Computational Methods in Drug Design: Modeling G Protein-Coupled Receptor Monomers, Dimers, and Oligomers

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ABSTRACT

G protein-coupled receptors (GPCRs) are membrane proteins that serve as very important links through which cellular signal transduction mechanisms are activated. Many vital physiological events such as sensory perception, immune defense, cell communication, chemotaxis, and neurotransmission are mediated by GPCRs. Not surprisingly, GPCRs are major targets for drug development today. Most modeling studies in the GPCR field have focused upon the creation of a model of a single GPCR (ie, a GPCR monomer) based upon the crystal structure of the Class A GPCR, rhodopsin. However, the emerging concept of GPCR dimerization has challenged our notions of the monomeric GPCR as functional unit. Recent work has shown not only that many GPCRs exist as homo- and heterodimers but also that GPCR oligomeric assembly may have important functional roles. This review focuses first on methodology for the creation of monomeric GPCR models. Special emphasis is given to the identification of localized regions where the structure of a GPCR may diverge from that of bovine rhodopsin. The review then focuses on GPCR dimers and oligomers and the bioinformatics methods available for identifying homo- and heterodimer interfaces.

KEYWORDS: GPCR modeling, GPCR dimer, GPCR oligomer

INTRODUCTION

G protein-coupled receptors (GPCRs) are membrane proteins that serve as very important links through which cellular signal transduction mechanisms are activated. Many vital physiological events such as sensory perception, immune defense, cell communication, chemotaxis, and neurotransmission are mediated by GPCRs. Not surprisingly,

GPCRs are major targets for drug development today. The total number of GPCRs with and without introns in the human genome has been estimated to be ~950, of which 500 are odorant or taste receptors and 450 are receptors for endogenous ligands.¹ Many of these GPCRs have been classified as orphan receptors because their endogenous ligands have not yet been identified. Based upon sequence homology, GPCRs have been classified into 6 families/classes (A-F).² Alternatively, the GPCRs have been broken down into a numerical system (1-5)³ or the GRAFS (Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2, and Secretion) system.⁴ The rhodopsin family is the largest and forms 4 main groups with 13 subbranches. The rhodopsin-like family Class A (named 1 or rhodopsin in the GRAFS system) includes the cationic neurotransmitters and such receptors as the cannabinoid and EDG (Endothelial Differentiation Gene) receptors, which have lipid-derived endogenous ligands. It is this first family of receptors that has received the majority of attention thus far in the modeling literature. The other GPCR classes/families include the secretin-like Class B (or 2 or secretin) class/family; the metabotropic glutamate and pheromone Class C (3 or glutamate) family; the fungal pheromone Class D (or 4) family⁵; the cAMP receptor Class E family; and the frizzled/smoothed Class F (or 5 or frizzled) family.²⁻⁴

Ballesteros and Weinstein⁶ have proposed a universal numbering scheme for Class A GPCRs. In this numbering system, the most highly conserved residue in each transmembrane (TM) helix is assigned a locant of .50. This number is preceded by the TM number and followed in parentheses by the sequence number. All other residues in a TM helix are numbered relative to this residue. In this numbering system, for example, the most highly conserved residue in TM2 of bovine rhodopsin is D2.50(83). The residue that immediately precedes it is A2.49(82). The Ballesteros-Weinstein numbering system will be used throughout this review.

GPCRs are expressed on cell membranes and are constructed to recognize specific ligands that can range in size from small organic molecules, such as dopamine, to much larger ligands, such as the peptide hormones. As revealed by the crystal structure of bovine rhodopsin at 2.8 Å,⁷ 2.65 Å,⁸

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2.6 Å,⁹ and 2.2 Å resolution,¹⁰ the general topology of a GPCR includes (1) an extracellular N terminus; (2) 7 TM alpha helices arranged to form a closed bundle; (3) loops connecting TM helices that extend intra- and extracellularly; and (4) an intracellular C terminus that begins with a short helical segment (Helix 8) oriented parallel to the membrane surface. Ligand binding in GPCRs is thought to occur within the binding site crevice formed by the TM helix bundle, to extracellular loops, or to a combination of extracellular loop and binding site crevice residues. Agonists are thought to bind and produce a conformational change that initiates coupling to the G protein that is located inside the cell. An agonist-bound receptor activates an appropriate G protein that promotes dissociation of GDP (guanosine diphosphate). Although the interactions between receptor and G protein are poorly understood, both mutagenesis and biochemical experiments with a variety of GPCRs suggest that, first, ligand-induced receptor activation causes a change in the relative orientations of TM3 and TM6. This modification then affects the conformation of the G protein-interacting intracellular loops of the receptor and thus uncovers previously masked G protein binding sites.¹¹ Second, as GDP is buried within the G protein, the receptor–G protein interaction must, in addition, promote changes in interdomain interactions.¹² Thus, to activate the G protein, the receptor has to deliver *2 pieces of information*: the first for the formation of the receptor/G protein complex and the second to induce the exchange of bound GDP (guanosine diphosphate) for GTP (guanosine triphosphate) on the heterotrimeric G protein, resulting in the dissociation of the G protein into active G α -GTP and G $\beta\gamma$ subunits. Both the G α -GTP and G $\beta\gamma$ subunits then interact with effectors to transduce the signal initiated by agonist binding.

Rhodopsin is the dim-light photoreceptor and is a prototypical member of the Class A GPCR family.³ It consists of a 348-amino-acid protein, opsin, which binds the chromophore 11-cis-retinal via a protonated Schiff base linkage to Lys7.43(296), giving the ground state of the protein an absorption maximum at 498 nm.¹³ Absorption of a photon by 11-cis-retinal triggers its isomerization to the all-trans form, converting light energy into molecular movement. Rhodopsin then thermally relaxes through a series of distinct photointermediates, each with characteristic UV/visible absorption maxima (λ_{max}). The early photointermediates include bathorhodopsin (batho, λ_{max} = 543 nm), a blue-shifted intermediate, and lumirhodopsin (lumi, λ_{max} = 497 nm).¹⁴ An equilibrium is formed between the later photointermediates, metarhodopsin I (meta I, λ_{max} = 480 nm) and metarhodopsin II (meta II, λ_{max} = 380 nm). Meta II corresponds to the fully activated receptor, which binds to and activates the heterotrimeric G protein transducin.¹³

Rhodopsin has been crystallized in its ground state in 2 different space groups: a tetragonal P4₁^{7,10} crystal form and a

trigonal P3₁ packing arrangement.⁸ The structures determined by x-ray crystallography from these 2 crystal forms are very similar within the TM domains but show differences in the G-protein binding region of the cytoplasmic surface, where the location of the third intracellular loop (IC-3 loop) between TM5 and TM6 is highly variable. Several additional water molecules are visible in the P3₁ structure, including a water molecule that crosslinks the kinks in TM6 and TM7 and a water molecule that is part of the complex counterion of the Schiff base. EM data show that the IC-3 loop adopts the same conformation as seen in the P3₁ structure (see Figure 2d in Schertler¹⁵). Okada et al reported the rhodopsin structure with the highest resolution (2.2 Å) so far from tetragonal crystals.¹⁰ However, in the tetragonal P4₁ crystal, there is a crystal contact between the IC-3 loops in neighboring molecules in the crystal. This contact distorts the loop structure seen in the 2.2 Å structure (PDB, Protein Data Bank, accession number 1U19). For this reason, the 2.65 Å structure (PDB accession number 1GZM)⁸ may be the better structural template from which to start a homology model of a rhodopsin-like GPCR, particularly in the region of the IC-3 loop because there is no distortion of loop structures due to crystal packing in the 1GZM structure.

MODELING GPCR MONOMERS

Homology Modeling of the GPCR Inactive State

By far the most common way the inactive/ground state of a GPCR is modeled is by homology modeling based on the crystal structure of bovine rhodopsin.⁷⁻¹⁰ The first step in homology modeling would be sequence alignment with the bovine rhodopsin sequence. There are numerous sequence alignment programs available (see <http://helix.nih.gov/apps/bioinfo/msa.html> and <http://www.hku.hk/bru/hk/sgaln.html> for a sampling). One of the most commonly used alignment programs is ClustalW (<http://www.ebi.ac.uk/clustalw/>). ClustalW is a general-purpose multiple sequence alignment program for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences and lines sequences up so that the identities, similarities, and differences can be seen. Evolutionary relationships can be seen via viewing cladograms or phylograms. However, a word of caution is in order. Such automatic sequence alignment programs work best when the GPCR sequence one wishes to align with bovine rhodopsin contains all of the highly conserved residues/sequence motifs across Class A GPCRs. Using the Ballesteros and Weinstein numbering system,⁶ these are N1.50 in TM1, D2.50 in TM2, (D/E)RY in TM3, W4.50 in TM4, P5.50 in TM5, CWXP in TM6, and NPXXY in TM7. Misalignment of a TM region can occur in a helix span for which the appropriate residue

or motif is missing or where more than one residue of the same type is located in proximity to each other. As an example, the cannabinoid CB1 and CB2 receptors, which are Class A receptors, lack the highly conserved proline at 5.50. As detailed in an early paper,¹⁶ the second most highly conserved residue in TM5 of most Class A GPCRs is a Tyr at position 5.58 in the C terminal portion of TM5. The CB1 and CB2 receptors have 2 Tyr in common in this region (CB1: YLMFWIGVTSVLLLFIVYAYMYILWKA; CB2: YLLSWLLFIAFLFSGIIITYGHVLWKA). Consequently, automatic alignment results for CB1/CB2 in the TM5 region can diverge from one another depending on the alignment program employed. To determine which of these would correspond to Tyr 5.58 in other GPCRs, we performed a manual "structural alignment."^{6,16} In such an alignment, residues are aligned based upon their predicted interior or surface-exposed character. First, the cytoplasmic end of TM5 was predicted using the criterion that Arg/Lys patches at the intracellular end of a TM helix face the lipid domain and may anchor the helix in the membrane by interaction with the negatively charged phospholipid head groups.¹⁷ The cytoplasmic end of TM5 predicted using the Arg/Lys criterion was used then to superimpose the predicted accessibility profile for TM5 of CB1 with the equivalent profile of the rest of the proteins used in the original alignment (see Bramblett et al¹⁶). By this method, Y294 in CB1 and Y210 in CB2 can be aligned with the highly conserved Tyr at 5.58 in other GPCRs. The Leu residue 8 residues back in the sequence, which normally is a Pro in Class A GPCRs, was assigned the locant 5.50 to preserve numbering system correspondence with other GPCRs.

Software such as MODELLER can be used for homology or comparative modeling of GPCR 3-dimensional structures.¹⁸ The user provides an alignment of a sequence to be modeled with known related structures, and MODELLER automatically calculates a model containing all nonhydrogen atoms. MODELLER implements comparative protein structure modeling by satisfaction of spatial restraints¹⁹ and can calculate loop segments for models of GPCRs.²⁰

The bovine rhodopsin sequence also has sequence motifs that dictate local structure that may be absent in another GPCR. Ballesteros et al have proposed that the overall structures of rhodopsin and of aminergic receptors are very similar, although there are localized regions where the structure of these receptors may diverge. Furthermore, they have proposed that several of the highly unusual structural features of rhodopsin are also present in aminergic GPCRs, despite the absence of amino acids that might have been thought critical to the adoption of these features. Thus, different amino acids or alternate microdomains can support similar deviations from regular α -helical structure, thereby resulting in similar tertiary structure. Such structural mimicry may be a mechanism by which a common ancestor

could diverge sufficiently to develop the selectivity necessary to interact with diverse signals, while still maintaining a similar overall fold. Through this process, the core function of signaling activation through a conformational change in the TM segments that alters the conformation of the cytoplasmic surface and subsequent interaction with G proteins is presumably shared by the entire Class A family of receptors, despite their selectivity for a diverse group of ligands.²¹ In the Class A aminergic GPCRs (with the exception of most of the muscarinic receptor family) there is a Pro at 2.59 in TM2. In bovine rhodopsin, no Pro exists in TM2, but TM2 does have a GGXTT motif that begins at G2.57(89) and ends at T2.61(93). This GGXTT motif causes a local distortion in the TM2 helix backbone that may be mimicked by P2.59 in most of the aminergic GPCRs.

But in other Class A GPCRs, there is neither a GGXTT motif nor a P2.59. This was the case with the cannabinoid CB2 receptor. We were drawn to the study of TM2 by substituted cysteine accessibility method data obtained by our collaborator, Dr. Zhao-Hui Song, which indicated that C2.59 in CB2 can be labeled by methanethiosulfonate (MTS) reagents, indicating that this residue is accessible from within the binding site crevice.²² Our original TM2 model had been modeled as a regular α -helix because CB2 TM2 lacked the GGXTT motif of rhodopsin. In this modeled helix, C2.59 faced lipid. So, we sought some other residue or motif in the CB2 sequence that could alter the conformation of CB2 TM2.

Both serines and threonines have been shown to be able to act as hinge residues to affect the conformation of an α helix via an intrahelical hydrogen bond between the O γ atom of the Ser or Thr (in a g^- or $+60^\circ \chi_1$) and the i-3 or i-4 carbonyl oxygen of the helix backbone. This is of particular significance for membrane proteins.²³ Using the biased Monte Carlo/simulated annealing method Conformational Memories,²⁴ we tested the hypothesis that S2.54(84) in a $g^- (+60^\circ) \chi_1$ forms an intrahelical hydrogen bond that produces an alteration from normal α -helicity in CB2 TM2. We found that S2.54(84) can indeed influence the backbone conformation of TM2. This influence was extracellular to S2.54(84), allowing the highly conserved residue, D2.50, to remain oriented as in rhodopsin. While we did not see a statistically significant difference in TM2 helix bend angles, we did see a statistically significant difference in another measure of helix geometry, wobble angle. So our Conformational Memories calculations suggested that S2.54(84) introduces a distortion from normal α -helicity in TM2. As illustrated in Figure 1, our Conformational Memories calculations predicted that the S2.54(84) effect on the TM2 conformation allowed a wobble in TM2 that places C2.59 in the TM2-TM3 interface, where it is accessible to MTS reagent. The corresponding residue in bovine rhodopsin, T2.59, faces lipid, as illustrated in Figure 1. This result suggests

Figure 1. A comparison of the relative positions of residue 2.59 in TM2 of Rho versus TM2 of CB2 (as predicted by Conformational Memories). TM2 of Rho and TM2 of CB2 have been superimposed at their intracellular ends to D2.50. It is clear here that the extracellular portions of TM2 of Rho versus CB2 differ significantly, resulting in a shift in the location of residue 2.59, for example. TM indicates transmembrane segment; CB, cannabinoid 2 receptor.²²

that there is a localized region in TM2 (extracellular to S2.54) where the structure of CB2 and rhodopsin diverge.

Even when the GPCR to be modeled has the highly conserved GPCR motifs, there still may be sequence-dictated variability from the rhodopsin structure. The CWXP motif in TM6 is thought to act as a flexible hinge, permitting TM6 to straighten during GPCR activation.²⁵ So can one expect that the flexibility and the helix geometry of TM6 is the same for all helices that have the CWXP motif? A study of TM6 in the cannabinoid receptors is offered here to illustrate that one cannot assume a common geometry. Both the CB1 and the CB2 receptors have the CWXP motif in TM6. In CB1, the sequence is CWGP, and in CB2, it is CWFP. We undertook a Conformational Memories study of CB1 versus CB2 TM6 in isolation to better understand the conformations possible for this important helix.²⁶ The Conformational Memories method employs multiple Monte Carlo/simulated annealing random walks and the Amber* force field. Conformational Memories has been shown to achieve complete sampling of the conformational space of flexible molecules, to converge in a very practical number of steps, and to be capable of overcoming energy barriers efficiently.²⁴ When Conformational Memories is used, the conformational properties of a helix can be fully characterized by the free energy of each conformation that the helix can adopt. This property includes not only the intrinsic energy of each conformational state but also the probability that the helix will adopt each conformation relative to all other ones accessible in an equilibrated thermodynamic ensemble. The calculation is performed in 2 phases. In the first phase, repeated runs of Monte Carlo/simulated annealing are performed to map the entire conformational space of the helix. In the second phase, new Monte Carlo/simulated annealing runs are performed in only the populated regions

identified in the first phase of the calculation. The final output is 100 structures at 310K. Conformers are grouped using X-Cluster (Schrödinger, Portland, OR) according to their increasing root mean square (rms) deviation from the first structure output at 310K. Because X-Cluster rearranges the conformers so that the rms deviation between nearest neighbors is minimized, any large jump in rms deviation is indicative of a large conformational change and hence identifies a new conformational family or cluster.

The Conformational Memories results for TM6 in wildtype (WT) CB1 and CB2 indicated a dramatic difference in the range of conformations possible for each receptor subtype (Figure 2A and 2B). Conformers were superimposed at their extracellular ends. For WT CB1 (Figure 2A), 2 major clusters were identified (yellow and magenta in Figure 2). For WT CB2 (Figure 2B), a single cluster was identified (green in Figure 2). The average proline kink and SD for all 100 conformers generated by Conformational Memories at 310K are given at right in each figure. For WT CB1, 2 clusters of conformers resulted. For all 100 conformers of WT CB1 generated by Conformational Memories, the average kink angle was 40.9° (SD ±16.9°; Figure 2A). For CB2, a single cluster of less kinked structures resulted. The average kink angle for all 100 structures was 24.6° (SD ± 4.3°; Figure 2B). The flexibility of WT CB2 TM6 was very

Figure 2. CM results for (A) WT CB1, (B) WT CB2, (C) CB1 G6.49F, and (D) CB2 F6.49G TM6. Conformers have been superimposed at their extracellular ends. For (A) and (D), 2 major clusters were identified. These are colored yellow and magenta. For (B) and (C), a single cluster was identified by CM. These are colored green here. The average proline kink and SD for all 100 conformers generated by CM at 310K are given to the right in each figure. It is clear here that WT CB1 TM6 has greater flexibility than WT CB2 TM6. The swap mutation results—(C) and (D)—suggest that this flexibility is due to the presence of a Gly at position 6.49 immediately preceding the Pro in TM6 of WT CB1. CM indicates Conformational Memories; WT, wildtype; CB, cannabinoid; TM, transmembrane segment.²⁶

consistent with the flexibility reported for TM6 in the β 2-adrenergic receptor.²⁵ However, the results for WT CB1 suggested that CB1 TM6 has increased flexibility. We hypothesized that this difference in flexibility may be due to the size of the residue that immediately precedes P6.50 (ie, residue 6.49) in each receptor subtype. In the more flexible TM6 (CB1), residue 6.49 is small in size, a glycine. In the less flexible TM6 (CB2), residue 6.49 is much larger in size, a phenylalanine. To test this hypothesis, Conformational Memories was used to compare the range of conformations possible for the “switch mutants,” CB1 G6.49F and CB2 F6.49G. The results appear in Figure 2C and 2D. Here, conformers have been superimposed at their extracellular ends. For the CB1 G6.49F mutant, one population of moderately kinked helices with an average kink angle of 25.3° (SD $\pm$ 5.7°; Figure 2C) resulted. These results are similar to those for WT CB2 (Figure 2B). For the CB2 F6.49G mutant, 2 clusters resulted. The average kink angle for all 100 conformers was 44.3° (SD $\pm$ 21.4°; Figure 2D). These results are similar to those obtained for WT CB1 (Figure 2A). Taken together, these results suggest that TM6 in CB1 has been engineered to have greater flexibility and ability to bend because of the small Gly residue in the flexible hinge motif, CWGP, in CB1. Clearly, many of the helices output by Conformational Memories for WT CB1 are too bent to be able to be accommodated in the TM bundle. We were able to use this output to choose an inactive state TM6 for CB1 (Pro kink angle = 53.1°) and an activated state (see below) TM6 (Pro kink angle = 21.8°). Another important outcome of the Conformational Memories studies was that while the wobble angle of CB2 TM6 is similar to that in rhodopsin (placing the extracellular end of TM6 close to TM5 in the context of the TM bundle), the wobble angle of TM6 in CB1 is quite different (pointing the extracellular end of TM6 toward TM3). As illustrated in our R model for CB1 in Figure 3 below, the flexibility of TM6 and its different wobble angle enables a salt bridge to form between K3.28 and D6.58.

Figure 3. (Top) An extracellular view of the CB1 TM bundle model of the inactive (R) state. In the R state, the wobble angle of TM6 causes the extracellular end to be close to TM3. As a result, a salt bridge is possible between D6.58 and K3.28. (Inset) A salt bridge between R3.50 and D6.30 brings the intracellular ends of TM3 and TM6 close in the inactive state. (Bottom) An extracellular view of the CB1 TM bundle model of the active (R*) state. In the R* state, TM6 has straightened and both TM3 and TM6 have rotated counterclockwise. (Inset) At the intracellular end, the salt bridge between R3.50 and D6.30 has broken. CB indicates cannabinoid; TM, transmembrane segment.

models of the dopamine D2 and the β 2-adrenergic receptors.^{34,35} These de novo methods may be particularly useful for modeling non-Class A GPCRs that have divergences from the well-known sequence motifs present in rhodopsin and other Class A receptors.

Creating Models of GPCR Activated State Conformation(s)

Recent electron microscopy (EM) studies have allowed the investigation of the metarhodopsin I intermediate, revealing evidence about the early changes during the photolysis process.³⁶ Comparison of this map with x-ray structures of the ground state⁷⁻¹⁰ reveals that metarhodopsin I formation does not involve large rigid-body movements of helices, but there is a rearrangement close to the bend of TM6, at the level of the retinal chromophore. There is no gradual buildup of the large conformational change known to accompany metarhodopsin II formation (see below). The protein remains in a conformation similar to that of the ground state until late in the photobleaching process.

De Novo Modeling of the GPCR Inactive State

Before the publication of the bovine rhodopsin crystal structure in 2000,⁷ many GPCR models were created de novo^{6,16} using a Fourier transform analysis of sequence periodicity in a set of highly homologous sequences.^{27,28} This approach permitted the identification of helical segments within the sequence and, through calculation of moment vectors, the correct orientation of each helix within the bundle.^{29,30} Helix tilts were arranged based on the projection structure of rhodopsin available at that time.^{31,32} Today there are methods available that create de novo models in much this same way. The Goddard group has developed a method, called Membstruk,³³ that has been used to create de novo

To date, no crystal or EM structure of rhodopsin in its fully activated, Meta II state has been published. Yet knowledge of the structure of this state is critical for modeling studies. Pharmacological studies show that agonists, antagonists, and inverse agonists do not bind to a single receptor conformation.³⁷ Many models of GPCRs fail to distinguish this fact, opting to dock agonists, antagonists, and inverse agonists in the same receptor model, usually based on the rhodopsin (inactive state) crystal structure. In contrast, pharmacological, biophysical, and structural data, and data on constitutively active receptors, all demonstrate that GPCRs exist in distinguishable conformations and, hence, *a single model for any receptor can never be adequate*.

So, how can one obtain a model of the activated state of a GPCR of interest? It would seem that one way to approach obtaining a model of the activated state would be via molecular dynamics (MD) simulations that start with the agonist bound in the inactive state. The time scale for activation has been estimated to be milliseconds for light activation of rhodopsin³⁸ but seconds for activation of the β_2 -AR (adrenergic receptor) by its diffusible ligand.³⁹ It is not possible at the present time to perform MD simulations to study agonist-induced changes to the inactive (R) state to generate the activated (R*) state because GPCR activation takes much longer than the typical length of MD simulations (10-100 ns).

An alternate way to build an activated state model is to create a model that reflects the conformational changes suggested to occur during GPCR activation as deduced from biophysical studies. Such studies of rhodopsin activation and activation of the β_2 -adrenergic receptor have revealed important information about the conformational changes that occur upon activation of these GPCRs. These studies have suggested that activation is accompanied by rigid domain motions and rotations of TM helices 3 and 6 (counterclockwise from an extracellular perspective).³⁹⁻⁴¹ At their intracellular ends, TMs 3 and 6 in rhodopsin are constrained by an E3.49(134)/R3.50(135)/E6.30(247) salt bridge that limits the relative mobility of the cytoplasmic ends of TM3 and TM6 in the inactive state⁷ and acts like an “ionic lock.”^{42,43} During activation, P6.50 of the highly conserved CWXP motif in TM6 of GPCRs may act as a flexible hinge, permitting TM6 to straighten upon activation, moving its intracellular end away from TM3 and upward toward the lipid bilayer.⁴⁴ In our work, we have chosen to create models of the activated state of the cannabinoid receptors based upon these documented changes that occur during the R to R* transition.⁴⁵ Figure 3 illustrates our current CB1 R and CB1 R* bundles. The importance of the generation of such models has been discussed by Gouldson et al.⁴⁶

To deduce the sequence-specific rotamer “toggle switch” within the binding pocket that leads to the R* state of individual GPCRs, initial R* models (see above) can be refined through a combination of computational and experimental

studies. Klein-Seetharaman and coworkers⁴⁷ have reported that even in the dark (inactive) state of rhodopsin, only some strong constraints exist, whereas the majority of the molecule experiences conformational flexibility. Light activation of rhodopsin, therefore, does not require the breaking and forming of thousands of specific contacts within nanoseconds. Instead, activation requires only a few specific contacts restricting the inactive state, including indole side-chain contacts of tryptophan residues, to break on activation. These changes can then be transmitted through the entire membrane protein because of its dynamic plasticity. One of the tryptophan residues that Klein-Seetharaman and coworkers have reported to be restricted in rhodopsin is W6.48(265). In the dark (inactive) state of rhodopsin, the beta-ionone ring of 11-cis-retinal is close to W6.48(265) of the CWXP motif on TM6 and acts as a linchpin, constraining W6.48 in a $\chi_1 = g+$ conformation.⁷⁻⁹ In the light-activated state, the beta-ionone ring moves away from TM6 and toward TM4, where it resides close to A4.58(169).⁴⁸ This movement releases the constraint on W6.48(265), making it possible for W6.48(265) to undergo a conformational change. Lin and Sakmar⁴⁹ reported that perturbations in the environment of W6.48(265) of rhodopsin occur during the conformational change concomitant with receptor activation. This suggests that the conformation of W6.48(265) when rhodopsin is in its inactive/ground state (R; $\chi_1 = g+$) changes during activation (ie, W6.48(265) $\chi_1 g+ \rightarrow trans$).²⁵ In the Class A aminergic receptors, a highly conserved cluster of aromatic amino acids is found on TM6 that faces the binding site crevice bracketing W6.48 (F6.44, W6.48, F6.51, and F6.52).²⁵ Shi and coworkers used mutation studies of the β_2 -adrenergic receptor combined with the biased Monte Carlo technique of Conformational Memories to propose that a C6.47 *trans*/W6.48 *g+*/F6.52 *g+* $\rightarrow$ C6.47 *g+*/W6.48 *trans*/F6.52 *g+* transition is the key switch within the binding site crevice that leads to the R* state of the β_2 -AR (adrenergic receptor).²⁵

Restriction of W6.48 by a TM6 aromatic cluster is not possible in the cannabinoid receptors, as the CB1 receptor has leucines at 6.44, 6.51, and 6.52. Instead, the CB1 receptor contains a microdomain of aromatic residues that face into the ligand binding pocket in the TM3-4-5-6 region, including F3.25(189), F3.36(200), W4.64(255), Y5.39(275), W5.43(279), and W6.48(356). Singh and coworkers used the biased Monte Carlo technique of Conformational Memories combined with receptor modeling to suggest that the F3.36(200)/W6.48(356) interaction may act as a mimic of the 11-cis-retinal/W6.48 interaction in the rhodopsin dark state and may serve as the toggle switch for CB1 activation, with F3.36(200) $\chi_1 trans$ /W6.48(356) $\chi_1 g+$ representing the inactive (R) and F3.36(200) $\chi_1 g+$ /W6.48(356) $\chi_1 trans$ representing the active (R*) state of CB1.⁵⁰ Figure 4 illustrates this, with F3.36 and W6.48 engaged in a direct

Figure 4. The relationship between F3.36(201) and W6.48(357) in the inactive (R) and active (R*) states of CB1 as predicted by molecular modeling. The major view is from TM5 looking toward TM3/TM6. (Left) In the R state, W6.48(356) adopts a g^+ χ_1 , whereas F3.36(200) adopts a $trans$ χ_1 . In this arrangement, W6.48(356) and F3.36(200) are engaged in an aromatic stacking interaction that stabilizes the R state. By analogy with Rho, the CB1-inactive state is also characterized by a salt bridge between R3.50(214) and D6.30(338) at the intracellular side of CB1 that keeps the intracellular ends of TM3 and TM6 close. The TM6 kink extracellular to W6.48(357) permits a hypothesized salt bridge between K3.28(193) and D6.58(367) to form. This salt bridge is made possible by the profound flexibility in TM6 due to the presence of G6.49(357) in the CWXP motif of TM6. (Right) In the R* state, W6.48(356) and F3.36(200) have moved apart because of rotation of TM3 and TM6 during activation. W6.48(356) has adopted a $trans$ χ_1 and has moved toward the viewer, and F3.36(200) has adopted a g^+ χ_1 and has moved away from the viewer. The R3.50(214)/D6.30(338) salt bridge is broken, and the proline kink in TM6 has moderated. (Inset) An extracellular view of CB1. It is clear that in R, F3.36(200) and W6.48(356) are engaged in an aromatic stacking interaction, but in R*, F3.36(200) and W6.48(356) are no longer close enough to interact. CB indicates cannabinoid; TM, transmembrane segment.⁴⁵

aromatic stack in the R state and rotated away from each other in the R* state. A detailed functional analysis of mouse CB1 F3.36A and W6.48A mutants, undertaken to test this toggle switch hypothesis, showed statistically significant increases in ligand-independent stimulation of GTP γ S binding for a F3.36A mutant versus WT mCB1, while basal levels for the W6.48A mutant were not statistically different from WT mCB1. These results suggested that F3.36 may

function as a linchpin, restraining W6.48 from moving to an active state conformation in the CB1 receptor.⁴⁵

It is important to note that there is accumulating experimental evidence for the existence of more than one “activated” state for a GPCR, with conformation influenced by the type of agonist bound.^{51,52} So, the assumption of a single activated state for any GPCR is, admittedly, a simplifying assumption. However, in our hands, despite the fact that we may have created a single R* model for each cannabinoid receptor subtype in the absence of ligand, we see differences between agonist/R* complexes depending upon which agonist occupies the receptor (data not shown).

Modeling Loop Regions

In much of the literature, GPCR models have been built of the TM regions only, with the implicit assumption that agonist/antagonist interaction occurs with TM residues only and therefore models need not include the loop regions. This is a simplifying assumption that may need to be reconsidered in light of experimental results. For example, for the κ -opioid receptor, there is evidence that extracellular loop regions form part of the binding site for ligands.⁵³ Loop conformations can be generated using homology modeling, database searching, or *ab initio* computational methods. A comprehensive review of the loop modeling literature is beyond the scope of this article. However, some methods in use are discussed below.

One approach to adding loop segments to GPCR TM bundle models is to search the Protein Data Bank (<http://www.rcsb.org/pdb/>) to identify loop conformations in the database with the highest sequence homology with each loop segment of the receptor to be modeled. Another approach is to use a protein structure predictor such as PredictProtein (<http://www.predictprotein.org>). PredictProtein is a service for sequence analysis and structure prediction. Users submit protein sequences, and PredictProtein retrieves similar sequences in the database and predicts aspects of protein structure.

Tosatto and coworkers have described an algorithm that uses a database of precalculated lookup tables, which represent a large set of possible conformations for loop segments of variable length. The target loop is recursively decomposed until the resulting conformations are small enough to be compiled analytically. The algorithm generates a ranked set of loop conformations.⁵⁴

As mentioned earlier, the Sali lab has developed MODELLER, which can be used to add loops to GPCR models.^{18,20} The Honig lab has developed a program for protein loop prediction, the *loopy* program (<http://honiglab.cpmc.columbia.edu/programs/loop/intro.html>). The program can also perform sequence mutation and addition of a

missing protein segment.⁵⁵ If the segment to be predicted already exists, *loopy* will delete the original segment and predict a new one to assemble onto the stems. If the segment to be predicted does not exist, the residue sequence of the missing segment must be provided and *loopy* will predict a segment with those residues and assemble it onto the 2 end stems. The *loopy* program first builds multiple initial conformations using an *ab initio* method. Each of the conformations is then closed using a random tweak method. Fast energy minimization in torsional angle space is then performed and the side chain is assembled using the side-chain prediction program (scap). Colony energy is used to sort out the best predictions.⁵⁵

Mehler and Weinstein^{56,57} have developed an *ab initio* approach for loop segment modeling that consists of a 2-step procedure that requires only knowledge of the structure (experimental or model) of the domains to be connected. The method employs simulated annealing Monte Carlo simulations performed on the loop segment starting from a completely extended structure, combined with a biased scaled collective variables Monte Carlo technique designed to complete the closure of the segment. This method is based on the assumption that loop regions have an intrinsic propensity for a particular set of conformations based on their amino acid sequence, but this intrinsic folding has to be disrupted for the best fit of the loop segment within the tertiary structure of the native protein. Thus, the final folding of the loops in the context of the protein is a compromise between 2 opposite effects: the intrinsic tendency to adopt a specific folding pattern dictated by the amino acid sequence, and the partial unfolding that is imposed by the inclusion of the loop in the native conformation of the protein. The sampling methodology uses simulated annealing Monte Carlo simulations to find conformations that are representative of the segment structure in solution, as encoded in the primary sequence, and subsequently forces a slow unfolding of the segment to fit the final protein conformation using an adjustable force constant scheme and Monte Carlo simulations with a scaled collective variables technique. The scaled collective variables technique allows the Monte Carlo simulation to improve the efficiency of the search. Finally, since an accurate force field for the study of peptide and protein conformational preferences must account for the hydrophobic and electrostatic effects of the solvent, the method also uses a continuum electrostatic model developed by Hassan and coworkers. This method is based on screened Coulomb potentials and has been validated in several systems ranging in size from small molecules to large.⁵⁸

GPCR DIMERS AND OLIGOMERS

While it has been common to build models of GPCRs as monomers and some recent studies support monomeric

GPCRs as functional units,⁵⁹ the emerging concept of GPCR dimerization has begun to challenge this notion. Recent work has shown not only that many GPCRs exist as homo- and heterodimers but also that GPCR oligomeric assembly may have important functional roles.^{60,61} Terrillon and Bouvier have described the 5 stages of the GPCR life cycle that could be affected by dimerization: ontogeny, ligand-promoted regulation, pharmacological diversity, signal transduction, and internalization.⁶⁰ Studies have suggested that dimerization may be important in receptor maturation, as dimerization appears to occur early after biosynthesis. Dimerization may mask specific retention signals or hydrophobic patches that would cause receptor retention in the endoplasmic reticulum (ER).⁶² The importance of dimerization to ontogeny has been clearly shown for the GPCR family C metabotropic γ -aminobutyric acid b receptor (G_bR), which is composed of 2 subunits, G_bR1 and G_bR2.⁶³ When expressed alone, G_bR1 is retained intracellularly as an immature protein because it has a carboxy-terminal ER retention motif,⁶⁴ whereas G_bR2 reaches the cell surface but is not functional. Following their coexpression, heterodimerization masks the G_bR1 ER retention signal (RXR(R) in the C terminus), allowing the proper targeting of a functional heterodimeric G_bR to the plasma membrane.⁶⁴

Experiments based on fluorescence resonance energy transfer (FRET) and bioluminescence energy transfer reveal that many GPCRs exist as oligomers, or at least as closely packed clusters, in the membranes of living cells.^{60,65} Once a receptor has reached the cell surface, its oligomeric state could be dynamically regulated by a ligand. Whether receptor activation can promote or inhibit dimerization and/or favor exchanges between protomers is a key question with wide implications for the mechanisms of receptor activation and regulation. Unfortunately, there is no general consensus yet. Several studies suggest that ligand binding can regulate the dimer by either promoting or inhibiting its formation (see, eg, Roess and Smith⁶⁶ and Latif et al⁶⁷); others conclude that homodimerization and heterodimerization are constitutive processes that are not modulated by ligand binding (see, eg, Terrillon et al⁶⁸). In any case, the structural data available strongly suggest that at least some GPCRs can form dimers in the absence of ligand stimulation. For example, Palczewski and coworkers used atomic force spectroscopy to show that rhodopsin and opsin form constitutive dimers in dark-adapted native retinal membranes.^{69,70}

Jordan and Devi's work on coexpressed δ - and κ -opioid receptors was the first evidence presented in the literature that GPCR heterodimerization could play a role in pharmacological diversity.⁷¹ These investigators found that coexpression of both receptors led to the formation of a stable heterodimer with low affinity for δ - or κ -selective ligands

when administered alone, but high affinity when these 2 agonists were administered together. Since these experiments, positive or negative ligand binding cooperativity that occurs after receptor coexpression has been interpreted as resulting from receptor heterodimerization for many other GPCRs.⁷²⁻⁷⁶

The first evidence that GPCR dimerization could have a crucial effect on signal transduction came from studies of the GPCR family C metabotropic GbR.⁶⁴ Heterodimerization has also been proposed to be crucial for the formation of functional taste receptors.^{77,78} Heterodimer formation has been suggested to underlie signal potentiation in numerous receptors, including the chemokine CCR5/CCR2,⁷⁹ somatostatin SSTR5/dopamine D2,⁷⁵ and angiotensin AT1/bradykinin B2 receptors,⁸⁰ while signal attenuation has been described for other heterodimers such as adenosine A1/dopamine D1⁸¹ and somatostatin SST2a/SST3 receptors.⁸² Heterodimerization has also been proposed to promote changes in G protein selectivity. Kearn et al recently reported that a regulated association of cannabinoid CB1 and dopamine D2 receptors profoundly alters CB1 signaling, providing evidence that CB1/D2 receptor complexes exist, are dynamic, and are agonist regulated, with highest complex levels detected when both receptors are stimulated with subsaturating concentrations of agonist. The consequence of this interaction is a differential preference for signaling through a “nonpreferred” G protein. In this case, D2 receptor activation, simultaneously with CB1 receptor stimulation, results in the receptor complex coupling to Gs protein rather than to the expected Gi/o proteins.⁸³

The emerging concept of GPCR dimerization has also challenged assumptions that one receptor interacts with one G protein. Several points of contact between G protein (G- α and G- $\beta\gamma$ subunits) and receptor have been proposed.⁸⁴ However, the crystal structure of rhodopsin reveals that the receptor is too small to make all of these contacts simultaneously. It has been proposed that 2 receptors may be necessary to satisfy all proposed points of contact with G protein.^{70,84} Baneres and Parello have shown that activated leukotriene B4 receptor (BLT1) and G α 12 β 1 γ 2 corresponds to a pentameric assembly of one G heterotrimeric protein and one dimeric receptor.⁸⁵

Heterodimerization has also been suggested to affect agonist-promoted endocytosis. In many cases, stimulation of one of the protomers in a heterodimer promotes co-internalization of both receptors.^{82,86-88}

The functional consequences of GPCR dimerization have been commonly studied in heterologous expression systems. It is possible in such systems to coexpress receptors that are actually never expressed together in vivo or to express receptors at such high levels that spurious interactions occur. However, the pharmacological relevance of

heterodimerization has been demonstrated in cells that endogenously express the GPCRs under consideration. The potential physiological importance of heterodimerization is supported by studies in cells that endogenously coexpress the GPCRs under consideration. For example, blockade of either the AT1 receptor or the β 2-adrenergic receptor with selective antagonists inhibits the signaling of both receptors simultaneously in freshly isolated mouse cardiomyocytes,⁸⁹ a phenomenon linked to the ability of the 2 receptors to heterodimerize.

Proposed Physical Models of Dimerization

Despite the fairly extensive literature on dimerization in GPCRs, there has been little discussion on the nature of the dimers. Two basic modes of dimerization have been proposed, contact dimers and domain-swapped dimers.

Contact Dimers

In *contact dimerization*, a dimer forms between 2 different TM bundles (monomers) with separate binding sites by packing at an interface that would face the lipid environment in the monomeric receptor (ie, by “touching” specific lipid faces). Experimental support for *contact dimers* can be found in the literature.^{69,70,90-94} One example comes from Javitch and coworkers, who used cysteine cross-linking of the endogenous cysteine residue C4.58(168) to show that TM4 forms a symmetrical dimer interface in the dopamine D2 receptor.⁹⁵ More recently,⁹⁶ this same group mapped the homodimer interface in the dopamine D2 receptor over the entire length of TM4 by crosslinking of substituted cysteines. Residue susceptibilities to crosslinking were found to be differentially altered by the presence of agonists and inverse agonists. The TM4 dimer interface in the inverse agonist-bound conformation was consistent with the dimer of the inactive form of rhodopsin modeled with constraints from atomic force microscopy.⁷⁰ Crosslinking of a different set of engineered cysteines in TM4 was slowed by inverse agonists and accelerated in the presence of agonists; crosslinking of the latter set locks the receptor in an active state. These results suggest that a conformational change at the TM4 dimer interface is part of the receptor activation mechanism.⁹⁶

Domain-Swapped Dimers

If, on the other hand, during dimerization, a hinge loop opens out, the domains could exchange to form a *domain-swapped dimer*. Gouldson and coworkers report that *domain-swapped dimers* are less common than *contact dimers* but have the major advantage that the interactions between the domains already present in the monomers can be reused to form the dimers; thus, domain swapping is an efficient way

of forming dimerization interfaces. The length of the hinge loop is important in this process. For GPCRs, the hinge loop has been proposed to be IC-3, which connects TM5 and TM6 because it is frequently the longest loop in GPCRs.⁹⁷ The concept of domain-swapped dimers has also received experimental support.⁹⁸⁻¹⁰⁵ One example is from the work of Maggio and coworkers, who found that muscarinic receptor heterodimerization was inhibited when the IC-3 loop was shortened. Maggio and coworkers concluded that their data suggested that an intermolecular interaction between muscarinic receptors, involving the exchange of amino-terminal (containing TM domains 1-5) and carboxyl-terminal (containing TM domains 6 and 7) receptor fragments (ie, the formation of a *domain-swapped dimer*), depended on the presence of a long IC-3 loop.¹⁰⁶

Methodology for the Prediction of Dimer/Oligomer Interfaces

Bioinformatics techniques used to predict dimer and oligomer interfaces begin with multiple sequence alignments and share the assumption that proteins that are evolutionarily related might exhibit common structural and functional features corresponding to detectable patterns in their sequences. As a result, a suitable representation of the evolutionary relationships between proteins under study is an essential requirement for the prediction of dimer/oligomer interfaces.¹⁰⁷

Correlated Mutation Analysis

The correlated mutation analysis (CMA) method is based upon sequence analyses and has been shown to provide information about interdomain contacts.¹⁰⁸ The correlation is the result of the tendency for amino acid positions in a sequence to mutate in a coordinated manner if the interface has to be preserved for structural or functional reasons. Sequence changes occurring over evolutionary time at the dimerization interface of monomer A would be compensated for by changes at the interacting face of monomer B to preserve the interaction interface.¹⁰⁷ Gouldson and coworkers recently used the CMA method to analyze candidate residues for the subtype-specific heterodimerization observed for the chemokine, opioid, and somatostatin receptors.¹⁰⁹

Subtractive Correlated Mutation Method

Although CMA is a powerful bioinformatics tool that was demonstrated from specific tests to identify residues that are functionally essential, the original CMA method in itself does not usually achieve specific identification of the residue composition of the dimerization interfaces of GPCRs (eg, results of Gouldson et al,¹⁰⁹ which essentially show

correlated mutations in all 7 TM helices). Filizola and coworkers have enhanced the CMA approach with filtering algorithms to enable identification of the likely hetero- and homo-oligomerization interfaces of family A GPCRs.^{110,111} The new method, termed the subtractive correlated mutation (SCM) method,¹¹⁰ consists of a modified version of an earlier algorithm¹¹² that provides a means to filter out the intramolecular pairs of correlated residues within each interacting monomer from the complete list of intra- and intermolecular pairs of correlated residues. Multiple sequence alignment of concatenated monomeric sequences of the 2 different GPCRs, obtained from the same organisms, is used to identify these correlated mutations. Concatenation is essential for the identification of the heterodimerization interface.¹¹⁰ A similar approach was developed independently by Pazos et al.¹⁰⁸ Additional stringency criteria have been added for the application to GPCR homodimerization, to achieve reliable predictions of the dimerization/oligomerization interface of GPCRs.¹⁰⁷ To increase the chance of obtaining correctly predicted contacts, correlated pairs with a correlation index ≤ 0.7 are purged from the list. With the information contained in the crystal structure of rhodopsin,⁷ residues are screened for lipid exposure. Only pairs of correlated residues where both positions have a surface exposure (to lipid) of more than 45 Å² are considered as candidates for intermolecular contacts (the implicit assumption is that association of GPCRs occurs only via contact dimers/oligomers). Finally, only residues that are part of an interacting neighborhood (defined as at least 3 residues on the lipid face of the helix within $i+7$ of each other) are retained.¹⁰⁷

We recently used the SCM method to identify the most likely homodimerization interface for the cannabinoid CB1 receptor. TM1 and TM4 contained the most predicted residues, but the residues identified for TM4 formed a continuous lipid-facing ridge (interaction neighborhood). Therefore, TM4 was chosen as the most likely homodimer interface for CB1. Figure 5 illustrates a side view and an extracellular view of this proposed homodimer, with the gray residues contoured at their Van der Waals radii representing the residues identified by the SCM method. Only the TM regions of the CB1 dimer are illustrated here.¹¹³

Evolutionary Trace Method

The evolutionary trace (ET) method is another approach for determining functional sites in a protein given its 3-dimensional structure and a multiple sequence alignment. The ET method was first described by Lichtarge and coworkers as an approach to predict functionally important residues in proteins of known structure.¹¹⁴⁻¹¹⁶ Based on the earlier hierarchical analysis of residue conservation in proteins developed by Livingstone and Barton,¹¹⁷ the ET method has some

Figure 5. A side view (top) and an EC view (bottom) of the CB1 homodimer predicted by the subtractive correlated mutation method. Residues predicted to be part of the homodimer interface at TM4 are shown in gray and are contoured at their Van der Waals radii. To determine the possible interface orientations for the homodimer, we looked for patches of continuous residues, or “interaction neighborhoods.” Both TM1 and TM4 contained the most predicted residues. However, TM4 contained a continuous exterior ridge formed by the predicted residues. The TM bundles are illustrated here without loops to simplify the display. EC indicates extracellular; CB, cannabinoid; TM, transmembrane segment.¹¹³

similarities with the CMA method, as evolutionary trace residues may also be correlated, but the ET method has the advantage that conserved residues are also included in the analysis.^{115,118} Built from the idea that proteins that have evolved from a common ancestor will show similar backbone structure,¹¹⁹ the ET method assumes that within a multiple sequence alignment, the protein family retains its fold. The method also assumes that the protein family should conserve the location of functional sites and have a distinctly lower mutation rate at these sites, punctuated by mutation events that cause divergence.¹¹⁵

Hidden-Site Class Model

Evolutionary relationships between proteins can be represented by a matrix indicating the rate at which every amino acid substitution occurs during evolution. Current models use a single substitution matrix for all locations in all

sequences. This is a limitation of these models because the probability that an amino acid substitution at a particular location in the protein sequence would produce a functional effect is not the same at all locations.¹⁰⁷ The hidden-site class model has been proposed to overcome this limitation by using different substitution matrices to represent amino acid substitutions at different locations in a protein sequence.¹²⁰⁻¹²² It has been demonstrated that this method attains better phylogenetic inferences by identifying locations in the sequences that are considered to be under similar selective pressure and by characterizing changes in selective pressure. Locations that are assigned to site classes with the slowest rate of substitution are expected to correspond to structurally or functionally important positions. This method has been applied recently to 199 Class A GPCR aminergic receptors. The method identified lipid-exposed evolutionarily conserved locations on TM4, TM5, and TM6 in different subfamilies.¹²³

In their recent review, Filizola and Weinstein¹⁰⁷ point out that both the assumptions underlying the computational algorithms and the selection of sequences in the alignment determine the nature of the answers returned by the algorithm. *The statistical nature of the tools makes their success in predicting dimer/oligomer interfaces highly dependent on the number of sequences available for a family and subfamily.* As more sequences become available with the completion of the sequencing of more genomes, the power of these approaches can be expected to increase.¹⁰⁷

GPCR Dimer Interface Predictions

Filizola and Weinstein have analyzed the occurrence of lipid-exposed residues in predictions from bioinformatics methods applied to search for dimerization/oligomerization interfaces of GPCRs (see Figure 3 in Filizola and Weinstein¹⁰⁷).

These authors report that residues most frequently identified cluster in TM4, TM5, and TM6, indicating that the prediction of dimerization/oligomerization interfaces of GPCRs with various computational methods has thus far pointed to a specific role for the lipid-exposed regions of these 3 helices. Among the residues identified within each of these 3 helices, 4.58, 5.48, and 6.42 have the greatest number of occurrences. In TM6, residue 6.30 (at the boundary between TM6 and the IC-3 loop) had nearly the same frequency of occurrence as 6.42. The high occurrence of residue 6.30 in dimer/oligomer analyses can be explained by an involvement of this residue in a broader oligomerization scheme of GPCRs. This is suggested by the atomic force microscopy map of rhodopsin in native membranes,⁷⁰ which indicates that the cytoplasmic loop connecting TM5 and TM6 facilitates the formation of rows of rhodopsin dimers.

CONCLUSIONS

The x-ray crystal structure of the Class A GPCR, rhodopsin, in its inactive state has catalyzed the construction of computational models of many other GPCRs. Models of the activated states of GPCRs are now emerging in the literature based upon evidence from biophysical studies of the conformational changes that occur upon the activation of rhodopsin and other GPCRs. These models are proving useful as hypothesis generators for experimental studies aimed at deducing ligand binding sites and ligand-receptor activation mechanisms. Recent experimental work has shown not only that many GPCRs exist as homo- and heterodimers but also that GPCR oligomeric assembly may have important functional roles. In response to these findings, a whole new computational literature is emerging to address the creation of homodimer, heterodimer, and oligomeric models of GPCRs. The new dimeric and oligomeric models that emerge from computational studies should prove very valuable as hypothesis generators for studies of the functional significance of GPCR dimerization and oligomerization.

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