Trends in Pharmacological Sciences

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Review

The Potential of ^{19}F NMR Application in GPCR Biased Drug Discovery

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Although structure-based virtual drug discovery is revolutionizing the conventional high-throughput cell-based screening system, its limitation is obvious, together with other critical challenges. In particular, the resolved static snapshots fail to represent a full free-energy landscape due to homogenization in structural determination processing. The loss of conformational heterogeneity and related functional diversity emphasize the necessity of developing an approach that can fill this space. In this regard, NMR holds undeniable potential. However, outstanding questions regarding the NMR application remain. This review summarizes the limitations of current drug discovery and explores the potential of ^{19}F NMR in establishing a conformation-guided drug screening system, advancing the cell- and structure-based discovery strategy for G protein-coupled receptor (GPCR) biased drug screening.

Highlights

Studies indicate that G protein-coupled receptor (GPCR) activation is a multistate transition process instead of a simple switch, suggesting the possibility of modulating the GPCR function through tuning of these individual states.

A biased ligand was proposed to specifically target one signaling pathway over others in a multiple-signaling coexistence system. However, despite the progress in structural biology, a detailed mechanism of how this is achieved remains elusive.

Research has indicated that different GPCR conformations interact with different downstream partners, dictating various pharmacological outputs.

^{19}F NMR has been demonstrated to be extremely promising in delineating GPCR conformations, attributable to its quantitative properties and high sensitivity toward electrostatic changes of the probe microenvironment. Thus, there is tremendous potential for biased drug discovery by establishing a rigorous correlation between individual receptor conformations and the pharmacological consequence upon ligand binding.

Biased Agonism in the GPCR

Biased agonism is a ligand-based signaling preference [1] observed when multiple signal pathways coexist in a signaling process. Since its introduction in the 1990s [2], the study of biased agonism has overwhelmingly centered on **G protein-coupled receptors (GPCRs)** (see Glossary) and, in particular, focused on two classical signal pathways: G protein and β-arrestin [3] (Figure 1). As a matter of fact, the concept of biased agonism can be applied to any signaling where the ligand-activated molecule (usually the receptor) is capable of interacting with multiple downstream partners [4] but selectively directs the downstream signal based on **biased ligand** binding.

Research has indicated that full agonist-type drugs often activate multiple signal pathways, resulting in off-target side effects; such ligands are also referred to as **balanced agonists** [5]. In the GPCR regime, balanced agonists often trigger both G protein and β-arrestin signaling [6]. The study of biased agonism is also primarily centered on determining whether the ligand is G protein or β-arrestin biased. However, as 16 Gα subunits ($G\alpha_s$, $G\alpha_i$, $G\alpha_o$, $G\alpha_q$, $G\alpha_{12/13}$, etc.) have been discovered, along with 5 Gβ and 12 Gγ proteins in human [7], a large combination pool of heterotrimeric G proteins exist. Multiple heterotrimeric G proteins have been reported for the same receptor [8], as well as β-arrestins [9]. Although the concept of biased agonism has been proposed for more than two decades, we are still at the early stage of exploring a suitable and practical approach that can rationally design a biased drug. This goal remains a grand challenge for cell- and structure-based drug discovery systems.

Overall Challenges of Biased Drug Designing

Lack of Molecular Understanding of Biased Signaling

While significant progress has been made in linking distinct pharmacological phenomena to various ligand bindings and their signaling effectors, such as taking advantage of approaches like bioluminescence resonance energy transfer (BRET) [10,11], the following has not yet been fully established: (i) techniques for quantitative analyses of ligand functional selectivity [12] in

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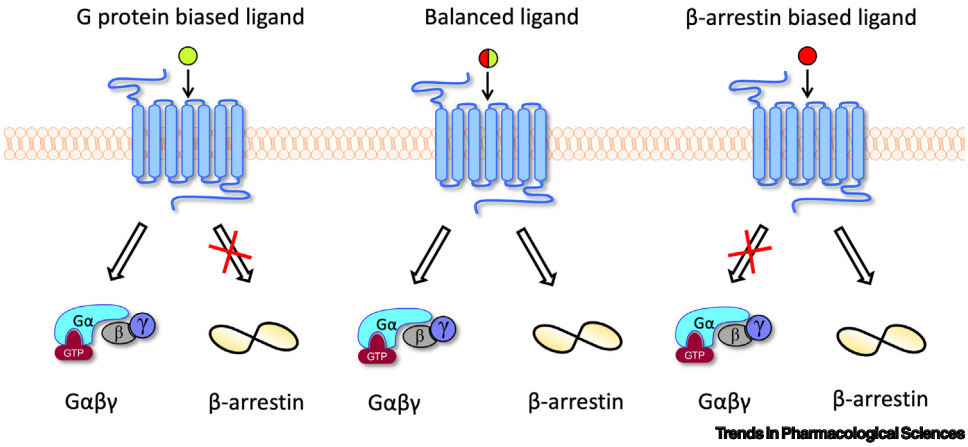

Figure 1. Classical Biased Signaling for G Protein and β-arrestin Pathways in G Protein-Coupled Receptors (GPCRs).

order to identify the roles of individual signal pathways in a multisignaling system; (ii) techniques for predicting the physiological response for any given known structure according to its pharmacophores; and, ultimately, (iii) how to rationally design an efficacious biased drug for a given disease that results from a specific signaling dysfunction [13]. This could be attributed to an incomplete understanding of the two following prerequisites:

(i) Limited molecular understanding of ligand bias. Perhaps the best-known aspect of molecular-based biased agonism is that each ligand stabilizes a specific receptor conformation [14,15]. While conformational ensembles have been preliminarily quantified for several key receptors, including β2-adrenergic receptor (β_2AR) [16,17], adenosine A2A receptor (A_{2A}R) [18,19], and the leukotriene B4 receptor BLT2 [20], we have yet to establish quantitative correlations between ligand structure, receptor conformation, and pharmacological output. First, an approach that can quantitatively differentiate the receptor conformations induced by ligand bindings is yet to be developed with the application for drug discovery, though significant progress has been made in developing various techniques, including **NMR** [16,18,20–24], double electron–electron resonance [25], BRET [26], and fluorescence resonance energy transfer (FRET) [27]. Second, a comprehensive connection between the ligand pharmacophore, the responsive receptor conformation(s), the ligand functional selectivity, and its pharmacological output, is still lacking. Furthermore, conformational dynamics and kinetics are also not fully linked to signaling efficiency and efficacies [18,24], though the concept of 'pluridimensional' efficacy [28] has been advanced so that receptor activation potency was not merely driven by ligand off-rate but also driven by ligand-induced conformation differences for both receptor and G proteins, along with their interaction efficiencies [29].

(ii) The current *in vivo* 'ligand bias' assay is often thwarted by 'system bias' and signal promiscuity. Technically speaking, a biased signaling response results from the combination of two distinct phenomena: ligand bias and system bias [30]. System bias, or 'apparent' bias, reflects inherent differences in biochemical measurements as well as in biological systems. Ligand bias, or 'true' biased agonism, refers to differential signaling caused by a molecular variation that governs the interaction between ligand and transduction proteins [18,22,31]. The biggest challenge of biased drug discovery is to create a correct target product profile, which is then used to determine how effective each ligand of interest is on each associated signal pathway [12]. However, the pharmacological output is often affected by undesirable signaling crosstalk due to the signal promiscuity among the various receptors. For instance, in mammalian

Glossary

Balanced agonist: a drug that increases activities of multiple signaling pathways.
Biased ligand: a drug that increases the activity of a specific signaling pathway.
Cryo-electron microscopy (cryo-EM): a procedure that deep-freezes a sample and uses electrons to make an image of protein structure.
G protein-coupled receptor (GPCR): a class of seven-transmembrane proteins that transmit extracellular to intracellular signals and are triggered by a wide range of factors, including light, compounds, peptides, and proteins.
NMR: nuclear magnetic resonance, a spectrometer used for studying the behavior of different nuclei, such as ^{1}H, ^{13}C, ^{15}N, and ^{19}F, etc.
Paramagnetic relaxation enhancement (PRE): an NMR technique whereby paramagnetic ions such Mn^{2+} are introduced to a system in order to enhance the relaxation of spins in the proximity.
Pseudo-contact chemical shift (PCS): an additional nuclear chemical shift effect on a nucleus in spatial vicinity due to partial polarization.
X-ray crystallography: a technique of using X-rays passing through crystallized proteins to determine structures.

systems, many GPCRs coexist and interplay with downstream transducers, leading to an inaccurate evaluation of bias-degree in a drug candidate.

Missing Parts in Current Drug Screening Systems

With differential ligand binding, GPCRs can interact selectively with various intracellular partners, resulting in different downstream signaling and pharmacological effects. Although studies have advanced our understanding of the GPCR activation process, the functional relevance of each individual conformation and their roles in the signaling process remains elusive. The increased number of protein structures and the advancement of computational tools such as pharmacophore models [32], molecular docking [33], *de novo* ligand design [34], and molecular dynamics (MD) simulations [35], in particular, a ligand docking platform HADDOCK incorporated with NMR data [36], have revolutionized structure-based drug discovery. As a consequence, several GPCR agents have entered into clinical trials [37]. Compared with the cell-based drug discovery platform alone, structure-based drug discovery begins to fulfill its promise in ligand screening with affordable, super-fast computational clusters [38]. Large-scale virtual screening systems have been developed, including ZINC [39], SwissDock [40], and Enamine REAL [41], accessible to a broad audience of practitioners, in which 0.75 billion real compounds and 1.4 billion make-on-demand compounds are included in the ZINC15 and Enamine databases, respectively. The advent of machine-learning techniques is expected to further accelerate and significantly improve the accuracy of *in silico* screening efforts.

The GPCR signaling process can be described as a chain of protein–protein interactions (PPIs) initially triggered by a ligand, ranging from photons, odorants, ions, and small neurotransmitters to large peptide hormones and large proteins, etc. [42,43]. The final output of the signaling is derived from a unique order of events. As discussed, some proteins, especially receptors, are capable of binding to different signaling partners, thus conferring the diversity and specificity to the PPIs and different outputs. We do not know yet the exact molecular mechanism by which the PPIs govern this distinct and specific signaling. It has been increasingly reported that the receptors, especially GPCRs, adopt different conformations in these processes, providing the molecular foundation for the signaling diversity. According to this paradigm, a ligand-triggered signaling process can be depicted, as shown in Figure 2, where different ligands could

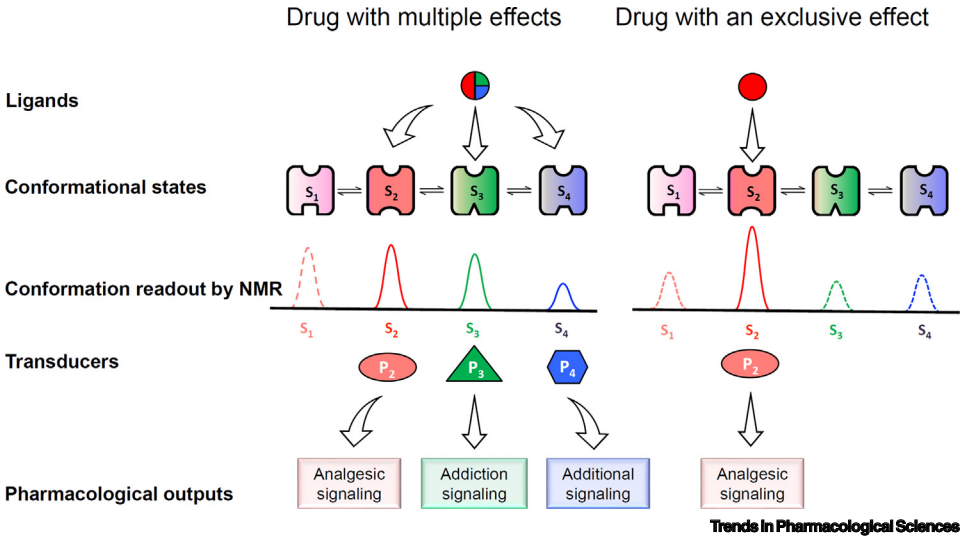

Figure 2. Schematic Correlations between Ligand, Receptor Conformation, Signaling, and Pharmacological Output Using Opioid Receptor as a Model Where Analgesics and Addiction Are Two Main Pharmacological Outputs.

preferentially select different receptor conformations [44–46]. This implies that any given specific conformation of the receptor has its own preferred transducer as well as a possibly responsive transducer conformation and, as such, a defined pharmacological signal or a set of signals that are triggered by a balanced ligand (such as morphine, buprenorphine, codeine, fentanyl, heroin) [47] in the opioid receptor exemplified in Figure 2. However, in our current drug discovery system, the detailed conformational information for the receptor or its downstream effectors are missing. Understanding the correlation between ligand pharmacophore, the receptor conformation, and correlated pharmacological output is critical for identifying biased signaling and such a strategy for rational biased drug design. We must therefore establish an approach to delineate receptor conformations and then define the pharmacological and physiological relatives of these different individual conformations, which in turn can guide purposeful design of a biased drug targeting a specific conformation (ensemble) that will lead to a desired signaling profile.

Limitation of Cell- and Structure-Based Drug Discovery Systems

With the advancement of **X-ray crystallography** and **cryo-electron microscopy (cryo-EM)**, structural biology has made tremendous progress. So far, over 370 structures of more than 70 GPCRs [48] have been resolved, providing unprecedented structural insights into receptor activation and allostery. Despite these, X-ray and cryo-EM are unable to elucidate dynamics of individual proteins or PPIs. Structural snapshots cannot capture a continuous conformational transition, extant structures associated with these processes, and the full spectrum of receptor functionality. Sample preparation for X-ray crystallography and cryo-EM homogenize samples, reducing their applicability in drug design. Furthermore, the intrinsic flexibility and plasticity of the GPCR often requires thermo-stabilization during sample preparation in order to facilitate structural determination (this includes replacing ICL3 with a thermo-stabilized T4 lysozyme or BRIL [49,50] proteins as well as fusing a stabilizer such as BRIL onto the N terminus [51], thermo-stabilized mutagenesis [52], nanobody-assisted stabilization, and engineered G protein [53,54]). Consequently, both structural heterogeneity and functional diversity are diminished. NMR, on the contrary, typically studies a wild-type receptor of interest used as-is or with minimal structure–function perturbation. This advantage is of paramount importance with respect to the study of the GPCR conformation, dynamics, kinetics of ligands and downstream effectors, and PPIs. Therefore, an NMR-based approach to biased drug discovery has unparalleled promise, as it is based on the conformational heterogeneity of the receptor.

In addition to the stand-alone molecular structure of the various GPCRs, more than ten GPCR-heterotrimeric G protein complex structures have been resolved to date, including β_2AR [55], calcitonin gene-related peptide (CGRP) [56], calcitonin receptor (CTR) [57], glucagon-like peptide-1 receptor (GLP-1R) [58], cannabinoid receptor (CB)1 and CB2 [59], CXCL8 [60], and parathyroid hormone receptor type 1 [61] in complex with G$\alpha\beta\gamma$. However, the mobility and plasticity of the interactions between the receptor and the G proteins creates challenges in revealing the intermediate complex structures using X-ray and cryo-EM in order to gain insights into the sequential interactions, emphasizing the need for the development of alternative approaches, including NMR and computational simulation methods. However, there are also inherent and technical limitations to MD simulations, most notably sampling issues, which can reduce their usefulness in the drug discovery process. Considering that most structures were resolved in complex with antagonists/inverse agonists, it is still extremely challenging to derive a complete picture of the receptor conformational ensemble and associated free-energy landscape starting from either the inactive state or active state structures. Furthermore, the missing parts of the experimentally resolved structures often include disordered loops or domains (e.g., intracellular domain III, unresolved extracellular domains, and missing orientations for H-bonds and side-chain residues), which all pose challenges of various degrees. Therefore, NMR exhibits undeniable

advantages in that the wild-type construct is used; the full energy landscape of the receptor conformation is maintained and can be presented at one time in a reconstitution system such as maltose–neopentyl glycol (MNG)-3 [62], nanodisc, or lipid bilayer systems [20,63,64]. On-going innovation of solid-state NMR technology, especially the dynamic nuclear polarization (DNP) technique [65] and its application in ^{19}F NMR [66], holds tremendous potential for profiling the receptor conformation faithfully in a genuine, native membrane environment or even in a living cell.

Prospects of ^{19}F NMR Application in Biased Drug Discovery

Progress of NMR Application in Receptor Conformational Delineation

It has been reported that the fluorine nucleus has a distinctly high gyromagnetic ratio and, thus, greatest sensitivity for NMR, next to tritium and ^{1}H nuclei [67]. ^{19}F NMR as a result exhibits a broad scope of chemical shifts over 1000 ppm [68], indicating a remarkable sensitivity to surrounding environmental changes, with potential to detect the subtle electrostatic changes associated with receptor activation. This provides a plausibility for rational design of biased drugs by delineating the receptor conformations and correlating each one to a specific pharmacological signaling using ^{19}F NMR. However, it is also true that the large chemical shift anisotropy (CSA) of ^{19}F, in contrast to that of ^{1}H [69], often leads to line width broadening. As a result, the signal/noise was dramatically affected and it may be difficult to distinguish overlapping conformations such as subtle conformational changes induced by ligands in some circumstances. In that case, line shape simulation would be required [16,18] to identify overlapped conformers as well as their exchange rates. Therefore, developing methods of further improving the sensitivity of the ^{19}F probe or identifying the most sensitive labeling site in the receptor are the alternatives of increasing the conformational resolution, in part compensating for defects arising from CSA effects.

Toward this effort, we discovered a higher electrostatic sensitive ^{19}F NMR probe, BTFMA, compared with two conventional chemical probes (e.g., BTFA and TFET) [16,67]. Taking advantage of this novel probe, together with an upgraded GPCR sample preparation system [70] and a judiciously selected ^{19}F labeling site [18] on the receptor, we were able to further break new ground by delineating A_{2A}R into four distinct conformational states in a detergent MNG-3 system via ^{19}F NMR on the basis of our previous efforts on β_2AR studies [16,21], including two inactive states ($S_{1\text{-}2}$) and two active states (S_3 and $S_{3'}$) (Figure 3). Besides ^{19}F NMR, 2D ^{1}H-^{13}C correlation spectra was also utilized to depict conformations of a typical GPCR, BLT2, in a reconstituted lipid bilayer through ^{13}C-labeled isoleucine and methionine [20]. Two distinct active conformations were also observed in angiotensin receptor for β-arrestin and G protein [71], respectively. The observation of the intermediate conformational state was meanwhile corroborated through computational simulation of β_2AR [46]. Furthermore, the biased signaling pathways of β_2AR was evaluated by another group through ^{19}F NMR, where the conventional TFET labeling strategy was used [22], though only two spectroscopic resonances were discerned in their study. The TFET labeling strategy was used for the A_{2A}R receptor as well [24], validating our previous observations [18], though a minor discrepancy existed, which could arise from different sample preparation and receptor constructs, ^{19}F probes (BTFMA versus TFET), and purification procedures [18,24].

Besides profiling the receptor conformation, ^{19}F NMR is also a powerful tool in fragment-based drug screening to assess promising hits [72], including screening fluorinated fragment libraries, measuring ligand binding affinities, and identifying the ligand binding mode. For instance, ligand dissociation constant (K_D) can be measured by ^{19}F or ^{1}H line width changes or the chemical shift of the investigated ligands through titration. The particular pulse sequences such as saturation transfer decay (STD) and WaterLOGSY experiments can also be used for the K_D

determination [73,74]. To this end, both binding affinity and preferred receptor conformation of the investigated ligand can be evaluated in one (set of) NMR experiment(s). We anticipate this kind of experiment could be done in a cell setting in the future, as depicted in Figures 3 and 4C. The development of unnatural amino acid (UAA) genetic incorporation techniques is paving the way by integrating a fluorinated conformation reporter into the receptor in either extracellular or intracellular domain. The related work is ongoing in our laboratory using *Pichia pastoris* as an expression system. Currently, the main hurdle for achieving this is still the intensity of NMR signal and how to further improve the heterogenous expression of the receptors instead of UAA incorporation itself.

Receptor Conformational Resolution Limitation and Solutions

Though dramatic progress has been made toward dissecting the receptor conformation ensemble, especially with NMR techniques, there remain outstanding issues. In particular, difficulty in resolving different conformational states is a major hurdle for its widespread practical application [18,22,24]. Insufficient resolution obscures the conformational transitions and dynamics of individual conformational states as well as the functions of individual states in response to a given ligand. Further efforts

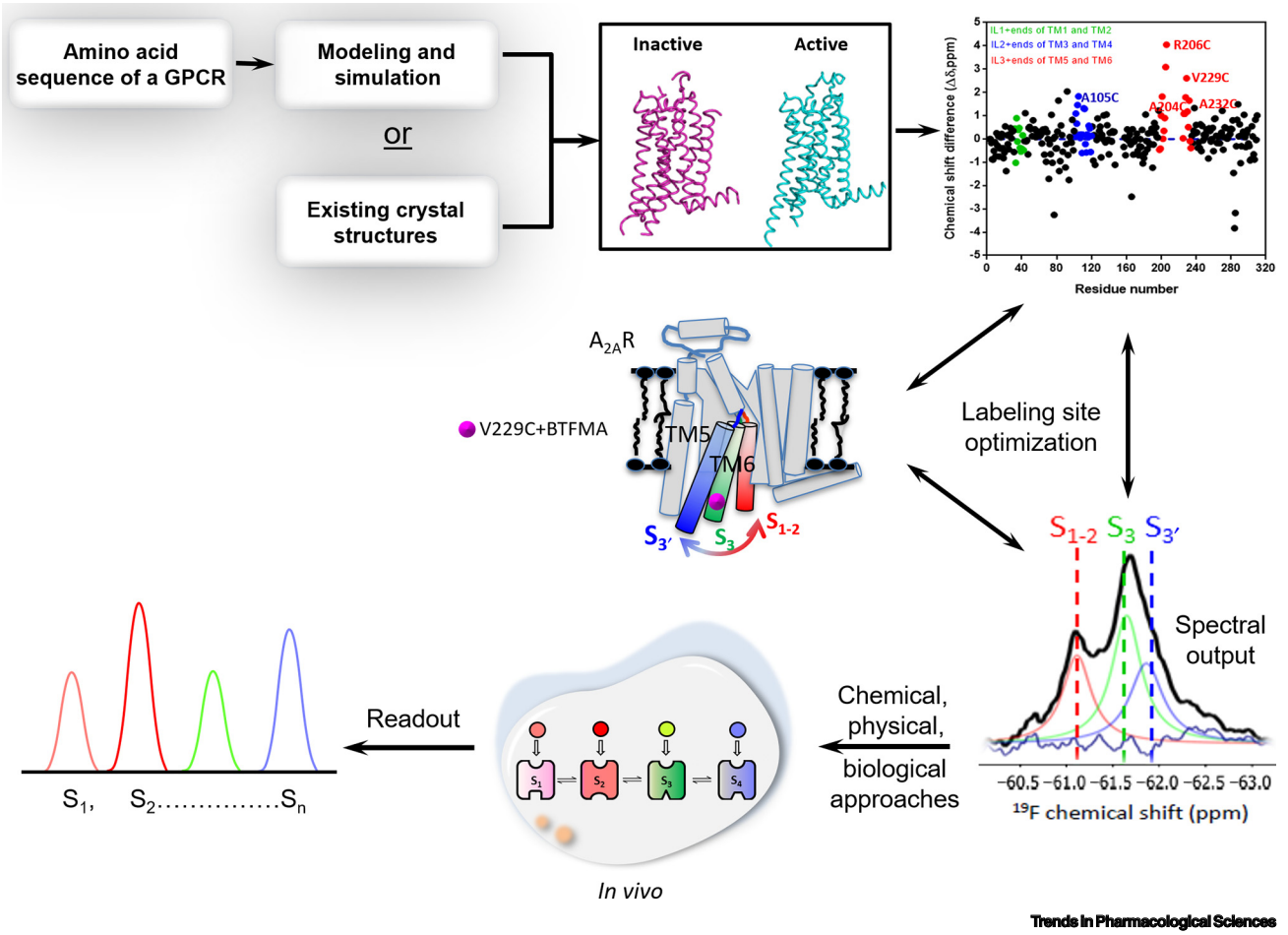

Figure 3. The Strategy of Profiling Receptor Conformation in High Resolution from Labeling Site Selection to the Approaches of Improving the Conformational Resolution. The adenosine A2A receptor ($A_{2A}R$) receptor labeling site of V229C was used as an example. Abbreviations: GPCR, G protein-coupled receptor; TM, transmembrane domain.

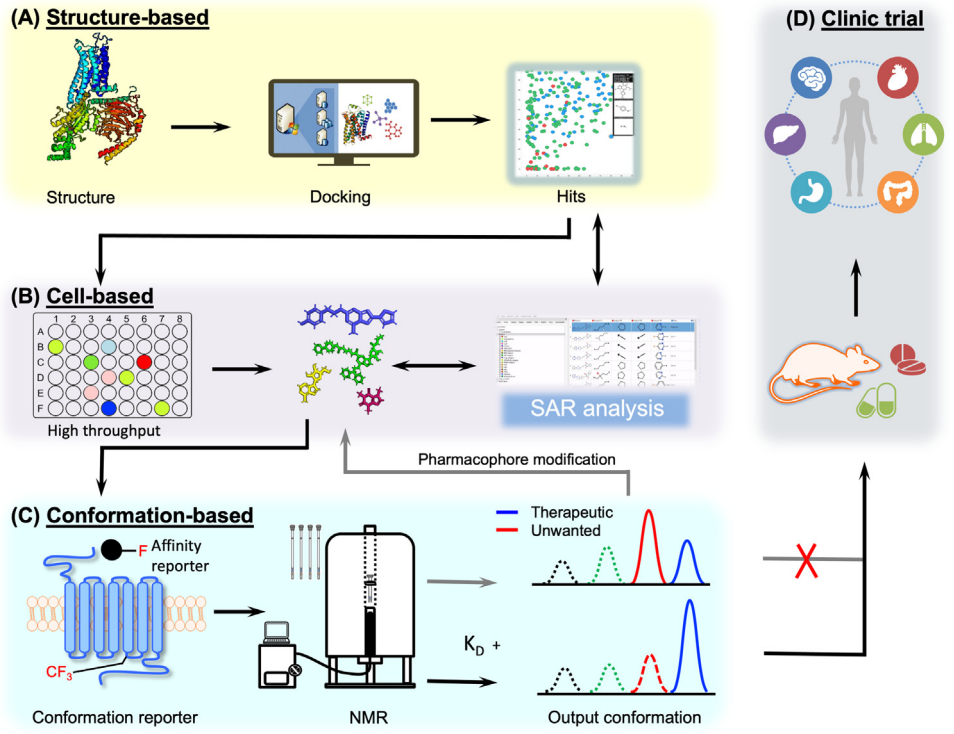

Figure 4. A Proposed Biased Drug Discovery Flowchart with Conformation Component Incorporated into a Cell- and Structure-Based Drug Screening System. (A) Structure based screening produces chemicals with potential activity (hits) that are then tested in (B) cell based screening. The hits that produce significant activity in cells are then run through (C) conformation based screening, where chemicals with significant unwanted effects are removed from the pool. Hits with this new layer of screening enter clinical trials (D), where they are tested in animal models for safety and efficacy. Abbreviation: SAR, structure activity relationship.

need to be made in order to establish a universal and applicable strategy for biased drug discovery and acquisition of a high conformational resolution profile is the first step. To address this, a combination of chemical, biological, and physical strategies could be implemented, including but not limited to:

(i) Continuing the search for a higher electrostatically sensitive ^{19}F probe: chemical screening is an endless quest, but the expectation is that it is possible to identify a new probe with even higher electrostatic sensitivity to microenvironmental changes than the current state-of-the-art probes (e.g., BTFMA) to use in future work; computational approaches will be of use in facilitating this screening process. MD simulations can in principle sample the relevant receptor conformational ensemble (possibly with the use of enhanced sampling techniques). Coupling this with quantum mechanical (QM) calculations to compute chemical shifts *in silico* of the dominant structural clusters, representing the intermediate receptor conformations, could predict ^{19}F probes and evaluate possible attachment sites in the receptor with maximal sensitivity. In our previous study, we established an empirical approach to examine the surrounding electrostatic changes for different chemical probes [18] in combination with QM calculations. Coupling this method with extensive MD simulations for conformational exploration will likely improve accuracy in predictions. In summary, computationally profiling the ^{19}F probe sensitivity by integrating candidate ^{19}F probes into the GPCR of interest

could be a more effective way to identify a specific labeling site for receptor conformational profiling, though an optimized computational protocol and a relatively large-scale computational capacity is required.

(ii) Shift reagent application: in NMR, lanthanides are a series of chemical elements that can facilitate **pseudo-contact chemical shift (PCS)** effects on other proximal nuclei. Thus, we can strategically attach a lanthanide metal to a specific location with a reasonable distance and orientation from the ^{19}F probe, which could result in a better resolution for different conformational states [75]. The disadvantage of the PCS approach is that most of the lanthanide metals also possess the **paramagnetic relaxation enhancement (PRE)** effects, causing undesirable line width broadening. Considering the difficulty and low yield of GPCR sample preparation, this line width broadening would be fatal. A macromolecular tag, such as DOTA chelated lanthanide attachment, could also affect the receptor functionality. However, expression system engineering could enable its application. The recent advance of yeast *P. pastoris* expression is promising in the large-scale preparation of functional receptor with a relatively low expense, especially in contrast to insect and mammalian expression systems where the cost is always a concern for most laboratories. In addition, the ease of isotopically labeling in the *P. pastoris* system under the control of the AOX promoter is another advantage where ^{13}C-methanol can be served as both inductor and carbon source to produce isotopically labeled receptors for NMR study.

(iii) New solvent system for receptor reconstitution: a major obstacle of the NMR application of membrane protein study is that usually a reconstitution system is applied for receptor solubilization. The increased rotational correlation time (new_tau]τ_c) caused by detergent, micelle, or nanodisc systems often leads to a shortened T2 value and, as such, a broadened and decreased NMR signal. Efforts are being made to develop nondetergent systems in hopes of increasing the τ_c. For instance, a completely detergent-free solvent system called nanoscale styrene-maleic acid (SMA) lipid particle (SMALP) has been developed, which also caused a remarkable increase in the thermostability of the receptor in comparison with n-dodecyl-β-d-maltopyranoside (DDM) [76]. Studies have indicated the promise of SMALP to reconstitute GPCR [77] and showed the engagement between the receptor and downstream partners [78]. Most recently, its applications have expanded into resolving the cryo-EM structures of alternative complex III in a supercomplex with cytochrome oxidase [79]. In comparison to nanodiscs and detergent systems, an overall smaller molecular weight assembly might offer favorable relaxation advantages for NMR application [80], though no NMR studies of SMALP-reconstituted GPCRs have been reported. An adaptable phospholipid membrane mimetic system was also developed for solution state NMR studies and it has been tested in several proteins, including GPCR [81]. A disulfide-containing detergent was also engineered for membrane structural biology studies using solution-state NMR [82].

(iv) Engineering an electrostatic environment around the ^{19}F probe: research has indicated that ^{19}F probes that manifested good conformational resolutions in various investigated receptors, including β_2AR [16,22,23], rhodopsin [83,84], and A_{2A}R [18,19], were often surrounded by aromatic amino acids. This finding offers the opportunity to increase conformational resolution by engineering the electrostatic surroundings of the ^{19}F probe.

(v) Labeling site expansion: it is possible to find a strategy of labeling residues in the hydrophobic domains in order to expand labeling sites, such as UAA incorporation, in the place of solvent-exposed domains [18,19]. A platform could be set up to provide a conformational profile with high resolution through an integrated effort using the methods mentioned earlier.

Concluding Remarks and Future Perspectives

The current drug discovery strategy is predominantly based on the measurement of dose-dependent downstream signaling such as the signaling levels of cAMP or Ca^{2+}. Therefore, receptor activation is typically described as an on/off two-state switch. As a result of this oversimplification, developed drugs based on the current system tend to overactivate or oversuppress

Outstanding Questions

How can an unbiased evaluation system, by which the individual receptor conformation can be linked to pharmacological outputs, be established?

How can a high throughput biased drug discovery system based on the delineated receptor conformational profile be established?

How can we achieve synergy between structure-based (X-ray and cryo-EM) and conformation-guided (NMR) drug discovery in the new era?

downstream signaling, resulting in concomitant side effects. A detailed mechanistic understanding of correlations between ligand, ligand-selected receptor conformation, and responsive pharmacological outputs would give scientists a critical understanding of how to rationally design a specific signaling biased drug by integrating the receptor-conformation component (see Outstanding Questions). As the technique develops, molecular cryo-EM, especially the newly developed cryo-electron tomography (cryo-ET), has shown its power to analyze repeating structures within larger pleomorphic objects using a process called cryo-ET subtomogram averaging and classification (cryoSTAC) with improved signal-to-noise ratio and map resolution [85,86]. These subtomograms can be even classified into multiple functional states or conformations [85,87]. However, the resolution is still limited to 2–4 nm, with a few cases having a resolution close to 1 nm, and cannot profile protein motion at the Ångström scale. Additionally, it is still practically impossible to get cryo-EM structures for all candidate drugs by analyzing their structures and conformations in a real drug screening process. Therefore, structure-based drug discovery systems have fundamental limitations that need complementary approaches to be successful. To that end, NMR spectroscopy is

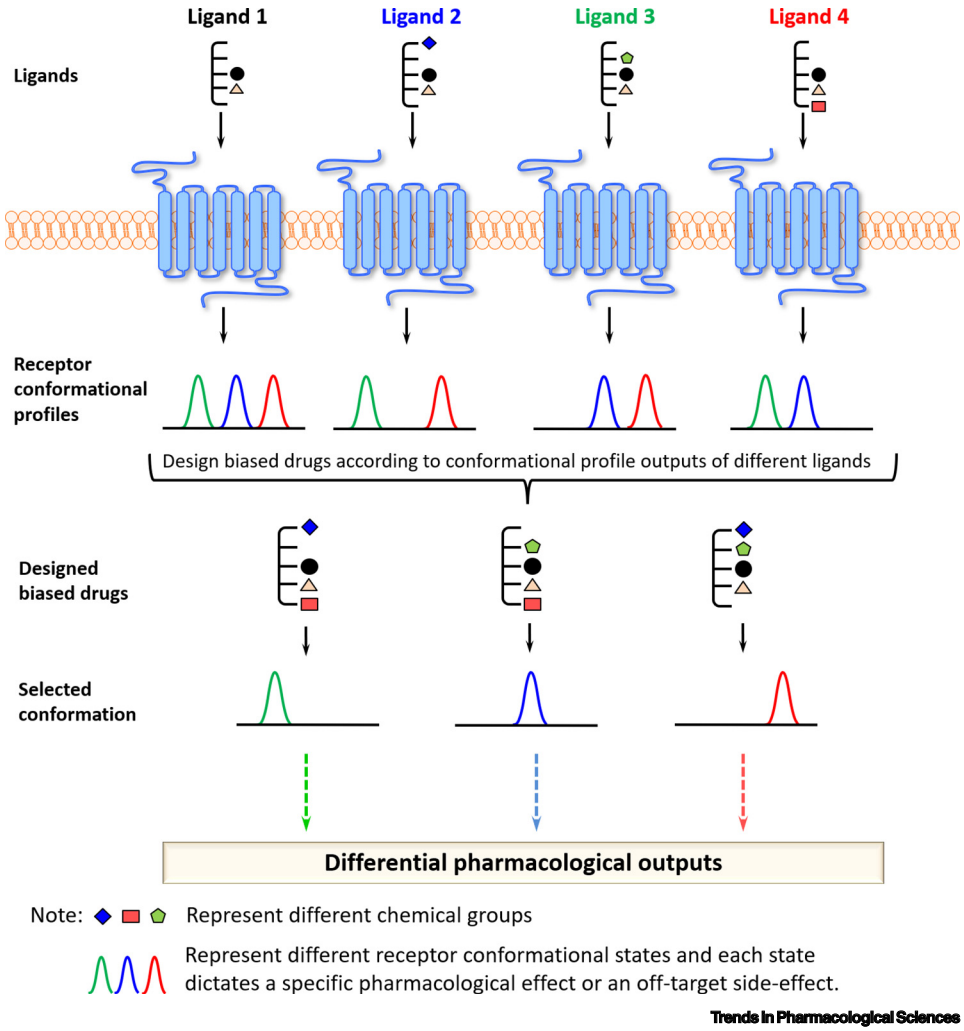

Figure 5. A Biased Drug Discovery Strategy That Correlates Receptor Conformation to the Functional Selectivity of Ligand. Note the resolution for different conformational states in this schematic merely illustrates the principle of activation by a biased drug.

poised to be an extremely powerful tool for gaining insights into the different conformations of a receptor, which can even be performed in a physiologically closed solution system for ligand competition experiments to be conducted. The dynamic behavior of individual conformational states can contribute to different downstream signaling pathways, which is impossible to capture in X-ray crystallography and cryo-EM.

We anticipate that, as the conformational resolution of receptors with NMR keeps improving, a platform correlating the ligand pharmacophore, ligand selected receptor conformation, and responsive functionality (Figure 5) could be established, enabling the rational design of biased drugs, elevating the current cell- and structure-screening system for high specific drug discovery with fewer side-effects (Figure 4). We note finally that the efficacy and efficiency of a particular signal pathway are also affected by the kinetics and dynamical aspects of the receptor, interaction effectors, and ligands; the comprehensive elaboration hereof is beyond the limited scope of this review.

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Disclaimer Statement

L.Y. is an independent principal investigator at University of South Florida as well as being affiliated with H. Lee Moffitt Cancer Center and Research Institute. None of the authors have conflicts of interest with their affiliations.

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