Published in final edited form as:
Biotechnol J. 2012 December ; 7(12): 1451–1461. doi:10.1002/biot.201200076.

Structure-function studies with G protein-coupled receptors as a paradigm for improving drug discovery and therapeutic development

Patrick M. McNeely[1], **Andrea N. Naranjo**[1], and **Anne Skaja Robinson**[1,2]

[1]Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE 19711

[2]Department of Chemical and Biomolecular Engineering, 300 Lindy Boggs Laboratory, Tulane University, New Orleans, LA 70118

Abstract

There are a great variety of human membrane proteins, which currently form the largest group of marketed drug targets. However, despite the advances in drug design, promiscuity between drug molecules and targets often leads to undesired signaling effects, which result in side effects from treatment. In this review, one family of membrane proteins – G protein-coupled receptors (GPCRs) – is used as a model to review experimental techniques that may be used to examine the activity of membrane proteins. As these receptors are highly relevant to healthy human physiology and represent the largest family of drug targets, they represent an excellent model for membrane proteins in general. We also review experimental evidence that suggests there may be multiple ways to target a GPCR – and by extension, membrane proteins – to more effectively target unhealthy phenotypes while reducing the occurrence and severity of side effects.

1. Membrane proteins as therapeutic targets

Membrane proteins reside at intracellular interfaces and serve in a wide variety of critical cellular functions, including signal relay, cell structure, binding, and transport of molecules across otherwise impermeable membranes. Given the critical roles these proteins play in human physiology, it is of little surprise that a plurality of drugs currently on the market attenuates disease phenotypes through membrane proteins. In a study of the DrugBank [1, 2], it was found that 44% of all drugs patented since 1982 primarily target receptors. Our own examination, linking the DrugBank and SIDER databases [3], finds roughly 526 distinct membrane-localized targets that are known to interact with a drug, summarized in Table 1.

For any particular protein, there may be many more known ligands than successfully marketed drugs. One well-described subfamily of membrane proteins, the adenosine receptors – belonging to the superfamily of G protein-coupled receptors (GPCRs) – is known to interact with 12 marketed drugs [2], yet is the target of 88 known natural and synthetic ligands [4]. This receptor subfamily alone has been proposed as targets in treatments for ischemia, neurodegenerative disorders, cancer, and diabetes [5], among other conditions. The “recognition promiscuity” of drugs with the adenosine receptors, and the GPCR family generally, frequently leads to unintended physiological responses – i.e. side

Correspondence to AS Robinson at Department of Chemical and Biomolecular Engineering, 300 Lindy Boggs Laboratory, Tulane University, New Orleans, LA 70118 PH: +1 504 865-5775 asr@tulane.edu.

The authors declare no conflict of interest.

effects, as shown in Table 1. These effects present an ongoing challenge to researchers, clinicians, and patients, but also represent an opportunity to improve future generations of drugs.

As indicated in Table 1, there appears to be great potential for improving the specificity of therapeutics in the future, thus reducing the occurrence of adverse effects. Given the complexity and diversity of membrane protein families, a comprehensive review of the various types of membrane proteins and their therapeutic potential is beyond the scope of this review. Here, we will focus on current techniques that may be used to improve the effectiveness of future drugs, using studies with GPCRs as a model. We will highlight these techniques while describing advances in understanding typical orthosteric ligand binding, as well as other modes of signal modulation that have received more attention in recent years, such as allosteric ligand binding, protein-protein interactions, and protein-membrane interactions. These various mechanisms of signal modulation are illustrated in Figure 1. The experimental techniques to isolate and identify these signaling effects are well known, but must be combined usefully to better inform drug design (Figure 2), both for efficacy and to reduce the occurrence and severity of adverse effects.

2 Molecular mechanisms of GPCR signaling: Enhancing therapeutic design through structure-function studies

The canonical means of modulating molecular signaling is through orthosteric effects of a receptor, either by direct ligand-binding interaction, or by restricting access to the ligand-binding region of the receptor. This is the mechanism by which most signal modulation is achieved [6]. However, in recent years a number of alternative mechanisms have been observed in a few membrane proteins, which may have a broad applicability to membrane protein drug targeting.

Allosteric ligands act away from the traditionally recognized binding site [7], and thus do not necessarily affect ligand binding at the orthosteric site. Ligands could modulate the pharmacological response to treatment by combining the effects of ortho- and allosteric binding, thus fine-tuning the effects of treatment using each signaling mechanism. This type of approach also could potentially take advantage of differential expression profiles [8] of a membrane protein among tissues to further alleviate side effects of a drug. The synergistic effects of ortho- and allosteric ligands with varied receptor levels among tissues are not well understood, but could prove to be a valuable tool in future [9].

Protein-protein interactions have been found to modulate receptor signaling as well [10]. However, the proteins involved in these interactions may themselves be further modified, e.g. through phosphorylation [11]. Some of these interactions are known *in vivo* (e.g. RGS (Regulator of G protein Signaling) proteins [11]); thus there is potential to modulate signaling through a route other than direct ligand binding [12], perhaps in ways that are not normally present in the native system.

The lipid environment of the plasma membrane also provides an interface that has been postulated to modify receptor activity [13, 14]. Cells derived from different tissue naturally have differing lipid compositions [15], and separate domains of lipid composition have been observed within single cells as well [16]. Lipid interacting domains have been speculated to exist in receptors [17], and specific lipid-receptor interactions may further modulate receptor signaling [18]. All of these methods of signal modulation may exist simultaneously *in vivo*, as in Figure 1. Using these methods singly or in conjunction to develop novel therapies for disease may help minimize the risks of side effects through greater specificity.

3 Research methods for structure-function analysis of GPCRs

3.1 Animal models

Signaling effects of a receptor are most naturally gauged by observing phenotypic effects of modulation in the native environment. Studies of this type have been performed in a number of multicellular organisms, particularly in *Drosophila melanogaster* and *Mus musculus*. These experiments have been highly illuminating, as knockout studies have revealed critical signaling pathways required for successful embryonic development [19], cardiological protective effects [20], and a variety of neuropathic phenotypes among other results [21]. Ledent *et al.* [22] demonstrated that the knockout of adenosine A_{2A} receptor led to a mouse phenotype exhibiting increased basal anxiety and aggressiveness, and decreased stimulation by caffeine. The protein-receptor interaction mouse model study reported by Ichikawa-Shindo *et al.* [23] examined adrenomedullin/receptor activity modifying protein 2 (AM/RAMP2) and showed non-lethal effects resulted from partial knockout; $RAMP2^{+/-}$ models showed increased vascular hyperpermeability and increased blood pressure. The full knockout model ($RAMP2^{-/-}$) did not survive through embryogenesis, indicating how important the presence of the receptor interaction was for the development of the animal [23].

An even more fundamental pathway was found in studies in fruit fly models [24]. The prototypical frizzled-type GPCR pathway was identified using this model; the frizzled family of receptor has since been found to play an outsized role in everything from embryogenesis through neurological development [25]. These studies demonstrated the breadth of importance of the GPCRs and associated proteins in support of healthy, functional phenotypes in the animals. However, development of these models requires substantial resources and time, rendering these techniques intractable for refining the molecular details of receptor signaling.

3.2 Cell culture models

Cell culture has been an invaluable vehicle for the engineering of receptors. The relatively high turnaround and ease of experimentation has led to a number of advances in the understanding of receptor activity *in vivo* [26, 27]. There are numerous means of examining the behavior of receptors in the cellular environment, several of which are highlighted here.

Lead experiments typically involve cloning the cDNA of the receptor into an appropriate vector that is then expressed in a cell line of interest [28, 29]. This type of overexpression allows the examination of trafficking, regulation, and signaling behavior of a single receptor type in the cellular environment. For example, in studies addressing GPCRs of the nervous system, the γ-aminobutyric acid (GABA) receptors bind to inhibitory ligand GABA and to the excitatory ligand glutamate, and proved to be complicated to study in the native system. Thus, this receptor was cloned in the early 90s, and subsequently found to be active as a dimer of the $GABA_{B1}$ and $GABA_{B2}$ subunits [30-33]. The availability and expression of a cloned receptor enabled the development of high-throughput drug discovery and lead compound identification, including those that enabled the development of cinacalcet for hypercalcemia [34], and LY487379 and other potential compounds for schizophrenia [35].

Pitfalls have been encountered using this technique, however. GPCRs, and generally membrane proteins, are often poorly expressed in heterologous hosts, greatly limiting the proteins available for study using heterologous expression. Substantial resources have been devoted solely to the expression of these receptors in heterologous hosts over the years, and a number of studies have collected and reviewed expression data from many hosts as a means of improving the expression of receptors in general [28, 29, 36]. Heterologous host systems frequently do not have a proteome similar to the native tissue a researcher is seeking

to emulate. Observation of the native-like behavior of the receptor may require more than expression; interacting species may not be present in the heterologous system, and may be required to develop a complete picture of the signaling activity of the receptor. In spite of these potential limitations, a number of experimental techniques taking advantage of heterologous expression have been used to examine *in vivo* effects of receptor signaling.

The discovery, characterization, and subsequent cloning of fluorescent proteins have allowed receptor tagging for observing trafficking and signaling activity in the cellular environment [37]. Using simple tagging by genetic engineering methods has enabled the examination of receptor fate within the cell. This basic method has been expanded to take advantage of advanced microscopy techniques [38, 39] and facilitated a more intricate understanding of receptor behavior in the cell. Total internal reflection fluorescence (TIRF) microscopy [40] and fluorescent photoactivation localization microscopy (FPALM) [41] have been used to examine the specific localization of tagged receptors within the plasma membrane. TIRF, fluorescence correlation spectroscopy (FCS) [42], and fluorescence lifetime imaging microscopy (FLIM) [43] have also been used to examine colocalization through particle tracking or overlapping fluorescence signals.

The latter techniques have also been used to examine the oligomerization state of receptors in cells. Many recent studies have found that receptors expressed at the plasma membrane frequently form dimers, trimers, or higher order oligomers [40, 44, 45]. In GPCRs, evidence has been found that this type of mechanism operates in some, though not all, receptors [46]. The degree to which this interaction influences signaling remains an open question.

To demonstrate oligomerization, the Förster resonance energy transfer (FRET) effect is frequently used in conjunction with bioluminescent or fluorescent proteins, as described in [47, 48]. Briefly, the excited fluorophore or reacting substrate resonantly donates energy to a separate fluorophore, which emits a photon characteristic of the acceptor emission spectrum, rather than the donor. This emission may be monitored by any fluorimetric method; transfer occurs when the donor and acceptor are within a range of roughly 1-10 nm, thus giving strong evidence of colocalization. This basic technique has been refined to further examine the proximity of each fluorophore, partial fluorophore, or luciferase fusion to the other markers present in the cells. In particular, bimolecular fluorescence complementation (BiFC) [49] and luciferase complementation imaging (LCI) [50] have been engineered to allow additional, persistent protein-protein interactions to be detected. Three-chromophore FRET has also been experimentally demonstrated [51], further expanding the degree of the interaction network that may be examined simultaneously.

However, given the nature of the experiment and the limited area of the membrane, it is difficult to establish the significance of these interactions. It may be challenging to eliminate the possibility of false positives in the measurements, particularly as the number of tagged proteins in the complex increases. Additionally, receptors are often overexpressed in engineered cell lines to collect this type of data. With many fewer receptors at the plasma membrane in native tissue, the burden of proof remains on the researcher to confirm the physiological relevance of oligomerization in native tissue [52]. The presence of confirmed inter-receptor interactions could introduce a novel therapeutic path for treatment; thus continued research into the interaction phenomenon is important.

Downstream signaling data may be used to experimentally determine the activation state of receptor-mediated pathways. A variety of methods to examine this activation have been developed. For example, commercially available kits based on intracellular Ca^{2+} or cAMP (cyclic adenosine monophosphate) accumulation are available to monitor downstream signaling activity resulting from GPCR activation, providing high throughput signaling

assays. These techniques require the chemical treatment of cells to obtain signaling data and thus may be time-intensive, but have been applied successfully with mammalian systems [53]. Additionally, endogenous downstream pathway activation in *Saccharomyces cerevisiae* may be linked to an observable signal; β-galactosidase activity [54], growth [55], or fluorescence-based [56] signaling assays have been used with live cells. Fluorescence and growth-based assays of this type, in particular, have improved the overall throughput of examining signaling activity in cells. As with any system, there are caveats to monitoring signaling activity in mammalian, insect, or fungal systems. Generally, insect or mammalian cells are capable of folding and expressing the receptor, but not successfully in all cases, and are expensive and time consuming to use [36]. Conversely, in lower eukaryotic systems, the receptors may express poorly and concerns remain over appropriate signal relay in the heterologous host.

Genetic mutation has also been used to examine receptor-signaling mechanisms. Directed and random mutagenesis studies have been used to identify residues of the receptor that are critical for ligand-binding activity, downstream signaling, or successful trafficking through the secretory pathway [57, 58]. Once a heterologous host organism has been identified, it is also possible to use scanning mutagenesis to identify thermostabilizing mutations for further study [59]. As described in Section 3.3, this technique has been used to identify stabilized forms of a given receptor for structural analysis [60], as well as for studying signal dynamics and to elucidate structural insight from biophysical characterization [61]. Mutagenic methods have thus become an invaluable tool for probing the structures of receptors in the cellular environment, as well as a tool for *in vitro* analysis.

3.3 Purification and structural analysis

Heterologous expression of GPCRs has pressed forward over the years as the most straightforward alternative to models in native tissues or animals, and remains the most frequent means of obtaining large quantities of protein for further characterization *in vitro*. Only rhodopsin has been crystallized from its native tissue with its intact sequence; all other GPCRs have required protein engineering to stabilize the receptor or facilitate crystallization [62]. Protein engineering and expression have been performed using a variety of model cell systems, including bacteria, yeast, insect, and mammalian cell lines [28, 63, 64]. Expression in all of these systems has yielded mixed results, leading to the continued use of trial and error approaches to determine the best expression system for any particular receptor [29]. When an adequate expression system is identified, these cell lines produce sufficient protein sample for further study using powerful *in vitro* methods.

Following expression, purification of membrane proteins from culture has been the major bottleneck preventing further characterization [63]. Structural crystallography and other biophysical studies require solubilization and purification from the cellular debris. Detergents must be tested for the solubilization and structural stabilization of the membrane protein [65, 66]. Detergent interaction could compromise the protein activity through direct interactions, or due to physical properties of micelles, as micelle dimensions are expandable and have a high degree of curvature [67]. These characteristics may not appropriately mimic the membrane environment and can pose challenges for crystal contact formation [61, 68]. The end goal of this lengthy process is to obtain a highly purified receptor that retains its native structure, dynamics and ligand-binding functionality in order to properly model *in vivo* signaling activity [67].

Historically, high-resolution structures have been the ideal paradigm for understanding the mechanism of protein interactions and how the structure relates to the biological function of membrane proteins. Most drug discovery projects against soluble protein targets have used medicinal chemistry, directed or guided by the crystal structures, at some stage of the project

[69]. Structure-based drug discovery methods have not been widely applied to GPCRs, and membrane proteins in general, due at least partially to the lack of crystal structures. There have been more challenges to structural resolution of membrane proteins than originally anticipated, in part due to the low expression level of membrane proteins in their native tissue [70], the instability of receptors in detergent solutions, and structural or conformational flexibility [67] of the purified protein. As of August 2012, there were 83,983 protein structures in the RCSB Protein Data Bank. Of these, less than 3% of the structures were of membrane-associated proteins and peptides [15, 71], corresponding to 355 unique membrane proteins (data obtained from Stephen White's website, http://blanco.biomol.uci.edu/mpstruc/listAll/list). Thus, structural knowledge for membrane proteins remains far underrepresented compared to that of soluble proteins [72].

However, the rate of the structural determination of membrane proteins has increased in the past twelve years, and there are now high-resolution crystal structures for fourteen different GPCRs, as part of the 355 aforementioned structures. For a recent review on high-resolution GPCR crystal structures, refer to [73]; since this review, the structures of four more GPCRs have been determined: Sphingosine 1-phosphate receptor, M2 and M3 human muscarinic acetylcholine receptors, κ-opioid receptor, μ-opioid receptor, δ-opioid receptor, and nociceptin/orphanin FQ receptor [74-79]. Some of these structures were determined with a variety of bound ligands (both agonist and antagonist), lipids, or the intracellular G protein [62, 80, 81]. These crystal structures revealed common and diverse features of the GPCRs, important characteristics of the ligand-binding pocket, GPCR motifs, possible allosteric sites, dimerization interfaces and structural conformational changes in the receptor important for G protein interaction [73, 80]. These structures, together with continuing advances in expression and crystallization of membrane proteins, will open the possibility to use structure-based methods for the identification and design of new pharmaceuticals targeted to these membrane proteins [69, 80].

The structural knowledge gained from these experiments has opened valuable insights into the activity of receptors while introducing new questions, as the complexity of the signaling pathway has become clearer. The emergence of new types of ligand-receptor-effector relationships [62], and the understanding of how lipids and oligomerization state modulate signaling have provided evidence for signaling mechanisms away from the orthosteric ligand binding site that may provide rich targets for therapeutics [73]. Many of these questions may be answered with more high-resolution crystal structures of membrane proteins, specifically capturing transitional structures as the receptor enters an active signaling state [82]; or through structural data of the receptors in native-like environments [67]. However, the mechanisms behind receptor signaling may also be elucidated by building upon structural information, with complementary dynamic observation methodologies.

3.4 Establishing a native-like environment in membrane-mimetics

Once the receptor is purified using detergents it may be reconstituted in lipids or other detergents to further stabilize the receptor. The choice of molecule for solubilizing and reconstituting the receptor is critical for the biophysical measurements to be taken, as different methods will leave the receptor in micelles, bicelles, liposomes, or in supported bilayers [83]. The geometry and composition of the membrane environment is critical not only for the characterization technique being used, but also for the membrane protein itself. There is growing evidence of the importance of the membrane environment for the modulation of membrane protein function [80]. Differential receptor activity has been reported in tissues or membranes with different lipid composition [80, 84], though in these cases it remains unclear whether the interaction is directed through specific lipid-protein

interactions or due to differences in the physical characteristics of the membrane (e.g. curvature, fluidity, and thickness) [80, 85].

Recent experiments have found that reconstitution of at least one receptor (β_2-adrenergic receptor) in lipid bilayers or micelles that mimic native conditions better recovers the native signaling properties of the receptor [86]. Purified adenosine receptor A2A loses its ligand binding activity when reconstituted in detergent, but when reconstituted in detergent with a cholesterol analogue, the receptor retains native ligand-binding affinity. When this micellar system was characterized using small-angle neutron scattering, the addition of cholesterol changed the micelle size (ellipsoidal with a thickness of 32 Å), and so better reflected the thickness of mammalian membranes [87]. Thus, it remains unclear whether the cholesterol is essential for signaling due to direct binding to the protein or due to the changes in the micellar environment.

3.5 Observing dynamic interactions of membrane proteins

By monitoring the dynamic response of the receptor during an experiment, a deeper understanding of the structural motions involved in signaling may be achieved. Subtle interactions among lipids, membrane proteins and the effector molecules sharing the membrane environment with the GPCR may be captured. Many techniques have been used to collect dynamic data regarding various regions within the receptor, and have the advantage that the receptor may be stabilized in native-like environments following purification and its function can be tested using biologically relevant conditions.

One of the most fundamental types of studies is to investigate binding of extracellular ligand to receptor. When designing experiments to investigate the receptor ligand-binding interaction, it is important to consider the desired ligand characteristics in order to choose the appropriate technique to capture these interactions. For small molecules, fluorescent [88, 89] or radiolabeled [52] ligands may be available and the ligand binding can be tested using purified or reconstituted receptors, membrane preparations, or whole cells [90, 91]. When labeled ligands are not available, downstream monitoring methods may be used, as discussed in Section 3.2 and in [91]. Additionally, surface plasmon resonance (SPR) methods have been used to examine the binding of receptor to known, though unlabeled, ligand [90, 92, 93]. Beyond observing ligand-binding behavior in well-established cases, there are orphan receptors – those with no known ligands – and there is much literature dedicated to classifying and de-orphaning these receptors [94, 95].

The changes in receptor conformation associated with ligand binding may be monitored by observing the intrinsic tryptophan fluorescence. A receptor with a tryptophan residue that is close to the binding pocket or in an area that undergoes a conformational change upon ligand-binding experiences a change in the local hydrophobicity of the environment; in turn, this may be quantitatively determined by changes in fluorescence intensity. When coupled with static structural data and the location of tryptophan in the protein, the dynamics of the receptor may be captured as the receptor changes conformation, e.g. upon addition of ligands. Many groups have used this technique to calculate the dissociation constant or to ensure that the purified receptor retains ligand-binding activity [96]. Environmentally sensitive probes and intrinsic fluorescence have also been used to monitor the interaction between receptors and G proteins; fluorescence was measured and conformational changes and binding rates were observed with various ligands and detergent and lipid compositions [97-99].

This principle has been extended to examine receptors using probes other than tryptophan, which are likewise sensitive to the local environment. Both Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) studies have taken advantage of these

effects to inspect the subtle conformational changes of the receptor during ligand-binding events, and have shown the ability to discriminate between the changes in signaling pathway activation using different agonists. For example, a particularly detailed and careful study of rhodopsin using FTIR combined with *p*-azido-L-phenylalanine probes enabled the analysis of electrostatic interactions during conformational transitions in rhodopsin [100]. Another study principally using NMR was able to determine mechanistic behavior of ligand signaling sensitivity in the β_2-adrenergic receptor [101]. Using local probes in these manners yields powerful insights into the conformational consequences of signaling activity, and so potentially identifies a means through which to modulate cellular activity with new drug designs.

The changes in receptor conformation associated with the membrane environment may also be traced with the methods mentioned above. It is also possible to study specific protein-lipid properties, such as the lipid binding site, protein affinity for lipids, lipid localization and interaction dynamics [102]. In particular, as the resolution of GPCR structures increases, specific lipid contacts may be identified. For example, one of the latest GPCR structures (PDB 4EIY) was determined at 1.8 Å resolution; 57 water, 2 cholesterol, and 23 lipid molecules were identified with the receptor [103].

Possible signal modulation by lipids has been observed *in vitro* using nanodiscs, where the addition of negatively charged lipids did not affect ligand binding activity; however, the lipid charge had a strong influence on the GPCR-G protein interaction [104]. Understanding the role of lipids in the function of membrane proteins will reveal important details about receptor segregation, trafficking, activation, and downstream signaling. Once we understand the lipid-receptor interactions we may be able to target the lipids of the membrane itself for therapy. For example, in native tissue, toxins may generate their effects via interactions with the membrane itself, thereby impairing the function of multiple membrane proteins [85]. Creating therapeutics to target a cell surface could thus be an effective option, in principle.

Furthermore, drug-targeting strategies may be designed to take advantage of specific membrane compositions, or to introduce novel interaction with receptors at the cell surface. The principle of such mechanisms has been demonstrated by engineering the surface of therapeutic cells or the membranes of target cells by coupling immune-inhibitory ligands or small molecules, expressing artificial receptors or insertion of polymers, peptides and proteins [105]. Drug design taking advantage of these mechanisms has the potential to redefine cell adhesion, migration, tissue homing, and cell-cell interaction [105].

3.6 Computational modeling

Over the last decade, advances in computer hardware and algorithm design have made molecular dynamic (MD) simulation much more tractable [106, 107]. All-atom MD simulations can allow the characterization of the motions of ligand-bound or ligand-free states, as well as interaction of the protein with the membrane environment (e.g. GPCR signaling with lipid dynamics has been modeled [108]). Multi-scale simulations, ranging from full atomistic to coarse-grained models, have been used to study membrane components at various levels of complexity, giving a balance between enough detail to describe the features of interest and limited computer power [83]. As more structural data becomes available, and the cost of computational power falls, MD should provide increasingly cost- and time-effective insight into signaling behavior.

Similarly to MD, docking simulations based on homology modeling have been performed as more crystal structures become available [109, 110]. One recent example of this approach utilized the crystal structure of β_2-adrenergic receptor to screen one million compounds,

followed by experimental testing of twenty-five compounds, yielding six active compounds, of which one obtained an affinity of 9 nM, suggesting great promise for this approach [111].

Another example involves the histamine receptor family: H1 has been the target of antihistamine drugs and H4 is being investigated as the target for drugs to treat asthma and itching. H1 antihistamine efficacy might be improved if H4 is targeted in conjunction, in order to reduce further itching and inflammation [112]. Using a structure-based virtual fragment screening protocol, de Graaf *et al.* [113] identified two fragments that could potentially target the H1 and H4 receptors. Taken together, these advances argue for the rational development of dual H1-H4 ligands with synergistic anti-allergenic, anti-itching, and anti-inflammatory properties. Though these studies are less rigorous than MD simulation, they are less computationally intensive and provide for higher drug screening throughput. Thus docking studies may provide a useful aid in future studies, in addition to MD [114].

4 Novel biopharmaceuticals targeting GPCR interfaces

As this review has shown, a plethora of analytical techniques has been used to determine the key components of receptor signaling, both *in vivo* and *in vitro*. The complexity of these signaling systems is exceptional, however, and using the correct tools to precisely determine the mechanism of signaling within tissue at the cellular level remains challenging. The basic mechanism – ligand binding, conformational change, and intracellular relay – through which receptors affect cell signaling are well understood, and yet this protein family is still yielding surprises today. As more is learned about the nuances of signaling, there will be more opportunities for improving the specificity of therapeutics, refining the effects of pharmaceuticals and perhaps alleviating unwanted side effects. As the interfacial phenomena of membrane proteins become better understood, the applicability of drugs targeting these regions of the receptors will only grow.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by funding from NSF CBET 1033268, NIH T32 GH08550 (PMM) and an NSF Graduate Research Fellowship (ANN).

References

[1]. Rask-Andersen M, Almen MS, Schioth HB. Trends in the exploitation of novel drug targets. Nat Rev Drug Discov. 2011; 10:579–590. [PubMed: 21804595]

[2]. Knox C, Law V, Jewison T, Liu P, Ly S, et al. DrugBank 3.0: a comprehensive resource for ‘omics’ research on drugs. Nucleic Acids Res. 2011; 39:D1035–1041. [PubMed: 21059682]

[3]. Kuhn M, Campillos M, Letunic I, Jensen LJ, Bork P. A side effect resource to capture phenotypic effects of drugs. Mol Syst Biol. 2010; 6:343. [PubMed: 20087340]

[4]. Muller CE, Jacobson KA. Recent developments in adenosine receptor ligands and their potential as novel drugs. Biochim Biophys Acta. 2011; 1808:1290–1308. [PubMed: 21185259]

[5]. Jacobson KA, Gao ZG. Adenosine receptors as therapeutic targets. Nat Rev Drug Discov. 2006; 5:247–264. [PubMed: 16518376]

[6]. Millar RP, Newton CL. The year in G protein-coupled receptor research. Mol Endocrinol. 2010; 24:261–274. [PubMed: 20019124]

[7]. Goudet C, Magnaghi V, Landry M, Nagy F, Gereau R. W. t. et al. Metabotropic receptors for glutamate and GABA in pain. Brain Res Rev. 2009; 60:43–56. [PubMed: 19146876]

[8]. Barrett T, Troup DB, Wilhite SE, Ledoux P, Rudnev D, et al. NCBI GEO: archive for high-throughput functional genomic data. Nucleic Acids Res. 2009; 37:D885–890. [PubMed: 18940857]

[9]. Goblyos A, Ijzerman AP. Allosteric modulation of adenosine receptors. Biochim Biophys Acta. 2011; 1808:1309–1318. [PubMed: 20599682]

[10]. Ritter SL, Hall RA. Fine-tuning of GPCR activity by receptor-interacting proteins. Nat Rev Mol Cell Biol. 2009; 10:819–830. [PubMed: 19935667]

[11]. Sjogren B. Regulator of G protein signaling proteins as drug targets: current state and future possibilities. Adv Pharmacol. 2011; 62:315–347. [PubMed: 21907914]

[12]. Kimple AJ, Bosch DE, Giguere PM, Siderovski DP. Regulators of G-protein signaling and their Galpha substrates: promises and challenges in their use as drug discovery targets. Pharmacol Rev. 2011; 63:728–749. [PubMed: 21737532]

[13]. Lee AG. Biological membranes: the importance of molecular detail. Trends Biochem Sci. 2011; 36:493–500. [PubMed: 21855348]

[14]. Chini B, Parenti M. G-protein-coupled receptors, cholesterol and palmitoylation: facts about fats. J Mol Endocrinol. 2009; 42:371–379. [PubMed: 19131499]

[15]. Lomize MA, Lomize AL, Pogozheva ID, Mosberg HI. OPM: orientations of proteins in membranes database. Bioinformatics. 2006; 22:623–625. [PubMed: 16397007]

[16]. Ziolkowska NE, Christiano R, Walther TC. Organized living: formation mechanisms and functions of plasma membrane domains in yeast. Trends Cell Biol. 2012; 22:151–158. [PubMed: 22245053]

[17]. Pucadyil TJ, Chattopadhyay A. Role of cholesterol in the function and organization of G-protein coupled receptors. Prog Lipid Res. 2006; 45:295–333. [PubMed: 16616960]

[18]. Fantini J, Barrantes FJ. Sphingolipid/cholesterol regulation of neurotransmitter receptor conformation and function. Biochim Biophys Acta. 2009; 1788:2345–2361. [PubMed: 19733149]

[19]. Matise MP, Wang H. Sonic hedgehog signaling in the developing CNS where it has been and where it is going. Curr Top Dev Biol. 2011; 97:75–117. [PubMed: 22074603]

[20]. Zhan E, McIntosh VJ, Lasley RD. Adenosine AA and AB receptors are both required for adenosine A receptor-mediated cardioprotection. Am J Physiol Heart Circ Physiol. 2011; 301:H1183–1189. [PubMed: 21743001]

[21]. Armentero MT, Pinna A, Ferre S, Lanciego JL, Muller CE, et al. Past, present and future of A(2A) adenosine receptor antagonists in the therapy of Parkinson's disease. Pharmacol Ther. 2011; 132:280–299. [PubMed: 21810444]

[22]. Ledent C, Vaugeois JM, Schiffmann SN, Pedrazzini T, El Yacoubi M, et al. Aggressiveness, hypoalgesia and high blood pressure in mice lacking the adenosine A2a receptor. Nature. 1997; 388:674–678. [PubMed: 9262401]

[23]. Ichikawa-Shindo Y, Sakurai T, Kamiyoshi A, Kawate H, Iinuma N, et al. The GPCR modulator protein RAMP2 is essential for angiogenesis and vascular integrity. J Clin Invest. 2008; 118:29–39. [PubMed: 18097473]

[24]. Lum L, Beachy PA. The Hedgehog response network: sensors, switches, and routers. Science. 2004; 304:1755–1759. [PubMed: 15205520]

[25]. Schulte G. International Union of Basic and Clinical Pharmacology. LXXX. The class Frizzled receptors. Pharmacol Rev. 2010; 62:632–667. [PubMed: 21079039]

[26]. Conner M, Hawtin SR, Simms J, Wootten D, Lawson Z, et al. Systematic analysis of the entire second extracellular loop of the V(1a) vasopressin receptor: key residues, conserved throughout a G-protein-coupled receptor family, identified. J Biol Chem. 2007; 282:17405–17412. [PubMed: 17403667]

[27]. Gsandtner I, Freissmuth M. A tail of two signals: the C terminus of the A(2A)-adenosine receptor recruits alternative signaling pathways. Mol Pharmacol. 2006; 70:447–449. [PubMed: 16707626]

[28]. Lundstrom K, Wagner R, Reinhart C, Desmyter A, Cherouati N, et al. Structural genomics on membrane proteins: comparison of more than 100 GPCRs in 3 expression systems. Journal of Structural and Functional Genomics. 2006; 7:77–91. [PubMed: 17120110]

[29]. Sarramegna V, Muller I, Milon A, Talmont F. Recombinant G protein-coupled receptors from expression to renaturation: a challenge towards structure. Cellular and Molecular Life Sciences (CMLS). 2006; 63:1149–1164. [PubMed: 16568239]

[30]. Kaupmann K, Malitschek B, Schuler V, Heid J, Froestl W, et al. GABA(B)-receptor subtypes assemble into functional heteromeric complexes. Nature. 1998; 396:683–687. [PubMed: 9872317]

[31]. Ng GY, Clark J, Coulombe N, Ethier N, Hebert TE, et al. Identification of a GABAB receptor subunit, gb2, required for functional GABAB receptor activity. J Biol Chem. 1999; 274:7607–7610. [PubMed: 10075644]

[32]. White JH, Wise A, Main MJ, Green A, Fraser NJ, et al. Heterodimerization is required for the formation of a functional GABA(B) receptor. Nature. 1998; 396:679–682. [PubMed: 9872316]

[33]. Margeta-Mitrovic M, Jan YN, Jan LY. A trafficking checkpoint controls GABA(B) receptor heterodimerization. Neuron. 2000; 27:97–106. [PubMed: 10939334]

[34]. Harrington PE, Fotsch C. Calcium sensing receptor activators: calcimimetics. Curr Med Chem. 2007; 14:3027–3034. [PubMed: 18220738]

[35]. Rudd MT, McCauley JA. Positive allosteric modulators of the metabotropic glutamate receptor subtype 2 (mGluR2). Curr Top Med Chem. 2005; 5:869–884. [PubMed: 16178732]

[36]. Chiu ML, Tsang C, Grihalde N, MacWilliams MP. Over-Expression, Solubilization, and Purification of G Protein-Coupled Receptors for Structural Biology. Combinatorial Chemistry & High Throughput Screening. 2008:439–462. [PubMed: 18673272]

[37]. Shaner NC, Steinbach PA, Tsien RY. A guide to choosing fluorescent proteins. Nat Methods. 2005; 2:905–909. [PubMed: 16299475]

[38]. Leung BO, Chou KC. Review of super-resolution fluorescence microscopy for biology. Appl Spectrosc. 2011; 65:967–980. [PubMed: 21929850]

[39]. Timpson P, McGhee EJ, Anderson KI. Imaging molecular dynamics in vivo--from cell biology to animal models. J Cell Sci. 2011; 124:2877–2890. [PubMed: 21878495]

[40]. Hern JA, Baig AH, Mashanov GI, Birdsall B, Corrie JE, et al. Formation and dissociation of M1 muscarinic receptor dimers seen by total internal reflection fluorescence imaging of single molecules. Proc Natl Acad Sci U S A. 2010; 107:2693–2698. [PubMed: 20133736]

[41]. Annibale P, Vanni S, Scarselli M, Rothlisberger U, Radenovic A. Quantitative photo activated localization microscopy: unraveling the effects of photoblinking. PLoS One. 2011; 6:e22678. [PubMed: 21818365]

[42]. Briddon SJ, Hill SJ. Pharmacology under the microscope: the use of fluorescence correlation spectroscopy to determine the properties of ligand-receptor complexes. Trends Pharmacol Sci. 2007; 28:637–645. [PubMed: 18001848]

[43]. Lukasiewicz S, Blasiak E, Faron-Gorecka A, Polit A, Tworzydlo M, et al. Fluorescence studies of homooligomerization of adenosine A2A and serotonin 5-HT1A receptors reveal the specificity of receptor interactions in the plasma membrane. Pharmacol Rep. 2007; 59:379–392. [PubMed: 17901566]

[44]. Kasai RS, Suzuki KG, Prossnitz ER, Koyama-Honda I, Nakada C, et al. Full characterization of GPCR monomer-dimer dynamic equilibrium by single molecule imaging. J Cell Biol. 2011; 192:463–480. [PubMed: 21300851]

[45]. Moriyama K, Sitkovsky MV. Adenosine A2A receptor is involved in cell surface expression of A2B receptor. J Biol Chem. 2010; 285:39271–39288. [PubMed: 20926384]

[46]. Gurevich VV, Gurevich EV. How and why do GPCRs dimerize? Trends Pharmacol Sci. 2008; 29:234–240. [PubMed: 18384890]

[47]. Albizu L, Cottet M, Kralikova M, Stoev S, Seyer R, et al. Time-resolved FRET between GPCR ligands reveals oligomers in native tissues. Nat Chem Biol. 2010; 6:587–594. [PubMed: 20622858]

[48]. Hein P, Frank M, Lohse MJ, Buenemann M. FRET analysis of receptor/G protein-interactions in intact cells. Biophysical Journal. 2005; 88:532A–533A.

[49]. Hu CD, Kerppola TK. Simultaneous visualization of multiple protein interactions in living cells using multicolor fluorescence complementation analysis. Nat Biotechnol. 2003; 21:539–545. [PubMed: 12692560]

[50]. Luker KE, Smith MC, Luker GD, Gammon ST, Piwnica-Worms H, et al. Kinetics of regulated protein-protein interactions revealed with firefly luciferase complementation imaging in cells and living animals. Proc Natl Acad Sci U S A. 2004; 101:12288–12293. [PubMed: 15284440]

[51]. Galperin E, Verkhusha VV, Sorkin A. Three-chromophore FRET microscopy to analyze multiprotein interactions in living cells. Nat Methods. 2004; 1:209–217. [PubMed: 15782196]

[52]. Fredholm BB, AP IJ, Jacobson KA, Linden J, Muller CE. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors--an update. Pharmacol Rev. 2011; 63:1–34. [PubMed: 21303899]

[53]. Stallaert W, Dorn JF, Westhuizen E, Audet M, Bouvier M. Impedance responses reveal beta-adrenergic receptor signaling pluridimensionality and allow classification of ligands with distinct signaling profiles. PLoS One. 2012; 7:e29420. [PubMed: 22242170]

[54]. Brown AJ, Dyos SL, Whiteway MS, White JH, Watson MA, et al. Functional coupling of mammalian receptors to the yeast mating pathway using novel yeast/mammalian G protein alpha-subunit chimeras. Yeast. 2000; 16:11–22. [PubMed: 10620771]

[55]. Pausch MH. G-protein-coupled receptors in Saccharomyces cerevisiae: high-throughput screening assays for drug discovery. Trends Biotechnol. 1997; 15:487–494. [PubMed: 9418303]

[56]. Ishii J, Matsumura S, Kimura S, Tatematsu K, Kuroda S, et al. Quantitative and dynamic analyses of G protein-coupled receptor signaling in yeast using Fus1, enhanced green fluorescence protein (EGFP), and His3 fusion protein. Biotechnol Prog. 2006; 22:954–960. [PubMed: 16889369]

[57]. Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc Natl Acad Sci U S A. 2007; 104:5163–5168. [PubMed: 17360345]

[58]. Sarkar CA, Dodevski I, Kenig M, Dudli S, Mohr A, et al. Directed evolution of a G protein-coupled receptor for expression, stability, and binding selectivity. Proc Natl Acad Sci U S A. 2008; 105:14808–14813. [PubMed: 18812512]

[59]. Magnani F, Shibata Y, Serrano-Vega MJ, Tate CG. Co-evolving stability and conformational homogeneity of the human adenosine A2a receptor. Proc Natl Acad Sci U S A. 2008; 105:10744–10749. [PubMed: 18664584]

[60]. Lebon G, Warne T, Edwards PC, Bennett K, Langmead CJ, et al. Agonist-bound adenosine A2A receptor structures reveal common features of GPCR activation. Nature. 2011; 474:521–525. [PubMed: 21593763]

[61]. Tate CG. Practical considerations of membrane protein instability during purification and crystallisation. Methods Mol Biol. 2010; 601:187–203. [PubMed: 20099147]

[62]. Salon JA, Lodowski DT, Palczewski K. The significance of G protein-coupled receptor crystallography for drug discovery. Pharmacol Rev. 2011; 63:901–937. [PubMed: 21969326]

[63]. Baneres JL, Popot JL, Mouillac B. New advances in production and functional folding of G-protein-coupled receptors. Trends Biotechnol. 2011; 29:314–322. [PubMed: 21497924]

[64]. Sarramegna V, Talmont F, Demange P, Milon A. Heterologous expression of G-protein-coupled receptors: comparison of expression systems from the standpoint of largescale production and purification. Cellular and Molecular Life Sciences (CMLS). 2003; 60:1529–1546. [PubMed: 14513829]

[65]. Columbus L, Lipfert J, Jambunathan K, Fox DA, Sim AY, et al. Mixing and matching detergents for membrane protein NMR structure determination. J Am Chem Soc. 2009; 131:7320–7326. [PubMed: 19425578]

[66]. Prive GG. Detergents for the stabilization and crystallization of membrane proteins. Methods. 2007; 41:388–397. [PubMed: 17367711]

[67]. Cross TA, Sharma M, Yi M, Zhou HX. Influence of solubilizing environments on membrane protein structures. Trends Biochem Sci. 2011; 36:117–125. [PubMed: 20724162]

[68]. Sonoda Y, Cameron A, Newstead S, Omote H, Moriyama Y, et al. Tricks of the trade used to accelerate high-resolution structure determination of membrane proteins. FEBS Lett. 2010; 584:2539–2547. [PubMed: 20394746]

[69]. Hubbard RE. Structure-based drug discovery and protein targets in the CNS. Neuropharmacology. 2011; 60:7–23. [PubMed: 20673774]

[70]. Wisedchaisri G, Reichow SL, Gonen T. Advances in structural and functional analysis of membrane proteins by electron crystallography. Structure. 2011; 19:1381–1393. [PubMed: 22000511]

[71]. Raman P, Cherezov V, Caffrey M. The Membrane Protein Data Bank. Cell Mol Life Sci. 2006; 63:36–51. [PubMed: 16314922]

[72]. Bowie JU. Solving the membrane protein folding problem. Nature. 2005; 438:581–589. [PubMed: 16319877]

[73]. Katritch V, Cherezov V, Stevens RC. Diversity and modularity of G protein-coupled receptor structures. Trends Pharmacol Sci. 2012; 33:17–27. [PubMed: 22032986]

[74]. Kruse AC, Hu J, Pan AC, Arlow DH, Rosenbaum DM, et al. Structure and dynamics of the M3 muscarinic acetylcholine receptor. Nature. 2012; 482:552–556. [PubMed: 22358844]

[75]. Haga K, Kruse AC, Asada H, Yurugi-Kobayashi T, Shiroishi M, et al. Structure of the human M2 muscarinic acetylcholine receptor bound to an antagonist. Nature. 2012; 482:547–551. [PubMed: 22278061]

[76]. Hanson MA, Roth CB, Jo E, Griffith MT, Scott FL, et al. Crystal structure of a lipid G protein-coupled receptor. Science. 2012; 335:851–855. [PubMed: 22344443]

[77]. Manglik A, Kruse AC, Kobilka TS, Thian FS, Mathiesen JM, et al. Crystal structure of the micro-opioid receptor bound to a morphinan antagonist. Nature. 2012

[78]. Wu H, Wacker D, Mileni M, Katritch V, Han GW, et al. Structure of the human kappa-opioid receptor in complex with JDTic. Nature. 2012

[79]. Thompson AA, Liu W, Chun E, Katritch V, Wu H, et al. Structure of the nociceptin/orphanin FQ receptor in complex with a peptide mimetic. Nature. 2012; 485:395–399. [PubMed: 22596163]

[80]. Oates J, Watts A. Uncovering the intimate relationship between lipids, cholesterol and GPCR activation. Curr Opin Struct Biol. 2011; 21:802–807. [PubMed: 22036833]

[81]. Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, et al. Crystal structure of the beta2 adrenergic receptor-Gs protein complex. Nature. 2011; 477:549–555. [PubMed: 21772288]

[82]. Choe HW, Kim YJ, Park JH, Morizumi T, Pai EF, et al. Crystal structure of metarhodopsin II. Nature. 2011; 471:651–655. [PubMed: 21389988]

[83]. Chan YH, Boxer SG. Model membrane systems and their applications. Curr Opin Chem Biol. 2007; 11:581–587. [PubMed: 17976391]

[84]. Smith AW. Lipid-protein interactions in biological membranes: a dynamic perspective. Biochim Biophys Acta. 2012; 1818:172–177. [PubMed: 21763269]

[85]. Phillips R, Ursell T, Wiggins P, Sens P. Emerging roles for lipids in shaping membrane-protein function. Nature. 2009; 459:379–385. [PubMed: 19458714]

[86]. Whorton MR, Bokoch MP, Rasmussen SG, Huang B, Zare RN, et al. A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. Proc Natl Acad Sci U S A. 2007; 104:7682–7687. [PubMed: 17452637]

[87]. O'malley MA, Helgeson ME, Wagner NJ, Robinson AS. The Morphology and Composition of Cholesterol-Rich Micellar Nanostructures Determine Transmembrane Protein (GPCR) Activity. Biophys J. 2011; 100:L11–L13. [PubMed: 21244820]

[88]. Kuder K, Kiec-Kononowicz K. Fluorescent GPCR ligands as new tools in pharmacology. Curr Med Chem. 2008; 15:2132–2143. [PubMed: 18781940]

[89]. Middleton RJ, Kellam B. Fluorophore-tagged GPCR ligands. Curr Opin Chem Biol. 2005; 9:517–525. [PubMed: 16125436]

[90]. Robertson N, Jazayeri A, Errey J, Baig A, Hurrell E, et al. The properties of thermostabilised G protein-coupled receptors (StaRs) and their use in drug discovery. Neuropharmacology. 2011; 60:36–44. [PubMed: 20624408]

[91]. Siehler S. Cell-based assays in GPCR drug discovery. Biotechnol J. 2008; 3:471–483. [PubMed: 18383022]

[92]. Chen K, Obinata H, Izumi T. Detection of G protein-coupled receptor-mediated cellular response involved in cytoskeletal rearrangement using surface plasmon resonance. Biosens Bioelectron. 2010; 25:1675–1680. [PubMed: 20044245]

[93]. Mizuguchi T, Uchimura H, Kataoka H, Akaji K, Kiso Y, et al. Intact-cell-based surface plasmon resonance measurements for ligand affinity evaluation of a membrane receptor. Anal Biochem. 2012; 420:185–187. [PubMed: 22005322]

[94]. G Protein-Coupled Receptors. Brit J Pharmacol. 2011; 164:5–113.

[95]. Chung S, Funakoshi T, Civelli O. Orphan GPCR research. Br J Pharmacol. 2008; 153(Suppl 1):S339–346. [PubMed: 18071299]

[96]. Langelaan DN, Ngweniform P, Rainey JK. Biophysical characterization of G-protein coupled receptor-peptide ligand binding. Biochem Cell Biol. 2011; 89:98–105. [PubMed: 21455262]

[97]. Jastrzebska B, Debinski A, Filipek S, Palczewski K. Role of membrane integrity on G protein-coupled receptors: Rhodopsin stability and function. Prog Lipid Res. 2011; 50:267–277. [PubMed: 21435354]

[98]. Kaya AI, Thaker TM, Preininger AM, Iverson TM, Hamm HE. Coupling efficiency of rhodopsin and transducin in bicelles. Biochemistry. 2011; 50:3193–3203. [PubMed: 21375271]

[99]. Yao XJ, Velez Ruiz G, Whorton MR, Rasmussen SG, DeVree BT, et al. The effect of ligand efficacy on the formation and stability of a GPCR-G protein complex. Proc Natl Acad Sci U S A. 2009; 106:9501–9506. [PubMed: 19470481]

[100]. Ye S, Huber T, Vogel R, Sakmar TP. FTIR analysis of GPCR activation using azido probes. Nat Chem Biol. 2009; 5:397–399. [PubMed: 19396177]

[101]. Liu JJ, Horst R, Katritch V, Stevens RC, Wuthrich K. Biased signaling pathways in beta2-adrenergic receptor characterized by 19F-NMR. Science. 2012; 335:1106–1110. [PubMed: 22267580]

[102]. Scott JL, Musselman CA, Adu-Gyamfi E, Kutateladze TG, Stahelin RV. Emerging methodologies to investigate lipid-protein interactions. Integr Biol (Camb). 2012; 4:247–258. [PubMed: 22327461]

[103]. Liu W, Chun E, Thompson AA, Chubukov P, Xu F, et al. Structural basis for allosteric regulation of GPCRs by sodium ions. Science. 2012; 337:232–236. [PubMed: 22798613]

[104]. Inagaki S, Ghirlando R, White JF, Gvozdenovic-Jeremic J, Northup JK, et al. Modulation of the interaction between neurotensin receptor NTS1 and Gq protein by lipid. J Mol Biol. 2012; 417:95–111. [PubMed: 22306739]

[105]. Stephan MT, Irvine DJ. Enhancing Cell therapies from the Outside In: Cell Surface Engineering Using Synthetic Nanomaterials. Nano Today. 2011; 6:309–325. [PubMed: 21826117]

[106]. Grossfield A. Recent progress in the study of G protein-coupled receptors with molecular dynamics computer simulations. Biochim Biophys Acta. 2011; 1808:1868–1878. [PubMed: 21443858]

[107]. Johnston JM, Filizola M. Showcasing modern molecular dynamics simulations of membrane proteins through G protein-coupled receptors. Curr Opin Struct Biol. 2011; 21:552–558. [PubMed: 21764295]

[108]. Lyman E, Higgs C, Kim B, Lupyan D, Shelley JC, et al. A Role for a Specific Cholesterol Interaction in Stabilizing the Apo Configuration of the Human A2A Adenosine Receptor. Structure. 2009; 17:1660–1668. [PubMed: 20004169]

[109]. Goddard WA 3rd, Kim SK, Li Y, Trzaskowski B, Griffith AR, et al. Predicted 3D structures for adenosine receptors bound to ligands: Comparison to the crystal structure. J Struct Biol. 2010; 170:10–20. [PubMed: 20079848]

[110]. Katritch V, Rueda M, Lam PC, Yeager M, Abagyan R. GPCR 3D homology models for ligand screening: lessons learned from blind predictions of adenosine A2a receptor complex. Proteins. 2010; 78:197–211. [PubMed: 20063437]

[111]. Kolb P, Rosenbaum DM, Irwin JJ, Fung JJ, Kobilka BK, et al. Structure-based discovery of beta2-adrenergic receptor ligands. Proc Natl Acad Sci U S A. 2009; 106:6843–6848. [PubMed: 19342484]

[112]. Westly E. Nothing to sneeze at. Nat Med. 2010; 16:1063–1065. [PubMed: 20930737]

[113]. de Graaf C, Kooistra AJ, Vischer HF, Katritch V, Kuijer M, et al. Crystal structure-based virtual screening for fragment-like ligands of the human histamine H(1) receptor. J Med Chem. 2011; 54:8195–8206. [PubMed: 22007643]

[114]. Koutsoukas A, Simms B, Kirchmair J, Bond PJ, Whitmore AV, et al. From in silico target prediction to multi-target drug design: current databases, methods and applications. J Proteomics. 2011; 74:2554–2574. [PubMed: 21621023]

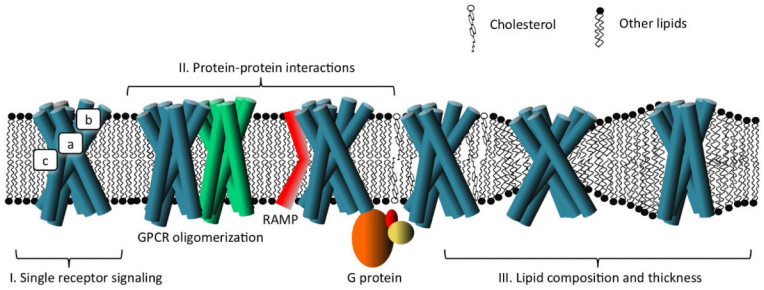

Figure 1.

Schematic of the membrane environment and GPCR signaling. (I) Single receptor molecules are generally capable of transducing a signal to the intracellular medium. Several regions of the receptor may modulate this signal. The most common means is modulation by orthosteric ligand binding, which is caused by the binding of agonists or antagonists (site a). Allosteric ligand binding sites may allow either soluble species (site b) or membrane-soluble species, including lipids, to access the receptor (site c). (II) Protein-protein interactions, here between two receptor molecules, a receptor and a heterotrimeric G protein, and a receptor activity modulating protein (RAMP). These inter-protein interactions may also modulate signaling behavior upon ligand binding to the receptor. As with all proteins in the cellular milieu, these are subject to regulatory proteins, which further modulate the overall signaling potential of receptors at the cell surface. There is also therapeutic potential in designing molecules, peptide or otherwise, that modulate signaling analogously to RAMP-like species. (III) The membrane itself has intrinsic mechanical properties the receptor must accommodate, in addition to specific interactions. These properties may shift the conformation of the receptor toward or away from active conformation.

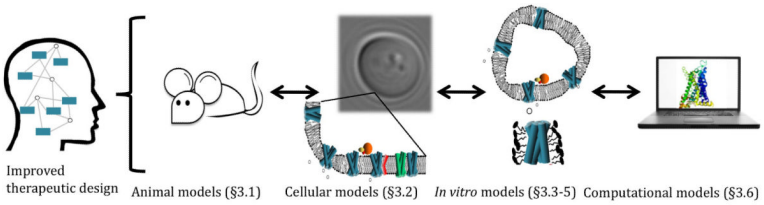

Figure 2.

Development of human therapeutics is by necessity built upon many experimental foundations. GPCR interactions with other proteins, ligands, or the cell membrane are best identified in isolation and then combined to form a holistic snapshot of receptor behavior. Animal models (manuscript section §3.1) show how tissues, or whole organisms, may react to treatment, but require substantial resources and studies are typically directed by results from cell cultures (§3.2). Therapeutic effects may be even more intensively examined through structural analysis (§3.3), as well as biophysical studies of protein in isolation in liposomes, micelles, or other biomimetic systems (§3.4-3.5). Finally, molecular dynamics and docking studies (§3.6) are used to study the movements of individual receptors. The collection of data from all of these experiments is required for the development of drugs that more specifically treat a condition, while minimizing the risk of adverse effects.

Table 1

The number of unique approved drugs that target various classes of membrane-localized proteins, as well as the total number of side effects resulting from those drugs. It may be possible to reduce the burden of a disease while also reducing side effects by better targeting of the protein and tissue affected by a disease. The method to obtain data is described in the Supporting Information and a complete listing of targets, target type, drugs, and side effects is given in the Table S1 of the Supporting Information.

Target type	Approved Drugs	Unique Associated Side Effects
Receptor	111	2278
Channel	43	1757
Transporter	38	1999
Enzymes	19	894
Other	9	307