvy, J. D. et al. Structure-inspired design of beta-arrestin-biased ligands for aminergic GPCRs. *Nat. Chem. Biol.* **14**, 126–134 (2018).
33. Han, J. et al. Ligand and G-protein selectivity in the kappa-opioid receptor. *Nature* **617**, 417–425 (2023).
34. El Daibani, A. et al. Molecular mechanism of biased signaling at the kappa opioid receptor. *Nat. Commun.* **14**, 1338 (2023).
35. Qu, Q. et al. Insights into distinct signaling profiles of the microOR activated by diverse agonists. *Nat. Chem. Biol.* **19**, 423–430 (2023).
36. Kim, K. et al. Structure of a Hallucinogen-Activated Gq-Coupled 5-HT(2A) Serotonin Receptor. *Cell* **182**, 1574–1588 e1519 (2020).
37. McCorvy, J. D. et al. Structural determinants of 5-HT(2B) receptor activation and biased agonism. *Nat. Struct. Mol. Biol.* **25**, 787–796 (2018).
38. Chen, A., Su, C., Zhang, Z. & Zhang, H. Cryo-EM Structures and AlphaFold3 Models of Histamine Receptors Reveal Diverse Ligand Binding and G Protein Bias. *Pharmaceuticals* **18**, 292 (2025).
39. Kenakin, T. & Christopoulos, A. Signalling bias in new drug discovery: detection, quantification and therapeutic impact. *Nat. Rev. Drug Discov.* **12**, 205–216 (2013).
40. Xu, P. et al. Structural genomics of the human dopamine receptor system. *Cell Res.* **33**, 604–616 (2023).
41. Kroeze, W. K. et al. PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCRome. *Nat. Struct. Mol. Biol.* **22**, 362–369 (2015).
42. Fan, F. et al. Novel genetically encoded biosensors using firefly luciferase. *ACS Chem. Biol.* **3**, 346–351 (2008).
43. Zhang, R. & Xie, X. Tools for GPCR drug discovery. *Acta Pharm. Sin.* **33**, 372–384 (2012).
44. Kenakin, T. A Scale of Agonism and Allosteric Modulation for Assessment of Selectivity, Bias, and Receptor Mutation. *Mol. Pharm.* **92**, 414–424 (2017).
45. Cheng, J. et al. Design and Discovery of Functionally Selective Serotonin 2C (5-HT(2C)) Receptor Agonists. *J. Med Chem.* **59**, 9866–9880 (2016).
46. Manglik, A. et al. Structure-based discovery of opioid analgesics with reduced side effects. *Nature* **537**, 185–190 (2016).
47. Viscusi, E. R. et al. APOLLO-1: a randomized placebo and active-controlled phase III study investigating oliceridine (TRV130), a G protein-biased ligand at the micro-opioid receptor, for management of moderate-to-severe acute pain following bunionectomy. *J. Pain. Res.* **12**, 927–943 (2019).
48. Singla, N. K. et al. APOLLO-2: A Randomized, Placebo and Active-Controlled Phase III Study Investigating Oliceridine (TRV130), a G Protein-Biased Ligand at the mu-Opioid Receptor, for Management of Moderate to Severe Acute Pain Following Abdominoplasty. *Pain. Pr.* **19**, 715–731 (2019).
49. Wisler, J. W. et al. A unique mechanism of beta-blocker action: carvedilol stimulates beta-arrestin signaling. *Proc. Natl. Acad. Sci. USA* **104**, 16657–16662 (2007).
50. Pang, P. S. et al. Biased ligand of the angiotensin II type 1 receptor in patients with acute heart failure: a randomized, double-blind, placebo-controlled, phase IIB, dose ranging trial (BLAST-AHF). *Eur. Heart J.* **38**, 2364–2373 (2017).
51. Cotter, G. et al. Relationship between baseline systolic blood pressure and long-term outcomes in acute heart failure patients treated with TRV027: an exploratory subgroup analysis of BLAST-AHF. *Clin. Res Cardiol.* **107**, 170–181 (2018).
52. Thal, D. M., Glukhova, A., Sexton, P. M. & Christopoulos, A. Structural insights into G-protein-coupled receptor allostery. *Nature* **559**, 45–53 (2018).
53. Shpakov, A. O. Allosteric Regulation of G-Protein-Coupled Receptors: From Diversity of Molecular Mechanisms to Multiple Allosteric Sites and Their Ligands. *Int J. Mol. Sci.* **24**, 6187 (2023).
54. Quoyer, J. et al. Pepducin targeting the C-X-C chemokine receptor type 4 acts as a biased agonist favoring activation of the inhibitory G protein. *Proc. Natl. Acad. Sci. USA* **110**, E5088–E5097 (2013).
55. Krumm, B. E. et al. Neurotensin Receptor Allosterism Revealed in Complex with a Biased Allosteric Modulator. *Biochemistry* **62**, 1233–1248 (2023).
56. Slosky, L. M. et al. beta-Arrestin-Biased Allosteric Modulator of NTSR1 Selectively Attenuates Addictive Behaviors. *Cell* **181**, 1364–1379.e1314 (2020).
57. Leach, K., Sexton, P. M. & Christopoulos, A. Allosteric GPCR modulators: taking advantage of permissive receptor pharmacology. *Trends Pharm. Sci.* **28**, 382–389 (2007).
58. May, L. T., Leach, K., Sexton, P. M. & Christopoulos, A. Allosteric modulation of G protein-coupled receptors. *Annu Rev. Pharm. Toxicol.* **47**, 1–51 (2007).
59. Christopoulos, A. et al. International Union of Basic and Clinical Pharmacology. XC. multisite pharmacology: recommendations for the nomenclature of receptor allosterism and allosteric ligands. *Pharm. Rev.* **66**, 918–947 (2014).
60. Valant, C., Felder, C. C., Sexton, P. M. & Christopoulos, A. Probe dependence in the allosteric modulation of a G protein-coupled receptor: implications for detection and validation of allosteric ligand effects. *Mol. Pharm.* **81**, 41–52 (2012).
61. Salahpour, A. et al. BRET biosensors to study GPCR biology, pharmacology, and signal transduction. *Front Endocrinol. (Lausanne* **3**, 105 (2012).
62. DiBerto, J. F., Olsen, R. H. J. & Roth, B. L. TRUPATH: An Open-Source Biosensor Platform for Interrogating the GPCR Transducerome. *Methods Mol. Biol.* **2525**, 185–195 (2022).
63. Olsen, R. H. J. et al. TRUPATH, an open-source biosensor platform for interrogating the GPCR transducerome. *Nat. Chem. Biol.* **16**, 841–849 (2020).
64. Black, J. W. & Leff, P. Operational models of pharmacological agonism. *Proc. R. Soc. Lond. B Biol. Sci.* **220**, 141–162 (1983).
65. Jakubik, J., Randakova, A., Chetverikov, N., El-Fakahany, E. E. & Dolezal, V. The operational model of allosteric modulation of pharmacological agonism. *Sci. Rep.* **10**, 14421 (2020).
66. Katritch, V. et al. Allosteric sodium in class A GPCR signaling. *Trends Biochem Sci.* **39**, 233–244 (2014).
67. Pert, C. B., Pasternak, G. & Snyder, S. H. Opiate agonists and antagonists discriminated by receptor binding in brain. *Science* **182**, 1359–1361 (1973).

68. Simon, E. J. & Groth, J. Kinetics of opiate receptor inactivation by sulfhydryl reagents: evidence for conformational change in presence of sodium ions. *Proc. Natl. Acad. Sci. USA* **72**, 2404–2407 (1975).
69. Horstman, D. A. et al. An aspartate conserved among G-protein receptors confers allosteric regulation of alpha 2-adrenergic receptors by sodium. *J. Biol. Chem.* **265**, 21590–21595 (1990).
70. Fenalti, G. et al. Molecular control of delta-opioid receptor signalling. *Nature* **506**, 191–196 (2014).
71. Wang, S. et al. D(4) dopamine receptor high-resolution structures enable the discovery of selective agonists. *Science* **358**, 381–386 (2017).
72. Wang, M., Lyu, J. & Zhang, C. Single transmembrane GPCR modulating proteins: neither single nor simple. *Protein Cell* **15**, 395–402 (2024).
73. Wang, M. et al. Identification of MRAP protein family as broad-spectrum GPCR modulators. *Clin. Transl. Med* **12**, e1091 (2022).
74. Gingell, J. J. et al. An allosteric role for receptor activity-modifying proteins in defining GPCR pharmacology. *Cell Discov.* **2**, 16012 (2016).
75. Pioszak, A. A. & Hay, D. L. RAMPs as allosteric modulators of the calcitonin and calcitonin-like class B G protein-coupled receptors. *Adv. Pharm.* **88**, 115–141 (2020).
76. Kotliar, I. B. et al. Multiplexed mapping of the interactome of GPCRs with receptor activity-modifying proteins. *Sci. Adv.* **10**, eado9959 (2024).
77. Shrivastava, S., Pucadyil, T. J., Paila, Y. D., Ganguly, S. & Chattopadhyay, A. Chronic cholesterol depletion using statin impairs the function and dynamics of human serotonin(1A) receptors. *Biochemistry* **49**, 5426–5435 (2010).
78. Paila, Y. D., Tiwari, S., Sengupta, D. & Chattopadhyay, A. Molecular modeling of the human serotonin(1A) receptor: role of membrane cholesterol in ligand binding of the receptor. *Mol. Biosyst.* **7**, 224–234 (2011).
79. Paila, Y. D., Jindal, E., Goswami, S. K. & Chattopadhyay, A. Cholesterol depletion enhances adrenergic signaling in cardiac myocytes. *Biochim Biophys. Acta* **1808**, 461–465 (2011).
80. Hanson, M. A. et al. A specific cholesterol binding site is established by the 2.8 A structure of the human beta2-adrenergic receptor. *Structure* **16**, 897–905 (2008).
81. He, J. et al. ASD2023: towards the integrating landscapes of allosteric knowledgebase. *Nucleic Acids Res* **52**, D376–D383 (2024).
82. Shen, S. et al. Allosteric modulation of G protein-coupled receptor signaling. *Front Endocrinol. ((Lausanne))* **14**, 1137604 (2023).
83. Zhang, L., Mobbs, J. I., May, L. T., Glukhova, A. & Thal, D. M. The impact of cryo-EM on determining allosteric modulator-bound structures of G protein-coupled receptors. *Curr. Opin. Struct. Biol.* **79**, 102560 (2023).
84. Lu, S. & Zhang, J. Small Molecule Allosteric Modulators of G-Protein-Coupled Receptors: Drug-Target Interactions. *J. Med Chem.* **62**, 24–45 (2019).
85. Roth, B. L. & Krumm, B. E. Molecular glues as potential GPCR therapeutics. *Biochem Pharm.* **228**, 116402 (2024).
86. Chen, G. et al. Activation and allosteric regulation of the orphan GPR88-Gi1 signaling complex. *Nat. Commun.* **13**, 2375 (2022).
87. Kobayashi, K. et al. Class B1 GPCR activation by an intracellular agonist. *Nature* **618**, 1085–1093 (2023).
88. Duan, J. et al. GPCR activation and GRK2 assembly by a biased intracellular agonist. *Nature* **620**, 676–681 (2023).
89. Pinkerton, A. B. et al. Discovery of beta-Arrestin Biased, Orally Bioavailable, and CNS Penetrant Neurotensin Receptor 1 (NTR1) Allosteric Modulators. *J. Med Chem.* **62**, 8357–8363 (2019).
90. Palczewski, K. et al. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **289**, 739–745 (2000).
91. Swain, S. M., Shastry, M. & Hamilton, E. Targeting HER2-positive breast cancer: advances and future directions. *Nat. Rev. Drug Discov.* **22**, 101–126 (2023).
92. Granger, K., Gaffney, K. J. & Davis, J. A. Newly approved and forthcoming T-cell-redirecting bispecific antibodies for the treatment of relapsed/refractory multiple myeloma. *J. Oncol. Pharm. Pr.* **29**, 722–726 (2023).
93. Liu, L. & Krishnan, A. Talquetamab in multiple myeloma. *Haematologica* **109**, 718–724 (2024).
94. Imamura, F., Arimoto, I., Fujiyoshi, Y. & Doi, T. W276 mutation in the endothelin receptor subtype B impairs Gq coupling but not Gi or Go coupling. *Biochemistry* **39**, 686–692 (2000).
95. Lapadula, D. & Benovic, J. L. Targeting Oncogenic Galpha(q/11) in Uveal Melanoma. *Cancers ((Basel))* **13**, 6195 (2021).
96. Jager, M. J. et al. Uveal melanoma. *Nat. Rev. Dis. Prim.* **6**, 24 (2020).
97. Luttrell, L. M., Maudsley, S. & Bohn, L. M. Fulfilling the Promise of "Biased" G Protein-Coupled Receptor Agonism. *Mol. Pharm.* **88**, 579–588 (2015).
98. Rodriguez-Espigares, I., Kaczor, A. A., Stepniewski, T. M. & Selent, J. Challenges and Opportunities in Drug Discovery of Biased Ligands. *Methods Mol. Biol.* **1705**, 321–334 (2018).
99. Kenakin, T. Know your molecule: pharmacological characterization of drug candidates to enhance efficacy and reduce late-stage attrition. *Nat. Rev. Drug Discov.* **23**, 626–644 (2024).
100. Tamura, T. et al. Identification of an orally active small-molecule PTHR1 agonist for the treatment of hypoparathyroidism. *Nat. Commun.* **7**, 13384 (2016).
101. Peddibhotla, S. et al. Discovery of ML314, a Brain Penetrant Non-Peptidic beta-Arrestin Biased Agonist of the Neurotensin NTR1 Receptor. *ACS Med Chem. Lett.* **4**, 846–851 (2013).
102. Moore, M. N. et al. Design of allosteric modulators that change GPCR G protein subtype selectivity. *bioRxiv*. https://doi.org/10.1101/2024.11.20.624209 (2024).
103. Vigneron, S. F. et al. Docking 14 million virtual isoquinuclidines against the mu and kappa opioid receptors reveals dual antagonists-inverse agonists with reduced withdrawal effects. *bioRxiv*. https://doi.org/10.1101/2025.01.09.632033 (2025).
104. Liu, F. et al. Large library docking identifies positive allosteric modulators of the calcium-sensing receptor. *Science* **385**, eado1868 (2024).


## Acknowledgements

The work summarized in this perspective was funded in part from the National Institute of Mental Health, National Institutes of Health, grant NIH R01MH112205 to BLR. Figures were created with BioRender.com.

## Author contributions

B.E.K. wrote and edited the paper and figures. B.L.R. wrote and edited the paper and the figures.

## Competing interests

B.L.R. is currently on the SAB of Epiodyne and Septerna Pharmaceuticals. UNC has licensed technologies from BLR's lab to many pharmaceutical and Biotechnology companies. All other authors declare no competing interest.

## Additional information

**Correspondence** and requests for materials should be addressed to Bryan L. Roth.