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The Cytoplasmic Rhodopsin-Protein Interface: Potential for Drug Discovery

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Abstract

The mammalian dim-light photoreceptor rhodopsin is a prototypic G protein coupled receptor (GPCR), interacting with the G protein, transducin, rhodopsin kinase, and arrestin. All of these proteins interact with rhodopsin at its cytoplasmic surface. Structural and modeling studies have provided in-depth descriptions of the respective interfaces. Overlap and thus competition for binding surfaces is a major regulatory mechanism for signal processing. Recently, it was found that the same surface is also targeted by small molecules. These ligands can directly interfere with the binding and activation of the proteins of the signal transduction cascade, but they can also allosterically modulate the retinal ligand binding pocket. Because the pocket that is targeted contains residues that are highly conserved across Class A GPCRs, these findings imply that it may be possible to target multiple GPCRs with the same ligand(s). This is desirable for example in complex diseases such as cancer where multiple GPCRs participate in the disease networks.

Keywords

G protein coupled receptors; allostery; conformational changes; docking; protein-protein interactions

1. INTRODUCTION

Rhodopsin is a prototypic G protein coupled receptor (GPCR). GPCRs are membrane receptors that share a common architecture of helices crossing the plasma membrane seven times, forming a bundle that has an extracellular, a transmembrane and a cytoplasmic (CP) domain (Fig. 1). Ligands typically bind in the extracellular or transmembrane domains. In rhodopsin, the ligand is *11-cis* retinal, covalently attached to Lys296 in the transmembrane domain, towards the extracellular side (depicted in red and rendered as spheres in Fig. 1). Light causes isomerization of the *11-cis* isomer to *all-trans* retinal, triggering activation. The light-activated conformation of rhodopsin is referred to as Metarhodopsin II. Throughout the remainder of the text, we will refer to the inactive, dark-adapted conformation of rhodopsin as R and the active light conformation as R*. In other GPCRs, the event equivalent to light activation is ligand binding to the empty receptor. The ligand binding signal in GPCRs in general or retinal isomerization signal in the case of rhodopsin is transmitted to the CP domain, which undergoes conformational changes that initiate two opposing signaling cascades. Sensitization of rhodopsin is achieved by binding and activation of the G protein,

transducin (Gt). Desensitization begins with phosphorylation of the C-terminus by rhodopsin kinase (RK). RK (gene symbol GRK1) is the “official” kinase to phosphorylate rhodopsin, but many other related kinases including protein kinase c will also do so. The phosphorylated C-terminus is recognized by arrestin, which binds and prevents further activation of G proteins. The combined action of RK and arrestin quenches the R* signal.

All of the above proteins bind to the CP surface of rhodopsin and competition between them for this surface is a major mechanism for regulation of function. For example, it was recently shown that single-photon responses of mouse rod photoreceptors are directly responsive to the competition between arrestin and RK as measured by varying the concentrations of the two proteins: arrestin tunes the kinetics of rhodopsin phosphorylation by competing with RK, thus controlling the active life-time of rhodopsin [1]. This importance of the fine-tuning of the concentrations of cytoplasmic receptor interaction partners is not unique to rhodopsin, but it also found in other GPCRs where in fact aberrant tuning has been associated with disease [2–6]. It has therefore been proposed that tissue-specific modulation of the activity of competing proteins is a novel avenue for GPCR-related drug discovery [1].

The rhodopsin system itself has clinical relevance because mutations in each of the above proteins are associated with diseases: rhodopsin, arrestin and RK in Retinitis Pigmentosa; Gt and rhodopsin in congenital stationary night blindness and arrestin and RK in Oguchi disease [7]. Due to the large amount of knowledge for the rhodopsin system, this is also a model for GPCRs in general and novel avenues for drug discovery can be pursued in this system. The perspective of this review will be to look at the potential for drug discovery by targeting protein-protein interactions involving rhodopsin. Thus, we will first review the current state of structural knowledge on the protein-protein interaction interfaces involving the rhodopsin CP domain and its cognate partners: the subsequent sections 2, and 3 will describe the best studied binding partner, Gt, starting with early experimental work on the rhodopsin-Gt interaction in Section 2, followed by a survey of recent structures, docking and molecular dynamics simulations of this interaction using Gt $\alpha\beta\gamma$ complex and peptides representing the Gt C-terminus in Section 3. We will review rhodopsin’s interactions with RK in Section 4, with arrestin in Section 5 and with other proteins in Section 6. We will summarize these studies with a comparison of the overlap between the CP binding interfaces in Section 7. We will then venture into describing recent findings of small molecules binding to rhodopsin in Section 8, followed by an analysis of the overlap between the binding pocket targeted by these ligands and the protein-protein interaction interfaces described in the earlier sections, with particular emphasis on Gt because the largest amount of information is available for this interface. We will then discuss the potential of these findings for drug discovery in Section 9. We propose that a major opportunity lies in multi-targeting GPCRs by exploiting the disruption or modulation of the CP protein-protein interaction interface by small molecules. Finally, we will end with a summary in Section 10.

2. EARLY EXPERIMENTAL WORK ON RHODOPSIN-G PROTEIN INTERACTION

The best studied interaction of rhodopsin is that with Gt. Early on it was discovered that the alpha-subunit (Gt α) was the minimal subunit necessary for its activity in the presence of R*, and noted that the other functional subunit, Gt β , served to increase the activity of Gt α by promoting its binding to R* [8]. The author compared the interaction of Gt with phospholipid vesicles containing R* to those containing R, finding interestingly, that both bound Gt, albeit with different affinity: the phospholipid vesicles containing R* bound approximately 2–2.5 times more Gt than those containing R [8]. Control experiments with vesicles containing no rhodopsin essentially did not bind any Gt, demonstrating that the

binding was rhodopsin-mediated, thus establishing that dark-state binding was not an artifact of unspecific interactions of Gt with the lipids. Furthermore, Hamm *et al.* showed in 1987 that an antibody capable of blocking Gt binding to purified R* in the absence of phospholipids also removes Gt from rod cell membranes in the dark [9]. Not only did this study show that Gt binds to dark-state rhodopsin in rod cell membranes, but the fact that the monoclonal antibody should theoretically only be specific to a single binding site also suggested that the binding mode for the R-Gt complex is similar to that of the R*-Gt complex.

Other early work performed on the nature of the R-Gt complex involved simple kinetic measures, and provides estimates on binding affinity. Liebman and Sitaramayya measured the binding affinity of Gt for rod cell membranes by recording pH changes in the cells, as the conversion of cGMP to GMP in the signal transduction cascade results in the release of a proton [10]. They were able to report in 1984 that Gt had a binding affinity of $1 \times 10^6 \text{ M}^{-1}$ for these cell membranes, implying a tight anchoring to the membrane [10]. The study also showed saturation on binding reciprocal plots at about one Gt per four rhodopsin molecules, and comparison of their binding data with activation rates previously measured by Liebman and Pugh led them to believe that Gt binds to rhodopsin by lateral diffusion across the membrane rather than “hopping” from the membrane to rhodopsin [10]. Bennett and Dupont expanded on this work in 1985 by calculating dissociation constants for the binding of GDP to Gt in the dark, GDP to Gt in the light, and the binding of Gt for R*, with and without GTP [11]. They reported that GDP binds tightly to Gt in the dark, but will dissociate from Gt readily in the presence of R*, and that the binding of Gt to R* is greater in the absence of GDP – affinities approximated 200nM with GDP compared to 2nM without [11]. These results led Bennett and Dupont to conclude that Gt does not bind to R (in contrast to the work done earlier by Fung) and therefore retains its GDP until binding to R*, remaining associated with R* until binding GTP. It was not until 1996 that researchers measured the binding affinity of Gt for R as approximately 60 nM [12].

In 1989, König *et al.* published a report describing an experiment that could indicate more precisely which areas of the CP domain of rhodopsin bind to Gt. A spectroscopic assay was used to measure changes in R* induced by Gt binding in the absence and presence of synthetic peptides comprising various regions of rhodopsin [13]. The experiment identified three regions of the CP domain, each with the ability to compete with R* for Gt binding: the second CP loop between helices III and IV, the third CP loop between helices V and VI, and a fourth CP loop between helix VII and an anchoring to the lipid bilayer [13]. In addition, any two of the competitors showed a synergistic effect when mixed than when assayed separately [13]. The reverse experiment using peptide sequences from Gt $_{\alpha}$ showed that the carboxy terminal domain of this subunit is largely responsible for binding to R* [14]. This was a major step in understanding the interface between the two proteins. This data also provided a starting point for mutagenesis experiments to further refine at the residue level the contacts such as residue R135 of the E/DRY motif on helix III of R* and residue F350 on the carboxy terminal domain of Gt that are critical for the activation of Gt.

3. RECENT STRUCTURES, DOCKING AND MOLECULAR DYNAMICS SIMULATIONS OF THE RHODOPSIN-G PROTEIN INTERACTION

3a. Docking Gt to R and Structural Models of R*

The ability to obtain crystal structures of rhodopsin [15] and Gt [16] has enabled detailed predictions as to the structural basis for the interaction between the two proteins, and to define the protein-protein interaction interface. Francesca Fanelli conducted a detailed protein-protein docking study of R-Gt using the ZDOCK algorithm applied to a monomeric

R crystal structure (PDB: 1U19) [17]. The goal of the study was to test the hypothesis that R has the potential to recognize and precouple with Gt in a position that would facilitate activation [17] consistent with the experimental findings described above in Section 2. Energy minimization placed Arg135 of the D/ERY motif and Phe350 of Gt α in remarkably close proximity with a C α -C α distance of 7.9 Å [17], indicating that the D/ERY motif is accessible to the C-terminus of Gt α already in R.

Recently, Sgourakis and Garcia have performed all-atom molecular dynamics (MD) simulations to predict the equilibrium fluctuations of Fanelli's docked R-Gt complex [17, 18]. They observed that the interface is highly dynamic, sampling patterns of interdomain interactions that were not in their entirety present in the initial docked conformation, but which contributed significantly to the stability of the complex [18]. Four distinct interdomain orientations were found that interconvert within 10 to 100 ns. The dynamics of the interface were proposed to be the basis for the molecular recognition between the two proteins [18]. In particular, the atomic motions of the known ionic partners associated with light activation of rhodopsin indicate that the dark-state crystal structure has access to an ionic lock interaction characteristic of activation, suggesting that dark-state dynamics may help to mediate the structural transition that occurs upon activation [18]. This observation is also in line with coarse-grained calculations for rhodopsin dynamics using anisotropic network modeling (ANM) (see section 3b) [19]. The ANM studies also suggested that the conformational changes observed upon light-activation are accessible to R.

While the study of Gt binding to R is important, the functionally more relevant interaction is the one with R*. Thus, the earliest publication that used the crystal structure of R to predict the R*-Gt interface actually altered the R structure to incorporate the major conformational changes thought to be characteristic of R* [20]. The Gt α subunit crystal structure (PDB: 1GOT) was physically placed in contact with a rhodopsin dimer model where helix 6 of one of the monomers was manually moved and rotated by ~90° to mimic the R* structure, setting known contacts, performing energy minimizations, then adding the Gt β subunits [20]. The study's emphasis was on the significance of dimeric and tetrameric rhodopsin structures in native disc membranes for Gt binding. For the tetrameric rhodopsin complex it was predicted that the Gt α subunit should have an interacting surface of 18.6 nm² with one R* subunit and a surface of 11.7 nm² with an adjacent subunit [20]. Further, it was concluded that the Gt β subunit should interact with all four of the tetramer subunits, and it was hypothesized that a tetrameric rhodopsin complex might be more energetically favorable when compared to a monomeric configuration [20]. Fanelli and coworkers re-addressed the question on the role of oligomeric rhodopsin by R-Gt docking studies. Using the same R model as Filipek et al. [20], but without the adjustments in helix VI, the docking of Gt to monomeric R was compared to that with dimeric and tetrameric R [21]. In contrast to the 4:1 R-Gt model proposed by Filipek and coworkers, their study concluded that there were little differences between the 2:1 and 4:1 predictions, and a 1:1 R:Gt stoichiometry resulted in the most reliable docking prediction, implying that the monomeric complex may represent the functional unit for the complex [21].

3b. Docking Studies of Gt α C-terminal Fragments

Complementing the docking studies presented in section 3a using full-length Gt, a number of computational studies have been carried out using the C-terminal fragment of Gt α (residues 340–350) and several analogs. To model the R*-Gt interaction interface Garland R. Marshall and coworkers have conducted studies by constructing biophysically favorable models using the Gt α C-terminal peptide (IKENLKDCGLF) without incorporating previous experimental data as *a priori* constraints [22, 23]. These studies were carried out with an ensemble of low-energy conformers of CP loops of an R* model generated by rotating helix 6 along its long axis [24] instead of a full-length model of R*. When comparing different

conformers CP loop 3 was found to move the most, inverting from an inward to an outward position. To capture the experimentally observed opening of the helical bundle, the conformations after minimization were chosen such as to maximize the “openness” of the domain. In the absence of an actual structure of R*, the Gt peptide was then docked to the two most different conformers with respect to the position of CP loop 3. Favorable interactions of the peptide with the rhodopsin CP loop domain were considered while allowing the side-chains of the CP loop conformers to be flexible and fixing the backbone atoms [22, 23]. The resulting predictions were analyzed with respect to what are the important contacts formed in the R*-Gt α complex. These results agreed well with published experiments [22, 23]. A tentative 3D model was generated by overlaying the X-ray crystal structure of Gt $\alpha\beta\gamma$ with the bound conformation of the Gt peptide and by aligning the CP loops to the tetrameric structure of R. Distance calculations on the significant contacts between Gt and R predicted using this model [22] were compared to those computed previously [17, 20, 25]. It was found that only one of the three previously generated models [17, 20, 26], namely the one proposed by Fanelli *et. al.* [17] satisfies two out of the four characteristic C α -C α distances i.e, distances between Lys313, Glu337 on Gt α to Ser240, Ser144 on monomer A of R*, respectively, and Leu19 on Gt α and Pro63 on Gt γ to Ser240, Cys316 of monomer B of R*. These distances are known from experiments to play a role in interacting with Gt [22]. They suggested that such differences in residue-residue contacts between the different models could be arising due to the rotation of the Gt $\alpha\beta\gamma$ complex with respect to the binding surface on the rhodopsin CP loops and also the interaction of Gt α with different rhodopsin monomers in a dimeric or tetrameric complex [22]. Furthermore, molecular modeling studies with Gt α C-terminal peptide (IKENLKDCGLF) indicated that different binding modes are possible with R*. The isolated Gt α fragment may not necessarily bind to rhodopsin in the same binding mode as the full Gt α subunit [22]. Next, modeling studies of Gt α C-terminal fragment and its analogs were performed using R* CP loops generated previously [24], to identify if there is a common binding site on R* [23]. Docking was conducted using several lowest energy conformers of R* CP loops but after addition of TM stems of length 2 amino acids at each end of a loop. Analysis of the residue-residue contacts indicated a common site of interaction on R* for the different analogs of Gt α [23].

In the Gt peptide docking studies described above, lowest energy conformers of CP loops after rotation in helix VI were used to mimic the R* conformation. Another approach has been to generate a full-length model of R* based on anisotropic network modeling (ANM) [19]. ANM is an elastic network that predicts global/slow and local/fast modes of vibrational motions of a macromolecule [27, 28]. While the slow modes usually aid in identifying global conformational changes in the protein, which are relevant to a protein's function, the fast modes on the other hand provide information regarding protein stability [19, 29]. ANM studies of rhodopsin suggested that the dynamics of rhodopsin can facilitate in transferring the signal from the retinal binding site to the CP domain and the predicted conformational changes could explain well experimental biophysical data validating the approach for rhodopsin [19]. We therefore docked the high affinity analogue of the Gt C-terminal peptide (VLEDLKSCGLF) [30, 31] to both R (PDBID: 1L9H:A) [15] and the ANM structure of R* (ANM-R*) [19] using ClusPro docking software [32]. The results are shown in Fig. (3A, B). In each case, the top 10 best scoring conformations were considered for analysis, and 90% of the time they were docked in the CP domain of rhodopsin. The electrostatic and desolvation energies of the peptide-rhodopsin complexes were estimated using FastContact analysis [33]. The electrostatic energy of peptide binding to ANM-R* was estimated at -14.1kcal/mol, as compared to -6.7kcal/mol for binding to R. This result is validated by the experimental observation that the peptide does not appreciably bind to R. The desolvation free energies calculated for the binding of peptide to R and ANM-R* were +1.1kcal/mol and -1.7kcal/mol, respectively, again supporting preferred binding to R*. The predicted peptide binding pocket residues are in general agreement with previously published data of the Gt

C-terminal peptide (IRENLKDSGLF) [23]. Specific differences in the poses of different peptides with the different models of R are described in more detail in light of the recently published opsin-Gt peptide complex, in Section 3c, below. After the release of a structural model for a deprotonated R* intermediate (PDB: 2I37) [34], Fanelli and Dell'Orco followed up on their earlier study [17] by comparing Gt docking to this R* intermediate with the original R structure [35]. Given that this R* structure is almost identical to R, not surprisingly a comparison of the docking to the photoactivated deprotonated R* structure with that of previously published R structures yielded essentially identical results [35]. There were subtle differences between the two recognition modes, consisting mainly of increased solvent accessibility in the CP loops of R*-Gt [35]. However, it is likely that crystal contacts prevented the conformational changes leading to R* in solution and generally it is assumed that the more recent opsin structure [36] – despite lacking the retinal – is more representative of R*. This is because this structure shows translation and rotation of helix VI generally thought characteristic of light-activation [36].

3c. Docking Studies using the Opsin Crystal Structure

The recent break-through of a 3.2Å Gt peptide bound opsin crystal structure by Oliver Ernst and coworkers [37] allows us to compare the different R/R*-Gt complex models generated earlier (see Section 3b) in light of an actual structure, even if it can be expected that the actual R*-Gt peptide complex may not be identical. The good agreement of the opsin conformation with the large number of biophysical studies of R* [36] in solution indicates that this comparison is particularly meaningful. In the opsin structure [37], the Gt peptide adopts a helical conformation as expected and binds at a location between TM helices 5 and 6 and leads to the rearrangement of the kink between TM helices 7 and 8 [37]. In line with numerous biochemical and biophysical studies and the dockings described above, the C-terminal region of the Gt peptide interacts with the D/ERY and NPxxY motifs [37]. Remarkably, however, the binding mode of the peptide observed in the Gt peptide bound opsin crystal structure is not possible with the R structure, suggesting that the details of the R*-Gt binding interface may differ from those of the R-Gt binding interface.

In comparing the previous docking studies to the opsin-Gt peptide crystal structure complex, the following fundamental differences in emphasis of the studies should be noted. First, the interaction of full-length Gt will clearly involve aspects not currently amenable to validation until the full-length protein is crystallized in complex with R or R*. Second, addressing the stoichiometry of interaction between R and Gt [17, 20, 35] is not possible, because this discussion also involves full-length Gt. Furthermore, the current opsin-Gt peptide structure is monomeric and the functional significance of the dimeric state of the opsin structure remains to be established. Thirdly, the docking studies involving only the CP loops as a binding interface [22, 23] suffer from the drawback that some of the residues that make up the binding pocket of the Gt peptide are located in the CP ends of helices rather than the CP loops themselves. In particular, CP loop 3 adopts a more extended helical conformation in opsin as compared to R and R* structures. In general, however, the Gt peptide (Fig. 3C) with respect to helices 3, 6, 7 and 8 was oriented with the N-terminus of the peptide pointing towards helix 3, and the C-terminus towards helices 6, 7, 8. This was regardless of whether R [17], photo-activated deprotonated R* [35], CP-loop R* [22], ANM-R* (Fig. 3B) or R* with moved helix 6 [20] was used for docking. In each case, the peptide was consistently predicted to be in a very similar orientation to that observed in the opsin-Gt peptide structure (Fig. 3C). The modeling studies that are most consistent with the opsin-Gt peptide complex are those involving the R* model generated by physically moving the helix 6 of R and Gt_α high affinity peptide studies using ANM-R* [19]. These models did account for the conformational changes in R upon activation.

More specifically, the physical changes that were made to obtain the R*-Gt model (rotation and movement in helix 6 followed by a slight rearrangement of neighboring helices) by Filipek *et al.* [20] described above in section 3a overlaps with the conformational changes observed in opsin-Gt peptide crystal structure (outward tilt in helix 6 and a rearrangement of helices 7 and 8). The predicted binding mode of the Gt α c-terminus was located between helices 2, 3, 6, and 7 in this model as compared to helices 3, 5, 6 and 7 in the opsin-Gt complex. While the predicted binding mode is not completely identical to that of the experimentally determined Gt peptide bound structure of opsin, ~ 50% of the predicted binding pocket residues overlapped with that of the Gt peptide binding site in the opsin structure.

The R*-Gt α peptide studies involving the ANM-R* model (Fig. 3B) also show similarities to the Gt bound opsin crystal structure (Fig. 3C). An overlay of the Gt peptide (ILENLKDCGLF) bound opsin structure and the R*-Gt α (VLEDLKSCGLF) model is shown in Fig. (3D). From the comparison it is clear that despite the differences in sequence, the C-terminus of the Gt peptide adopts a similar conformation regardless of the structure it is bound to, while the N-terminus part is the most variable. While the exact conformation of the bound state of the Gt peptide does not fully overlap with that seen in the crystal structure, the contacts made especially by the C-terminus of the peptide highly overlap. For example, Phe350 is shown to be in close proximity to Glu242 and Ala246 on helix 6 and the model also predicts both of these residues to be in close proximity, strongly supporting the utility of using computational modeling studies. However, structural details regarding the intermediates of R*, oligomerization of R/R* and the interaction interface of full-length Gt $\alpha\beta$ with R/R* is still missing and predictions need to be interpreted with caution.

Finally, the Gt bound opsin crystal structure was used to propose a mechanism of how conformational changes in the rhodopsin CP domain are propagated to the GDP binding site of Gt [38]. Two possible modes of interaction, referred to as S- and I-interaction between Gt and opsin were proposed [38]. The S-interaction is characterized by contacts between the peptide and the D/ERY and NPxxY motifs similar to those observed in the Gt peptide bound opsin crystal structure [37]. In contrast, Gt α -helix 5 in the I-interaction mode displays distinct contacts with the NPxxY motif [38].

4. RHODOPSIN – RHODOPSIN KINASE INTERACTION

The first step in desensitization of the rhodopsin activation signal is the phosphorylation of rhodopsin's flexible C-terminus by RK, a process that has been studied for over 30 years [39]. It is only recently, however, that the structural basis for this process is becoming elucidated [40, 41].

RK is catalytically inactive, unless it is bound and activated by R*. Activated RK is subsequently active in phosphorylating both, native rhodopsin as well as peptides representing the C-terminus of rhodopsin that is being phosphorylated. The K_m value for R* as a substrate is in the micromolar range, while the peptides are only in the millimolar range [42–45]. The catalytic efficiency is highest for rhodopsin, but once RK has bound to R*, it is also able to better phosphorylate the peptide substrates [44–46]. After activation by binding to R*, RK is active with a half-life of ~2 min [47]. RK was recently crystallized in different ligand-bound states [41] providing structure-based hypotheses for these functional properties of RK. The study showed that nucleotide binding alone is not sufficient to induce the conformational changes required to bring the kinase domain into an active state. Binding to the CP surface of R* is required to mediate the closure of the catalytic domain and thus activate the kinase [41]. This interpretation is supported by previous data that have favored the interpretation that R* binds to an allosteric site on RK [40, 44, 48].

The allosteric site forming the interface with R* comprises both, the RK N- and C-termini. The interaction of the N-terminal domain is independent of light-activation, while that of the C-terminal domain occurs only with R* [49]. The evidence for the involvement of the N-terminal domain in kinase activity regulation includes mutagenesis [50], binding of recoverin, a protein that functions as an inhibitor of kinase activity ([51, 52], see below) and inhibition of R* phosphorylation by an antibody directed against the N-terminus [53]. Like the Gt C-terminal residues involved in the interaction with rhodopsin (see above), the N-terminal region is predicted (and has been experimentally confirmed recently by NMR spectroscopy [54]) to form an α -helix [40]. Evidence for the interaction of the C-terminal domain comes from deletion studies [55]. Recent mutagenesis experiments specified a proline-rich motif within the C-terminal conserved region of RK and related kinases as the key element involved in the interaction of this domain with R* [49]. The positioning of the N- and C-terminal tails with respect to the kinase structure make it likely that the full interface will involve residues in other parts of the structure as well. A structure- and sequence-analysis based mutagenesis study in particular identified additional residues in the small and large lobes of the kinase as part of this expanded interface [40].

The complementary site of the interface on rhodopsin involves primarily the third and to some extent second and first CP loops [44, 56–59]. Mastoparan, which is considered an analogue of the third CP loop enhances phosphorylation [44]. The conformational changes in the CP domain of rhodopsin upon light-activation leading to a relative displacement of helices III and VI are critical for binding and activation of RK, because their restriction by disulfide cross-linking impairs phosphorylation [60]. On the other hand, tethering the C-terminal tail to the second CP loop enhances phosphorylation [60].

Taking all the data together, a qualitative model has been proposed that places the rhodopsin second and third CP loop into proximity of the N- and C-termini as well as the supporting surface from the small and large lobes of the kinase domain, while the rhodopsin C-terminus, i.e. the substrate of phosphorylation, is captured in a hydrophobic groove on the kinase domain [40, 41], analogous to other kinase family members [61]. In fact, rhodopsin, like other GPCRs, is promiscuous in the kinases that it can bind to and serve as a substrate for. In particular, GRK2, 5, 6 and Protein kinase c- α have all been shown to interact with rhodopsin. However, a detailed structural model of the complex with any kinase remains to be proposed, and is hampered by the large size of RK and other kinases which makes computational docking difficult. Furthermore, the conformation representing full closure of the kinase has not yet been observed in any of the RK (GRK1) or the other (GRK2, GRK6) crystal structures. In particular, the current structures do not show density for the C-terminal tails and the N-terminal tail has so far only been observed in one crystal structure, that of RK in complex with ATP [41]. Thus, the two regions on RK that are critical for its interaction with R* are highly dynamic and structural knowledge is limited.

5. RHODOPSIN – ARRESTIN INTERACTION

Arrestin binds phosphorylated rhodopsin with high-affinity and unphosphorylated rhodopsin with low affinity [62–67]. The literature on the rhodopsin-arrestin interactions has been reviewed recently [68]. While there is debate as to whether a single arrestin interacts with a single GPCR, there are three key events that must take place in order for activated, phosphorylated Meta II state rhodopsin to interact with visual arrestin, thereby terminating signaling [69]. These events include (1) modulation of the polar core of basal arrestin by phosphorylated rhodopsin, thereby allowing new salt bridges to form between arrestin and rhodopsin, (2) disruption of the three-element interaction in basal arrestin, and (3) displacement of the C-terminal region from the N-domain of arrestin mediated by the hinge region amino acids 179–191 [68, 70–73]. Visual arrestin was crystallized as a tetramer [70,

71]. However, it is only observed to associate with rhodopsin in monomeric form. Thus, it was proposed that in order for visual arrestin to interact with rhodopsin specific changes in the C-terminal region of arrestin were required, thus dissociating the basal tetramer in a cooperative fashion [74, 75]. Up to nine phosphates may be added to rhodopsin by RK [76]. *In vivo*, however, only the C-terminal serines Ser343, Ser338 and Ser334 are preferentially and sequentially phosphorylated by RK. These three phosphoserines are key to the interaction with visual arrestin [77]. RK has a much higher affinity for unphosphorylated rhodopsin than arrestin, thereby defining the sequence of interaction events [78]. Because fully phosphorylated RK binds tightly to rhodopsin but less to phosphorylated rhodopsin, whereas unphosphorylated RK binds both, phosphorylated and non-phosphorylated rhodopsin, it is thought that RK dissociates from rhodopsin upon RK autophosphorylation and R* phosphorylation, allowing the interaction with arrestin to occur [78].

It has been shown that the specific C-terminal site of phosphorylation in rhodopsin may not be as important in the fidelity of the response to single-photon events as compared to the number of phosphorylated residues, suggesting that the comprehensive phosphorylated state of rhodopsin governs interaction and affinity for arrestin [79]. Once the C-terminus of rhodopsin has accumulated three phosphates, this phosphorylated rhodopsin species is considered to have greatest affinity for visual arrestin – additional phosphorylation of rhodopsin reduces the affinity for visual arrestin, and may increase the rate of retinal release [80]. Structural studies on a C-terminal region of rhodopsin (amino acids 330–348) suggest that this disordered region adopts a helix-loop fold when in the presence of arrestin, adding to the evidence that disordered regions of both arrestin and rhodopsin become ordered upon interaction [81]. With regards to the interaction between visual arrestin and activated rhodopsin, a recent report from Feuerstein *et al.*, suggests that independent of the phosphorylation state of rhodopsin, a loop region (amino acids 67–77) of visual arrestin connection β -strands V and VI becomes ordered and helical (Glu70 to Val74) when rhodopsin is activated [82]. This builds upon previous work from Sommer *et al.*, suggesting the importance of the conformational flexibility of loop V–VI [83]. In addition, Hanson *et al.*, using site directed spin-labeling studies have shown that this loop in arrestin exhibits strong immobilizing interactions only upon interaction with phosphorylated R* [84]. These studies further suggests that this loop becomes a primary site of interaction for arrestin and phosphorylated R* [84]. When comparing this region to the basal crystal structure, one notes that while dynamic, this loop covers a region of the N-domain, postulated to be important in activated rhodopsin-visual arrestin interaction [68]. Thus, upon becoming ordered in the presence of activated rhodopsin, new surface contacts, specifically within the concave section of the N-domain, may become more accessible to the CP face of rhodopsin. Moreover, a region containing α -helix I (residues 100–111) in arrestin was also shown to play an indirect role in stabilizing the interaction between arrestin and R* [85]. However, there is also evidence suggesting that either the N-domain or C-domain of visual arrestin is capable of binding activated, phosphorylated rhodopsin [86]. Additionally, visual arrestin association with activated rhodopsin has been suggested to extend beyond simply shutting off signal transduction. Farrens and Sommer have suggested that visual arrestin is capable of trapping a retinal species within rhodopsin, maintaining the retinal Schiff-base, and may even interconvert a Metarhodopsin III-like species into a Metarhodopsin II-like species [87]. Thus, they hypothesize that arrestin release from rhodopsin is directly related to retinal release [88]. This observation may explain why arrestin deficiency or certain arrestin mutations contribute to pathologic states [87].

6. OTHER INTERACTIONS WITH RHODOPSIN'S CYTOPLASMIC DOMAIN

Phosphorylation sites on rhodopsin are removed by the action of protein phosphatases such as Protein phosphatase 2A- α [89]. Nothing is currently known about the putative interface between rhodopsin and phosphatases.

The phosphorylation of rhodopsin by RK is modulated by recoverin: recoverin is a protein that binds to RK in a calcium-dependent fashion and inhibits phosphorylation of rhodopsin by RK. The purpose is to sense the opening of cGMP-gated ion channels that cause a decrease in calcium concentration, indicating completion of the light-activated signaling cascade. The binding site for recoverin on RK is at the extreme N-terminus of RK (contained within residues 1–15, in particular residues 6, 12 and 15 [90]), but binding does not affect the catalytic activity of the kinase [52]. Thus, it was proposed that steric blockage of the interaction interface between rhodopsin and RK is the mechanism of inhibition [52]. Consistent with the hypothesis of a helical conformation for this region [40], it is believed that the nature of this helix is amphipathic and interacts with recoverin primarily through a hydrophobic interface [52]. A recent NMR structure of a peptide derived from residues 1–25 of RK in complex with recoverin confirms this hypothesis [54]. Although not as important for the interaction as the N-terminus, the C-terminus of RK has also been implicated in the interface with recoverin [90], further supporting the overlap between the binding surface for R* and for recoverin on RK. There is a possibility that the recoverin-RK complex may bind to rhodopsin in a ternary complex [91], and recoverin bound RK still binds to R* [92]. A triple complex could be eluted from concanavalin A columns [54]. However, this study was carried out with the RK peptide (residues 1–25). Recoverin alone was also found to co-elute with rhodopsin from concanavalin A columns, in a calcium-dependent fashion [54]. The myristoylation of recoverin is believed to target this protein to membranes [54] and R* immobilized on a sensor chip causes retention of myristoylated but not unmyristoylated recoverin [93]. However, the specificity of a direct interaction with rhodopsin still remains to be confirmed by further complementary evidence. Recently it was proposed that recoverin and RK may not compete directly for the same binding site on R* despite the overlap between the RK/R* and RK/recoverin interfaces, and a model favoring the role of recoverin binding in preventing the positioning of the rhodopsin C-terminus on the kinase has been proposed [93].

7. OVERLAP IN THE CYTOPLASMIC BINDING INTERFACES

The above sections on the binding sites of Gt, RK and arrestin on rhodopsin provide evidence that the binding sites highly overlap (also see [44, 56, 57, 66, 78, 94]). Furthermore, RK and Gt compete for binding to R* [95]. (Note that recoverin does not interfere with this competition [93]). For example, residues at position 72,73 in the first CP loop [94] and 142, 143 in the second CP loop [94] and 250 in the third CP loop [96] in rhodopsin were shown to play a role in interacting with arrestin, while mutations at residue positions 72 and 250 were also shown to strongly interfere with Gt activation. Similarly studies by Shi Wen *et al.* focused on identifying residues that were similar between the RK and Gt binding interfaces. Mutations introduced at residue positions 62–64 in the first CP loop and 147–149 in the second CP loop showed altered phosphorylation in rhodopsin by RK [57]. It has been shown that Arg147 plays a major role in binding to Gt [35]. In addition, residues at position 233–235 altered both phosphorylation by RK and Gt activation [57]. These studies highlight the fact that there are similarities between Gt, RK and arrestin binding interfaces in rhodopsin.

8. RHODOPSIN – SMALL MOLECULE STUDIES

Recently, we [97] and others [98] have shown that in addition to Gt and its peptide analogs, RK and arrestin, there are small organic molecule ligands that can bind at the CP domain of rhodopsin. Studies by Taylor *et al.* identified two compounds from a virtual screening against the NCI database as binding to the CP domain of rhodopsin. These compounds stabilized the Metarhodopsin II state and inhibited Gt from interacting with rhodopsin [98]. Using rhodopsin CP loops and a Gt α C-terminal peptide model from earlier studies [22], Taylor *et al.*, proposed a general method for discovering inhibitors that target the intracellular loops of activated GPCRs called MEDSET [98]. Computational modeling studies predict a binding pocket for these ligands at the CP center of the helical bundle (Fig. 2). The proposed binding pocket overlaps to a great extent with that of Gt and the recent Gt peptide bound opsin crystal structure [37]. Furthermore, we found that the natural plant colorants anthocyanins slightly inhibit the activation of Gt using [35 S]GTP γ S filter binding assays and enhance the regeneration of rhodopsin [97]. These studies strongly support the presence of a novel, allosteric ligand binding pocket in the CP domain of rhodopsin (Fig. 2B). The predicted CP binding site provides a plausible explanation for the observed effects these small molecules have on rhodopsin's conformation and Gt activation – it is located at the “heart of the action,” where the largest conformational changes are observed after light-activation [99, 100], and where critical residues are exposed to interact with Gt [99, 100]. The idea that binding of small molecules in this pocket may result in allosteric modulation of rhodopsin (either by accelerating regeneration [97] or by inhibiting the G protein [97, 98]) is intriguing, since allosteric modulation is an important concept in the GPCR field. Previously, there were no known allosteric modulators for rhodopsin, and all known modulators for other GPCRs bind in either the transmembrane or extracellular domains. Moreover, some of the residues in this pocket are highly conserved [101], such as Glu134 and Arg135 within the D/ERY motif, which is conserved close to 100% in Class A GPCRs [102]. It is possible that ligands binding in this pocket could be developed into universal modulators of GPCR activity, in such areas where multiple GPCRs are discovered to be responsible for pathologic states [103–106].

9. POTENTIAL FOR DRUG DISCOVERY: MULTI-TARGETING G PROTEIN COUPLED RECEPTORS

The small molecules discovered to bind to the CP domain of rhodopsin are not the only small molecules binding to GPCRs. The protease-activated receptors (PAR1, PAR2, PAR4) [107–109] and chemokine receptors (CXCR1, CXCR2, and CXCR4) [110] bind to pepducins, peptides corresponding to the CP loops (mostly CP loop 3) with a N-terminal lipid tag [111]. Pepducins are membrane anchored with an ability to penetrate the cell membrane and are specific to the receptor type and act as either agonists or antagonists of their cognate receptor [108]. These lipidated-peptides are shown to bind the receptor at the CP interface and interfere with Gt activation [108, 111]. This is an indication that CP domain targeting may be a general phenomenon for the GPCR family. Below, we discuss novel ways how targeting the CP domain of GPCRs may impact drug discovery.

The observation that there is a high degree of overlap between the protein-protein interaction interfaces described in Section 7 and the small molecule binding site described in Section 8 and above and that this site involves mostly highly conserved residues throughout class A GPCRs has important implications for drug discovery: it may be possible to target with the same molecules different GPCRs, and this targeting may affect interactions with multiple players of the GPCR signaling cascades. Multi-targeting GPCRs is in fact a major biomedical challenge in treating complex diseases. The GPCR family is a membrane protein family of superlatives: with at least 5000 unique members [112], it is the largest cell surface

receptor family, and even protein family discovered to date. GPCRs respond to very diverse ligands and most drugs are targeted at them, perhaps as many as 60% of current drugs on the market [113]. They range from very commonly used medicines such as anti-histamines (Claritin, Allegra, Pepcid and Zantac) for the treatment of allergies and cold symptoms or adrenergic receptor antagonists for the treatment of hypertension to schizophrenia, Parkinson's disease, migraines and other complex diseases, including cancer. GPCR activity is classically inhibited by a specific receptor antagonist, or activated by a specific receptor agonist. However, the therapeutic benefit of this strategy is limited in complex diseases. For example, tumor growth is simultaneously stimulated by multiple agonists binding to several different GPCR's [114]. Many GPCR agonists, including cyclooxygenase-3-derived eicosanoids, CXC chemokines, hormones and neuropeptides directly mediate cancer cell motility and invasiveness, tumor angiogenesis and survival of metastatic cancer cells [115]. Furthermore, GPCR agonists also act indirectly by activating the Epidermal Growth Factor Receptor (EGFR). For example, the GPCR ligands prostaglandin E2 (PGE2), bradykinin (BK), gastrin releasing peptide (GRP), lysophosphatidic acid (LPA) and thrombin can activate EGFR signaling [103–106]. This observation has been exploited for cancer chemotherapy development, in that cancer growth can be inhibited more effectively by co-administration of two antagonists, one against the EGFR and one against the GPCR, or against a GPCR downstream signaling partner, such as Src. However, there is no reason to believe that the above list of GPCRs is comprehensive, and there may be other GPCRs whose inhibition might be beneficial to prevent cancer growth and progression. Instead of targeting each GPCR with its own antagonist, an alternative strategy is to target the heterotrimeric G protein complex, kinase or arrestin, all being downstream effectors common to all GPCRs. Epitomizing on this fact, it was shown recently that the G protein inhibitor BIM-46174 inhibits growth of a large number of tumor cell types [115]. However, administration of BIM-46174 did not show effects on the angiogenic responses induced by tyrosine kinase receptors so far [115]. Thus, multi-targeting GPCRs remains an open and unsolved major biomedical challenge. Addressing this challenge may lead to improved approaches to treat complex diseases such as cancer more effectively. This implies the hypothesis that multi-targeting GPCRs through CP ligands may be a mechanism to treat cancer. Computational systems biology studies suggest that there are likely to be large numbers of undiscovered receptor crosstalk mechanisms [116] and that multi-targeting GPCRs will require simultaneous silencing/activating of more receptors or pathway intermediates than previously anticipated.

Based on these considerations, it may be possible that multi-targeting may best be carried out at the receptor activity level, rather than at a down-stream level and that this may lead to ways of prohibiting cancer growth (Fig. 4). We specifically propose that multi-targeting can be achieved through ligands that bind in a location that is highly conserved throughout the GPCR family, namely the center of the CP surface, formed by the CP ends of the seven transmembrane helices. As described in this review, an opening of the helical bundle at this site is the heart of the ligand-induced conformational changes in GPCRs, exposing sites critical for the binding and activation of the G protein, kinase and arrestin. Because this site is critical to GPCR function, it is highly conserved and ligands targeted at this site are likely to bind to more than one GPCR. This characteristic will be critical to the multi-targeting goal. Finally, we propose that because this location is distant to the natural ligand binding site – i.e. the CP ligands will be allosteric ligands - the effects of the CP ligands can be designed to be modulatory. Modulatory drugs have the advantage of taking effects only when the receptor pathway is being used, typically causing less side effects than direct agonists or antagonists.

10. SUMMARY

GPCRs are cell surface receptors that respond to very diverse ligands and account for the majority of current drug targets. GPCR activity is classically inhibited by a specific receptor antagonist, or activated by a specific receptor agonist. However, the therapeutic benefit of this strategy is limited in complex diseases. For example, tumor growth is simultaneously stimulated by various agonists binding to different GPCRs and several GPCR agonists also activate signaling by the EGFR, which is over-expressed in many tumor types. In this review, we proposed a novel strategy to simultaneously target multiple GPCRs to provide new avenues to develop more effective treatments against complex diseases such as cancer. Based on fluorescence, NMR spectroscopic and modeling studies with the prototypic GPCR, rhodopsin, we propose the hypothesis that there could be a conserved binding pocket for small molecule ligands at the CP surface of many GPCRs [101]. We reviewed the current knowledge of the interfaces formed between the proteins of GPCR signal transduction cascade, G protein, arrestin and kinase, for the specific and best studied GPCR rhodopsin. There is a high degree of overlap leading to competition between these proteins for the same binding site. The ability to modulate this competition and/or inhibit all of these interactions through small molecules binding at the CP pocket may have broad implications for GPCR drug discovery in general.

References

1. Doan T, Azevedo AW, Hurley JB, Rieke F. Arrestin competition influences the kinetics and variability of the single-photon responses of mammalian rod photoreceptors. *J Neurosci*. 2009 Sep 23; 29(38):11867–79. [PubMed: 19776273]
2. Hansen JL, Theilade J, Aplin M, Sheikh SP. Role of G-protein-coupled receptor kinase 2 in the heart--do regulatory mechanisms open novel therapeutic perspectives? *Trends Cardiovasc Med*. 2006 Jul; 16(5):169–77. [PubMed: 16781951]
3. Lombardi MS, Kavelaars A, Cobelens PM, Schmidt RE, Schedlowski M, Heijnen CJ. Adjuvant arthritis induces down-regulation of G protein-coupled receptor kinases in the immune system. *J Immunol*. 2001 Feb 1; 166(3):1635–40. [PubMed: 11160205]
4. Metaye T, Perdrisot R, Kraimps JL. GRKs and arrestins: the therapeutic pathway? *Med Sci (Paris)*. 2006 May; 22(5):537–43. [PubMed: 16687124]
5. Vroon A, Heijnen CJ, Kavelaars A. GRKs and arrestins: regulators of migration and inflammation. *J Leukoc Biol*. 2006 Dec; 80(6):1214–21. [PubMed: 16943386]
6. Lymperopoulos A, Rengo G, Funakoshi H, Eckhart AD, Koch WJ. Adrenal GRK2 upregulation mediates sympathetic overdrive in heart failure. *Nat Med*. 2007 Mar; 13(3):315–23. [PubMed: 17322894]
7. OMIM - Online Mendelian Inheritance in Man. [cited; Available from: <http://www.ncbi.nlm.nih.gov/omim>]
8. Fung BKK. Characterization of Transducin from Bovine Retinal Rod Outer Segments. *J Biol Chem*. 1983; 258(17):10495–502. [PubMed: 6136509]
9. Hamm HE, Deretic D, Hofmann KP, Schleicher A, Kohl B. Mechanism of Action of Monoclonal Antibodies That Block the Light Activation of the Guanyl Nucleotide-binding Protein, Transducin. *J Biol Chem*. 1987; 262(22):10831–8. [PubMed: 2440875]
10. Liebman PA, Sitaramayya A. Role of G-Protein-Receptor Interaction in Amplified Phosphodiesterase Activation of Retinal Rods. *Adv Cyclic Nucl Prot*. 1984; 17:215–25.
11. Bennet N, Dupont Y. The G-protein of Retinal Rod Outer Segments (Transducin). *J Biol Chem*. 1985; 260(7):4156–68. [PubMed: 3920215]
12. Salamon Z, Wang Y, Soulages JL, Brown MF, Tollin G. Surface Plasmon Resonance Spectroscopy Studies of Membrane Proteins: Transducin Binding and Activation by Rhodopsin Monitored in Thin Membrane Films. *Biophys J*. 1996; 71:283–94. [PubMed: 8804611]
13. Konig B, Arendt A, McDowell JH, Kahlert M, Hargrave PA, Hofmann KP. Three cytoplasmic loops of rhodopsin interact with transducin. *P Natl Acad Sci USA*. 1989; 86:6878–82.

14. Hamm HE, Deretic D, Arendt A, Hargrave PA, Koenig B, Hofmann KP. Site of G Protein Binding to Rhodopsin Mapped with Synthetic Peptides from the alpha Subunit. *Science*. 1988; 241(4867): 832–5. [PubMed: 3136547]
15. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, et al. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science*. 2000 Aug 4; 289(5480):739–45. [PubMed: 10926528]
16. Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB. The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature*. 1996 Jan 25; 379(6563):311–9. [PubMed: 8552184]
17. Fanelli F, Dell'Orco D. Rhodopsin Activation Follows Precoupling with Transducin: Inferences from Computational Analysis. *Biochemistry-US*. 2005; 44(45):14695–700.
18. Sgourakis NG, Garcia AE. The Membrane Complex between Transducin and Dark-State Rhodopsin Exhibits Large-Amplitude Interface Dynamics on the Sub-Microsecond Timescale: Insights from All-Atom MD Simulations. *J Mol Biol*. Feb 23.
19. Isin B, Rader AJ, Dhiman HK, Klein-Seetharaman J, Bahar I. Predisposition of the dark state of rhodopsin to functional changes in structure. *Proteins*. 2006 Dec 1; 65(4):970–83. [PubMed: 17009319]
20. Filipek S, Krzysko KA, Fotiadis D, Liang Y, Saperstein DA, Engel A, et al. A concept for G protein activation by G protein-coupled receptor dimers: the transducin/rhodopsin interface. *Photochem Photobiol Sci*. 2004 Jun; 3(6):628–38. [PubMed: 15170495]
21. Dell'Orco D, Seeber M, Fanelli F. Monomeric dark rhodopsin holds the molecular determinants for transducin recognition: Insights from computational analysis. *FEBS Lett*. 2007; 581:944–8. [PubMed: 17300784]
22. Nikiforovich GV, Taylor CM, Marshall GR. Modeling the Complex between Transducin and Photoactivated Rhodopsin, a Prototypical G-Protein-Coupled Receptor. *Biochemistry-US*. 2007; 46(16):4734–44.
23. Taylor CM, Nikiforovich GV, Marshall GR. Defining the Interface between the C-terminal Fragment of alpha-Transducin and Photoactivated Rhodopsin. *Biophys J*. 2007; 92(12):4325–34. [PubMed: 17351008]
24. Nikiforovich GV, Marshall GR. Modeling flexible loops in the dark-adapted and activated states of rhodopsin, a prototypical G-protein-coupled receptor. *Biophys J*. 2005 Dec; 89(6):3780–9. [PubMed: 16199504]
25. Slusarz R, Ciarkowski J. Interaction of class A G protein-coupled receptors with G proteins. *Acta Biochim Pol*. 2004; 51(1):129–36. [PubMed: 15094833]
26. Ciarkowski J, Witt M, Slusarz R. A hypothesis for GPCR activation. *J Mol Model*. 2005 Sep; 11(4–5):407–15. [PubMed: 15889287]
27. Atilgan AR, Durell SR, Jernigan RL, Demirel MC, Keskin O, Bahar I. Anisotropy of fluctuation dynamics of proteins with an elastic network model. *Biophys J*. 2001 Jan; 80(1):505–15. [PubMed: 11159421]
28. Eyal E, Yang LW, Bahar I. Anisotropic network model: systematic evaluation and a new web interface. *Bioinformatics*. 2006 Nov 1; 22(21):2619–27. [PubMed: 16928735]
29. Isin B, Schulten K, Tajkhorshid E, Bahar I. Mechanism of signal propagation upon retinal isomerization: insights from molecular dynamics simulations of rhodopsin restrained by normal modes. *Biophys J*. 2008 Jul; 95(2):789–803. [PubMed: 18390613]
30. Kisselev OG, Meyer CK, Heck M, Ernst OP, Hofmann KP. Signal transfer from rhodopsin to the G-protein: evidence for a two-site sequential fit mechanism. *Proceedings of the National Academy of Sciences of the United States of America*. 1999; 96(9):4898–903. [PubMed: 10220390]
31. Arimoto R, Kisselev OG, Makara GM, Marshall GR. Rhodopsin-transducin interface: studies with conformationally constrained peptides. *Biophysical Journal*. 2001; 81(6):3285–93. [PubMed: 11720992]
32. Comeau SR, Gatchell DW, Vajda S, Camacho CJ. ClusPro: an automated docking and discrimination method for the prediction of protein complexes. *Bioinformatics*. 2004 Jan 1; 20(1): 45–50. [PubMed: 14693807]
33. Camacho CJ, Zhang C. FastContact: rapid estimate of contact and binding free energies. *Bioinformatics*. 2005 May 15; 21(10):2534–6. [PubMed: 15713734]

34. Salom D, Lodowski DT, Stenkamp RE, Le Trong I, Golczak M, Jastrzebska B, et al. Crystal structure of a photoactivated deprotonated intermediate of rhodopsin. *Proc Natl Acad Sci U S A*. 2006 Oct 31; 103(44):16123–8. [PubMed: 17060607]
35. Fanelli F, Dell'Orco D. Dark and photoactivated rhodopsin share common binding modes to transducin. *FEBS Lett*. 2008; 582:991–6. [PubMed: 18307992]
36. Park JH, Scheerer P, Hofmann KP, Choe HW, Ernst OP. Crystal structure of the ligand-free G-protein-coupled receptor opsin. *Nature*. 2008 Jul 10; 454(7201):183–7. [PubMed: 18563085]
37. Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, et al. Crystal structure of opsin in its G-protein-interacting conformation. *Nature*. 2008 Sep 25; 455(7212):497–502. [PubMed: 18818650]
38. Scheerer P, Heck M, Goede A, Park JH, Choe HW, Ernst OP, et al. Structural and kinetic modeling of an activating helix switch in the rhodopsin-transducin interface. *Proc Natl Acad Sci U S A*. 2009 Jun 30; 106(26):10660–5. [PubMed: 19541654]
39. Maeda T, Imanishi Y, Palczewski K. Rhodopsin phosphorylation: 30 years later. *Prog Retin Eye Res*. 2003 Jul; 22(4):417–34. [PubMed: 12742390]
40. Huang CC, Yoshino-Koh K, Tesmer JJ. A surface of the kinase domain critical for the allosteric activation of G protein-coupled receptor kinases. *J Biol Chem*. 2009 Jun 19; 284(25):17206–15. [PubMed: 19364770]
41. Singh P, Wang B, Maeda T, Palczewski K, Tesmer JJ. Structures of rhodopsin kinase in different ligand states reveal key elements involved in G protein-coupled receptor kinase activation. *J Biol Chem*. 2008 May 16; 283(20):14053–62. [PubMed: 18339619]
42. Palczewski K, Arendt A, McDowell JH, Hargrave PA. Substrate recognition determinants for rhodopsin kinase: studies with synthetic peptides, polyanions, and polycations. *Biochemistry*. 1989 Oct 31; 28(22):8764–70. [PubMed: 2605220]
43. Onorato JJ, Palczewski K, Regan JW, Caron MG, Lefkowitz RJ, Benovic JL. Role of acidic amino acids in peptide substrates of the beta-adrenergic receptor kinase and rhodopsin kinase. *Biochemistry*. 1991 May 28; 30(21):5118–25. [PubMed: 1645191]
44. Palczewski K, Buczylo J, Kaplan MW, Polans AS, Crabb JW. Mechanism of rhodopsin kinase activation. *J Biol Chem*. 1991 Jul 15; 266(20):12949–55. [PubMed: 2071581]
45. Kim CM, Dion SB, Benovic JL. Mechanism of beta-adrenergic receptor kinase activation by G proteins. *J Biol Chem*. 1993 Jul 25; 268(21):15412–8. [PubMed: 8393441]
46. Chen CY, Dion SB, Kim CM, Benovic JL. Beta-adrenergic receptor kinase. Agonist-dependent receptor binding promotes kinase activation. *J Biol Chem*. 1993 Apr 15; 268(11):7825–31. [PubMed: 8096517]
47. Dean KR, Akhtar M. Novel mechanism for the activation of rhodopsin kinase: implications for other G protein-coupled receptor kinases (GRK's). *Biochemistry*. 1996 May 14; 35(19):6164–72. [PubMed: 8634260]
48. Fowles C, Sharma R, Akhtar M. Mechanistic Studies on the Phosphorylation of Photoexcited Rhodopsin. *FEBS Lett*. 1988; 238:56–60.
49. Gan X, Ma Z, Deng N, Wang J, Ding J, Li L. Involvement of the C-terminal proline-rich motif of G protein-coupled receptor kinases in recognition of activated rhodopsin. *J Biol Chem*. 2004 Nov 26; 279(48):49741–6. [PubMed: 15375171]
50. Yu QM, Cheng ZJ, Gan XQ, Bao GB, Li L, Pei G. The amino terminus with a conserved glutamic acid of G protein-coupled receptor kinases is indispensable for their ability to phosphorylate photoactivated rhodopsin. *J Neurochem*. 1999 Sep; 73(3):1222–7. [PubMed: 10461915]
51. Levay K, Satpaev DK, Pronin AN, Benovic JL, Slepak VZ. Localization of the sites for Ca²⁺-binding proteins on G protein-coupled receptor kinases. *Biochemistry*. 1998 Sep 29; 37(39):13650–9. [PubMed: 9753452]
52. Higgins MK, Oprian DD, Schertler GF. Recoverin binds exclusively to an amphipathic peptide at the N terminus of rhodopsin kinase, inhibiting rhodopsin phosphorylation without affecting catalytic activity of the kinase. *J Biol Chem*. 2006 Jul 14; 281(28):19426–32. [PubMed: 16675451]

53. Palczewski K, Buczylo J, Lebioda L, Crabb JW, Polans AS. Identification of the N-terminal region in rhodopsin kinase involved in its interaction with rhodopsin. *J Biol Chem*. 1993 Mar 15; 268(8):6004–13. [PubMed: 8383684]
54. Ames JB, Levay K, Wingard JN, Lusin JD, Slepak VZ. Structural basis for calcium-induced inhibition of rhodopsin kinase by recoverin. *J Biol Chem*. 2006 Dec 1; 281(48):37237–45. [PubMed: 17020884]
55. Gan XQ, Wang JY, Yang QH, Li Z, Liu F, Pei G, et al. Interaction between the conserved region in the C-terminal domain of GRK2 and rhodopsin is necessary for GRK2 to catalyze receptor phosphorylation. *J Biol Chem*. 2000 Mar 24; 275(12):8469–74. [PubMed: 10722682]
56. Kelleher DJ, Johnson GL. Characterization of rhodopsin kinase purified from bovine rod outer segments. *J Biol Chem*. 1990 Feb 15; 265(5):2632–9. [PubMed: 2303419]
57. Shi W, Osawa S, Dickerson CD, Weiss ER. Rhodopsin mutants discriminate sites important for the activation of rhodopsin kinase and Gt. *J Biol Chem*. 1995 Feb 3; 270(5):2112–9. [PubMed: 7836439]
58. Thurmond RL, Creuzenet C, Reeves PJ, Khorana HG. Structure and function in rhodopsin: peptide sequences in the cytoplasmic loops of rhodopsin are intimately involved in interaction with rhodopsin kinase. *Proc Natl Acad Sci U S A*. 1997 Mar 4; 94(5):1715–20. [PubMed: 9050844]
59. Shi W, Sports CD, Raman D, Shirakawa S, Osawa S, Weiss ER. Rhodopsin arginine-135 mutants are phosphorylated by rhodopsin kinase and bind arrestin in the absence of 11-cis-retinal. *Biochemistry*. 1998 Apr 7; 37(14):4869–74. [PubMed: 9538004]
60. Cai K, Klein-Seetharaman J, Hwa J, Hubbell WL, Khorana HG. Structure and function in rhodopsin: effects of disulfide cross-links in the cytoplasmic face of rhodopsin on transducin activation and phosphorylation by rhodopsin kinase. *Biochemistry*. 1999 Sep 28; 38(39):12893–8. [PubMed: 10504260]
61. Tesmer VM, Kawano T, Shankaranarayanan A, Kozasa T, Tesmer JJ. Snapshot of activated G proteins at the membrane: the Galphaq-GRK2-Gbetagamma complex. *Science*. 2005 Dec 9; 310(5754):1686–90. [PubMed: 16339447]
62. Burns ME, Mendez A, Chen CK, Almuete A, Quillinan N, Simon MI, et al. Deactivation of phosphorylated and nonphosphorylated rhodopsin by arrestin splice variants. *J Neurosci*. 2006 Jan 18; 26(3):1036–44. [PubMed: 16421323]
63. Gurevich VV, Benovic JL. Visual arrestin binding to rhodopsin. Diverse functional roles of positively charged residues within the phosphorylation-recognition region of arrestin. *J Biol Chem*. 1995 Mar 17; 270(11):6010–6. [PubMed: 7890732]
64. Gurevich VV, Gurevich EV. The molecular acrobatics of arrestin activation. *Trends Pharmacol Sci*. 2004 Feb; 25(2):105–11. [PubMed: 15102497]
65. Krupnick JG, Gurevich VV, Schepers T, Hamm HE, Benovic JL. Arrestin-rhodopsin interaction. Multi-site binding delineated by peptide inhibition. *J Biol Chem*. 1994 Feb 4; 269(5):3226–32. [PubMed: 8106358]
66. Raman D, Osawa S, Weiss ER. Binding of arrestin to cytoplasmic loop mutants of bovine rhodopsin. *Biochemistry*. 1999 Apr 20; 38(16):5117–23. [PubMed: 10213616]
67. Vishnivetskiy SA, Paz CL, Schubert C, Hirsch JA, Sigler PB, Gurevich VV. How does arrestin respond to the phosphorylated state of rhodopsin? *J Biol Chem*. 1999 Apr 23; 274(17):11451–4. [PubMed: 10206946]
68. Modzelewska A, Filipek S, Palczewski K, Park PS. Arrestin interaction with rhodopsin: conceptual models. *Cell Biochem Biophys*. 2006; 46(1):1–15. [PubMed: 16943619]
69. Hanson SM, Gurevich EV, Vishnivetskiy SA, Ahmed MR, Song X, Gurevich VV. Each rhodopsin molecule binds its own arrestin. *Proc Natl Acad Sci U S A*. 2007 Feb 27; 104(9):3125–8. [PubMed: 17360618]
70. Hirsch JA, Schubert C, Gurevich VV, Sigler PB. The 2.8 Å crystal structure of visual arrestin: a model for arrestin's regulation. *Cell*. 1999 Apr 16; 97(2):257–69. [PubMed: 10219246]
71. Granzin J, Wilden U, Choe HW, Labahn J, Krafft B, Buldt G. X-ray crystal structure of arrestin from bovine rod outer segments. *Nature*. 1998 Feb 26; 391(6670):918–21. [PubMed: 9495348]
72. Smith WC, Dinculescu A, Peterson JJ, McDowell JH. The surface of visual arrestin that binds to rhodopsin. *Mol Vis*. 2004 Jun 15; 10:392–8. [PubMed: 15215746]

73. Vishnivetskiy SA, Hirsch JA, Velez MG, Gurevich YV, Gurevich VV. Transition of arrestin into the active receptor-binding state requires an extended interdomain hinge. *J Biol Chem*. 2002 Nov 15; 277(46):43961–7. [PubMed: 12215448]
74. Hanson SM, Dawson ES, Francis DJ, Van Eps N, Klug CS, Hubbell WL, et al. A model for the solution structure of the rod arrestin tetramer. *Structure*. 2008 Jun; 16(6):924–34. [PubMed: 18547524]
75. Imamoto Y, Tamura C, Kamikubo H, Kataoka M. Concentration-dependent tetramerization of bovine visual arrestin. *Biophys J*. 2003 Aug; 85(2):1186–95. [PubMed: 12885662]
76. Wilden U, Kuhn H. Light-dependent phosphorylation of rhodopsin: number of phosphorylation sites. *Biochemistry*. 1982 Jun 8; 21(12):3014–22. [PubMed: 6980670]
77. Kennedy MJ, Lee KA, Niemi GA, Craven KB, Garwin GG, Saari JC, et al. Multiple phosphorylation of rhodopsin and the in vivo chemistry underlying rod photoreceptor dark adaptation. *Neuron*. 2001 Jul 19; 31(1):87–101. [PubMed: 11498053]
78. Buczylo J, Gutmann C, Palczewski K. Regulation of rhodopsin kinase by autophosphorylation. *Proc Natl Acad Sci U S A*. 1991 Mar 15; 88(6):2568–72. [PubMed: 2006192]
79. Doan T, Mendez A, Detwiler PB, Chen J, Rieke F. Multiple phosphorylation sites confer reproducibility of the rod's single-photon responses. *Science*. 2006 Jul 28; 313(5786):530–3. [PubMed: 16873665]
80. Vishnivetskiy SA, Raman D, Wei J, Kennedy MJ, Hurley JB, Gurevich VV. Regulation of arrestin binding by rhodopsin phosphorylation level. *J Biol Chem*. 2007 Nov 2; 282(44):32075–83. [PubMed: 17848565]
81. Kisselev OG, Downs MA, McDowell JH, Hargrave PA. Conformational changes in the phosphorylated C-terminal domain of rhodopsin during rhodopsin arrestin interactions. *J Biol Chem*. 2004 Dec 3; 279(49):51203–7. [PubMed: 15351781]
82. Feuerstein SE, Pulvermuller A, Hartmann R, Granzin J, Stoldt M, Henklein P, et al. Helix formation in arrestin accompanies recognition of photoactivated rhodopsin. *Biochemistry*. 2009 Nov 17; 48(45):10733–42. [PubMed: 19835414]
83. Sommer ME, Farrens DL, McDowell JH, Weber LA, Smith WC. Dynamics of arrestin-rhodopsin interactions: loop movement is involved in arrestin activation and receptor binding. *J Biol Chem*. 2007 Aug 31; 282(35):25560–8. [PubMed: 17606620]
84. Hanson SM, Francis DJ, Vishnivetskiy SA, Kolobova EA, Hubbell WL, Klug CS, et al. Differential interaction of spin-labeled arrestin with inactive and active phosphorhodopsin. *Proc Natl Acad Sci U S A*. 2006 Mar 28; 103(13):4900–5. [PubMed: 16547131]
85. Vishnivetskiy SA, Francis D, Van Eps N, Kim M, Hanson SM, Klug CS, et al. The role of arrestin alpha-helix I in receptor binding. *J Mol Biol*. 2010 Jan 8; 395(1):42–54. [PubMed: 19883657]
86. Skegrod D, Pulvermuller A, Krafft B, Granzin J, Hofmann KP, Buldt G, et al. N-terminal and C-terminal domains of arrestin both contribute in binding to rhodopsin. *Photochem Photobiol*. 2007 Mar–Apr; 83(2):385–92. [PubMed: 17132044]
87. Sommer ME, Farrens DL. Arrestin can act as a regulator of rhodopsin photochemistry. *Vision Res*. 2006 Dec; 46(27):4532–46. [PubMed: 17069872]
88. Sommer ME, Smith WC, Farrens DL. Dynamics of arrestin-rhodopsin interactions: arrestin and retinal release are directly linked events. *J Biol Chem*. 2005 Feb 25; 280(8):6861–71. [PubMed: 15591052]
89. Ohguro H, Rudnicka-Nawrot M, Buczylo J, Zhao X, Taylor JA, Walsh KA, et al. Structural and enzymatic aspects of rhodopsin phosphorylation. *J Biol Chem*. 1996 Mar 1; 271(9):5215–24. [PubMed: 8617805]
90. Torisawa A, Arinobu D, Tachibanaki S, Kawamura S. Amino acid residues in GRK1/GRK7 responsible for interaction with S-modulin/recoverin. *Photochem Photobiol*. 2008 Jul–Aug; 84(4): 823–30. [PubMed: 18266817]
91. Senin, Koch KW, Akhtar M, Philippov PP. Ca²⁺-dependent control of rhodopsin phosphorylation: recoverin and rhodopsin kinase. *Adv Exp Med Biol*. 2002; 514:69–99. [PubMed: 12596916]
92. Inglese J, Koch WJ, Caron MG, Lefkowitz RJ. Isoprenylation in regulation of signal transduction by G-protein-coupled receptor kinases. *Nature*. 1992 Sep 10; 359(6391):147–50. [PubMed: 1522899]

93. Komolov KE, Senin, Kovaleva NA, Christoph MP, Churumova VA, Grigoriev, et al. Mechanism of rhodopsin kinase regulation by recoverin. *J Neurochem.* 2009 Jul; 110(1):72–9. [PubMed: 19457073]
94. Raman D, Osawa S, Gurevich VV, Weiss ER. The interaction with the cytoplasmic loops of rhodopsin plays a crucial role in arrestin activation and binding. *J Neurochem.* 2003 Mar; 84(5): 1040–50. [PubMed: 12603828]
95. Pulvermuller A, Palczewski K, Hofmann KP. Interaction between photoactivated rhodopsin and its kinase: stability and kinetics of complex formation. *Biochemistry.* 1993 Dec 28; 32(51):14082–8. [PubMed: 8260489]
96. Kolobova EA, Klug CS, Buckheister SM, Kuznetzow AK, Hubbell WL, Gurevich VV. Identification of Elements Involved in Arrestin-rhodopsin Interaction by Site-directed Spin-labelling. *Invest Ophthalmol Vis Sci.* 2003;3176.
97. Tirupula KC, Balem F, Yanamala N, Klein-Seetharaman J. pH-dependent interaction of rhodopsin with cyanidin-3-glucoside. 2. Functional aspects. *Photochem Photobiol.* 2009 Mar–Apr; 85(2): 463–70. [PubMed: 19267871]
98. Taylor CM, Barda Y, Kisselev OG, Marshall GR. Modulating G-protein coupled receptor/G-protein signal transduction by small molecules suggested by virtual screening. *J Med Chem.* 2008 Sep 11; 51(17):5297–303. [PubMed: 18707087]
99. Oldham WM, Hamm HE. Heterotrimeric G protein activation by G-protein-coupled receptors. *Nature Reviews Molecular Cell Biology.* 2008; 9(1):60–71.
100. Janz JM, Farrens DL. Rhodopsin activation exposes a key hydrophobic binding site for the transducin alpha-subunit C terminus. *Journal of Biological Chemistry.* 2004; 279(28):29767–73. [PubMed: 15070895]
101. Yanamala N, Tirupula KC, Balem F, Klein-Seetharaman J. pH-dependent interaction of rhodopsin with cyanidin-3-glucoside. 1. Structural aspects. *Photochem Photobiol.* 2009 Mar–Apr; 85(2):454–62. [PubMed: 19192199]
102. Sakmar TP. Structure of rhodopsin and the superfamily of seven-helical receptors: the same and not the same. *Curr Opin Cell Biol.* 2002 Apr; 14(2):189–95. [PubMed: 11891118]
103. Thomas SM, Bhola NE, Zhang Q, Contrucci SC, Wentzel AL, Freilino ML, et al. Cross-talk between G protein-coupled receptor and epidermal growth factor receptor signaling pathways contributes to growth and invasion of head and neck squamous cell carcinoma. *Cancer Research.* 2006; 66(24):11831–9. [PubMed: 17178880]
104. Zhang Q, Bhola NE, Lui VW, Siwak DR, Thomas SM, Gubish CT, et al. Antitumor mechanisms of combined gastrin-releasing peptide receptor and epidermal growth factor receptor targeting in head and neck cancer. *Molecular Cancer Therapeutics.* 2007; 6(4):1414–24. [PubMed: 17431120]
105. Lui VW, Thomas SM, Zhang Q, Wentzel AL, Siegfried JM, Li JY, et al. Mitogenic effects of gastrin-releasing peptide in head and neck squamous cancer cells are mediated by activation of the epidermal growth factor receptor. *Oncogene.* 2003; 22(40):6183–93. [PubMed: 13679857]
106. Gschwind A, Prenzel N, Ullrich A. Lysophosphatidic acid-induced squamous cell carcinoma cell proliferation and motility involves epidermal growth factor receptor signal transactivation. *Cancer Research.* 2002; 62(21):6329–36. [PubMed: 12414665]
107. Kubo S, Ishiki T, Doe I, Sekiguchi F, Nishikawa H, Kawai K, et al. Distinct activity of peptide mimetic intracellular ligands (pepducins) for proteinase-activated receptor-1 in multiple cells/tissues. *Ann N Y Acad Sci.* 2006 Dec.1091:445–59. [PubMed: 17341635]
108. Covic L, Gresser AL, Talavera J, Swift S, Kuliopulos A. Activation and inhibition of G protein-coupled receptors by cell-penetrating membrane-tethered peptides. *Proc Natl Acad Sci U S A.* 2002 Jan 22; 99(2):643–8. [PubMed: 11805322]
109. Swift S, Leger AJ, Talavera J, Zhang L, Bohm A, Kuliopulos A. Role of the PAR1 receptor 8th helix in signaling: the 7-8-1 receptor activation mechanism. *J Biol Chem.* 2006 Feb 17; 281(7): 4109–16. [PubMed: 16354660]
110. Kaneider NC, Agarwal A, Leger AJ, Kuliopulos A. Reversing systemic inflammatory response syndrome with chemokine receptor pepducins. *Nat Med.* 2005 Jun; 11(6):661–5. [PubMed: 15880119]

111. Miller J, Agarwal A, Devi LA, Fontanini K, Hamilton JA, Pin JP, et al. Insider access: pepducin symposium explores a new approach to GPCR modulation. *Ann N Y Acad Sci.* 2009 Nov; 1180(Suppl 1):E1–12. [PubMed: 19925639]
112. Fredriksson R, Schiöth HB. The repertoire of G-protein-coupled receptors in fully sequenced genomes. *Mol Pharmacol.* 2005 May; 67(5):1414–25. [PubMed: 15687224]
113. Müller G. Towards 3D structures of G protein-coupled receptors: a multidisciplinary approach. *Curr Med Chem.* 2000 Sep; 7(9):861–88. [PubMed: 10911020]
114. Gutkind JS. The pathways connecting G protein-coupled receptors to the nucleus through divergent mitogen-activated protein kinase cascades. *J Biol Chem.* 1998 Jan 23; 273(4):1839–42. [PubMed: 9442012]
115. Prevost GP, Lonchampt MO, Holbeck S, Attoub S, Zaharevitz D, Alley M, et al. Anticancer activity of BIM-46174, a new inhibitor of the heterotrimeric G α /G β gamma protein complex. *Cancer Res.* 2006 Sep 15; 66(18):9227–34. [PubMed: 16982767]
116. Qi Y, Dhiman HK, Bhola N, Budyak I, Kar S, Man D, et al. Systematic prediction of human membrane receptor interactions. *Proteomics.* 2009 Dec; 9(23):5243–55. [PubMed: 19798668]
117. Berman H, Henrick K, Nakamura H. Announcing the worldwide Protein Data Bank. *Nat Struct Biol.* 2003 Dec.10(12):980. [PubMed: 14634627]
118. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, et al. The Protein Data Bank. *Nucleic Acids Res.* 2000 Jan 1; 28(1):235–42. [PubMed: 10592235]
119. Okada T, Fujiyoshi Y, Silow M, Navarro J, Landau EM, Shichida Y. Functional role of internal water molecules in rhodopsin revealed by X-ray crystallography. *Proc Natl Acad Sci U S A.* 2002 Apr 30; 99(9):5982–7. [PubMed: 11972040]

Fig. 1. Structure of rhodopsin and its cytoplasmic interaction partners, transducin, arrestin and rhodopsin kinase. Structures were downloaded from the pdb [117, 118], with pdb id's 1L9H [119], 1GOT [16], 1CF1 [70], 3C4W [41].

Fig. 2.

Overlap in binding interfaces between small molecule ligands and transducin on rhodopsin.

A. Peptide corresponding to the C-terminal helix in transducin (IRENLKDSGLF) docked to rhodopsin dark state crystal structure. **B.** Anthocyanin cyanidin-3-glucoside (C3G) docked to rhodopsin as described [101]. **C.** Transducin docked to rhodopsin dark state dimer crystal structure [20]. In C, transducin is not shown for clarity and only the residues that are at the binding interface are highlighted. All rhodopsin structures are rendered as surface representation using Connolly surface calculation. The C-terminal peptide is shown in cartoon representation in A, the anthocyanin C3G in B is shown in spacefill representation. The color code for surfaces on rhodopsin is as follows: red – residues found in all; blue – residues unique to each structure; yellow – found in both C3G and Gt-peptide; magenta – found in both C3G and transducin; orange – found in both Gt-peptide and transducin.

Fig. 3. Docking of C-terminal peptide to rhodopsin

Docking of Gt C-terminal peptide VLEDLKSCGLF (colored in magenta) to (A) dark-state and (B) ANM-activated model of rhodopsin [19] (C) X-ray crystal structure of opsin state in complex with Gt C-terminal peptide ILENLKDCGLF (colored in yellow) [37] and (D) overlay of (B) and (C). R crystal structure and R* activated model are represented as cartoon and colored using a blue-red spectrum in (A), and (B), respectively. Opsin is colored in cyan and rendered as cartoon in (C),(D) and ANM generated model of R* is colored in green in (D)

Fig. 4.

Schematic highlighting the potential for GPCR drug discovery associated with the finding of overlapping binding sites for small molecules and proteins of the signal transduction cascade in rhodopsin. Left: Rhodopsin serves as a prototypic receptor for GPCRs in general, where the activation by light is paralleled by binding of small molecule ligands. These ligands may bind in orthosteric (red) or allosteric (green) pockets on the GPCRs, and their binding affects directly or indirectly rhodopsin's binding partners, G protein, arrestin and rhodopsin kinase, and the equivalent proteins in other GPCRs. Right: In cases, where GPCRs cooperate with non-GPCRs in signaling, such as in the case of the Epidermal Growth Factor receptor (discussed in the text), the direct modulation of GPCR signaling analogous to the scenario shown on the left, will impact signaling more broadly, and may involve other GPCRs (indicated by the circle) or non-GPCR signaling proteins, in particular other receptors.