Structural Basis of Dopamine Receptor Activation

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Chapter 3
Structural Basis of Dopamine Receptor Activation

Irina S. Moreira, Lei Shi, Zachary Freyberg, Spencer S. Ericksen, Harel Weinstein, and Jonathan A. Javitch

Abstract G protein-coupled receptors (GPCRs) are seven transmembrane (TM) proteins representing the largest and most universally expressed cell surface receptors and are present in almost all species and in a wide variety of cells. Here we will focus our attention on the catecholamine-binding GPCRs and in particular on the dopamine receptors. The catecholamine-binding GPCRs form a group of rhodopsin-like GPCRs composed of adrenoceptors, which are endogenously activated by epinephrine and norepinephrine, and dopamine receptors. We review the different "molecular switches" involved in GPCR activation and we emphasize the importance of extracellular loop 2 (ECL2) in ligand binding. A better understanding of the functional role of ECL2 can be achieved after the release of the crystal structures of B2AR and rhodopsin, which are consistent with dopamine D2 receptor substituted cysteine accessibility method (SCAM) experimental data. Even though reconstituted GPCR monomers appear sufficient to activate a G protein, in the native setting their dimerization/oligomerization may modulate activation through changes at the dimerization interface or a larger-scale reorientation of the protomers. Therefore, the structural aspects of oligomerization and their importance for receptor activation and signaling are also addressed.

Keywords Catecholamine-binding GPCRs · Dopamine receptors · Binding site · ECL2 · GPCR oligomerization · GPCR–G Protein interaction · Activation · Structural rearrangements

3.1 Introduction

G protein-coupled receptors (GPCRs) are seven transmembrane (TM) proteins representing the largest and most universally expressed cell surface receptors. GPCRs

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are present in almost all species and in a wide variety of cells [1–8]. They play important roles in a broad array of cellular functions and in disease and represent the targets for a large fraction of existing drugs [9–12]. GPCRs are classified into three major classes based on the size of the N termini, on sequence homology, the identity of conserved residues within the seven TM domains that participate in ligand binding, mode of action, and pharmacology [13, 14]. The largest family is Class A (more than 90%), which comprises rhodopsin as well as receptors for biogenic amines, peptides, and odorants. Class B receptors are a much smaller group and include receptors for large peptides such as secretin, cytokines, thrombin, and glucagon. Class C receptors (comprised of approximately 12 members) include the γ-aminobutyric acid B receptor ($GABA_B$), eight metabotropic glutamate receptors, the Ca^{2+} sensing receptor, as well as some pheromone and taste receptors [15]. GPCRs, upon ligand binding, induce dissociation of G proteins into their G_α and $G_{\beta\gamma}$ components and ultimately modulate the activity of enzyme or ion channel effectors [5, 16–19].

Structurally, GPCRs are made up of seven TM segments connected by three intracellular and three extracellular loops (ICL, ECL), and Class A receptors share important functionally conserved sites identified as structural motifs that act as functional microdomains, such as the D(E)RY motif in TM3 and NPXXY in TM7 [8, 20–28]. The first GPCR structure, bovine rhodopsin, was solved in 2000 [29], and there was much anticipation that many other GPCR structures would be rapidly forthcoming. Although a number of different rhodopsin structures were solved, 7 years passed without any other GPCR structures, in support of the unique biochemical properties of rhodopsin, including its high abundance and its unusual stability, retaining function under conditions that denature other GPCRs, due to the covalently bound 11-*cis*-retinal, which maintains the receptor in an inactive conformation [30–32].

At the end of 2007, two new crystal structures of the human β2 adrenergic receptor (B2AR) were solved, including the wild-type receptor bound to an antibody fragment and an engineered receptor with T4 lysozyme inserted into the third intracellular loop [31–33]. Although the B2AR crystallographic structures are quite similar to rhodopsin with a root mean square deviation of 1.6 Å, there are some interesting differences, which impact on considerations of the structure of the dopamine receptor family, for which a structure is not yet available. Very recently two new structures of the β1 adrenergic receptor [34] and the adenosine A2 receptor [35] have been solved, and while there are interesting differences, the overall structures are again quite similar.

Here we will focus our attention on the catecholamine-binding GPCRs and in particular on the dopamine receptors. The catecholamine-binding GPCRs form a group of rhodopsin-like GPCRs composed of adrenoceptors, which are endogenously activated by epinephrine and norepinephrine, and dopamine receptors [36]. For the adrenoceptors, there are three main classes based on their pharmacological properties, amino acid sequences, and signaling mechanisms. These adrenoceptor classes were subsequently divided in humans into three subtypes each: α_1 (α_{1A}, α_{1B}, α_{1D}), α_2 (α_{2A}, α_{2B}, α_{2C}), and β (β_1, β_2, β_3). These receptors respond to the

neurotransmitters/hormones, norepinephrine and epinephrine, which play key roles in regulation of cardiovascular function, energy metabolism, and blood pressure [15]. In contrast to adrenoceptors, dopamine receptors in human are divided into two classes: D_1-like receptors (D_{1A} or D_1 and D_{1B} or D_5) and D_2-like receptors (D_2, D_3, and D_4). While sharing some common properties, each receptor displays unique properties including affinity for dopamine, specificity for G protein coupling and signaling, and specific neuronal distributions [37]. Furthermore, in the case of the D_2 receptor (D2R) subfamily, there are two isoforms: the long isoform D_{2L} and the short isoform D_{2S}, generated by alternative splicing of an 87-bp exon. This splicing event leads to an additional 29 amino acids in the ICL3 of the isoform D_{2L} [38].

3.2 Transmembrane Segments and Activation

TM segment interactions are a key determinant in the assembly and stability of the native structure of membrane proteins [39–41]. As the sequence conservation within the membrane-spanning regions is high, it is thought that class A GPCRs share a similar architecture [16, 17, 41, 42], which has been supported to date by the four different receptors for which we have crystal structures. The catecholamine-binding GPCRs share within their TM regions 20–26% sequence identity with rhodopsin [36]. For example, the sequence identity between the TM domains of rhodopsin and B2AR is 21%, between rhodopsin and D2R is 25%, and between D2R and B2AR is 38% [36].

Some of the most important features of the TM domains are the kinks and bends generated by prolines and glycines, respectively [23, 43–46]. Serines, threonines, and cysteines can also bend the α-helices that constitute the TM domains [24, 47]. In rhodopsin, TM1 possesses a proline-induced kink that bends it inward, toward the helix bundle. It was proposed that other GPCRs, which do not have this proline in TM1, including the D2R, might be packed somewhat differently with TM1 more distant from the bundle [8, 24, 48]. Consistent with such an orientation of TM1, the extracellular segment of TM1 of D2R did not seem to contribute to the binding site based on substituted cysteine accessibility method (SCAM) studies [8, 49]. The B2AR 3D structure validated this hypothesis because its TM1 is comparatively straight [32]. Moreover, although the TM segments in rhodopsin and B2AR have similar orientations, there are some differences: the angles between TM1, TM3, and TM6 and the membrane are different from their counterparts in rhodopsin, TM4 is translated away from the center of the receptor, and TM5 is translated closer to the center of the receptor [32].

It is hypothesized that GPCRs exist as an ensemble of various conformational states that are in a dynamic equilibrium, and that agonist binding and subsequent activation occur through a series of conformational intermediates [50, 51]. Ligands have the ability to stabilize or possibly induce specific conformations [52]. Mutations that disrupt stabilizing non-covalent interactions favor more active receptor conformations by increasing the movement of the TM segments relative to

each other [52]. Although there are different ligand-binding modes in the different GPCR classes, activation processes are thought to result from similar conformational changes involving the TM domain [53–59]. In particular, rotation and outward movement of TM6 likely open a crevice allowing for interaction with the C terminus of the G protein α-subunit and triggering GPCR activation [59–61].

Many GPCRs show a considerable amount of basal, agonist-independent activity, reflecting GPCR structural flexibility and the existence of conformational ensembles [52]. The study of constitutively active GPCRs has contributed to our understanding of the activation mechanism [57, 62–65]. Mutation of certain residues in GPCRs significantly increases their constitutive activation [66, 67] by breaking crucial intramolecular interactions between amino acid residues that normally constrain the receptor to its inactive state [21, 24, 62]. Many residues that produce constitutive activation when mutated are linked through packing interactions with residues that are essential for receptor activation by side chain rearrangement on adjacent TMs and/or by larger-scale TM movements [62, 68–70]. Some of the most well-known constitutively active mutants (CAMs) are those that disrupt the highly conserved (D/E)R(Y/W) amino acid sequence present in 72% of class A GPCRs. By contrast, an "ionic lock" is crucially involved in maintaining the inactive state of the receptor [21, 24, 71–73]. This is exemplified in a network of hydrogen bonding and charge interactions between Glu134$^{3.49}$ and Arg135$^{3.50}$ at the cytoplasmic end of TM3 and Glu247$^{6.30}$ and Thr251$^{6.34}$ at the cytoplasmic end of TM6 of rhodopsin (Ballesteros general number in the superscript [7]) [23, 24, 74]. In the B2AR structures, the "ionic lock" is broken, which may account for the residual basal activity of the B2AR bound to the inverse agonist carazolol [31, 33, 51]. Recent computational studies suggest that the ionic lock dynamically forms and unforms in association with conformational change in ICL2 [75].

Besides the "ionic lock," there are other "molecular switches" involving non-covalent intramolecular interactions that must be altered to achieve an active state. The "rotamer toggle switch" involves Phe290$^{6.52}$, which is accessible in the binding site crevice and serves as a "sensor," a change in the bend of TM6 at the highly conserved residue Pro288$^{6.50}$, and a change in the rotamer of Trp265$^{6.48}$ upon activation of rhodopsin and related family members [46]. Although carazolol does not directly interact with the "toggle switch" in the B2AR TM6, it seems to modulate the rotameric state of Trp286 indirectly by interacting with Phe289$^{6.51}$ and Phe290$^{6.52}$ [31–33, 76, 77]. These kinds of molecular switches can be studied experimentally and computationally [6, 25, 71, 72].

3.3 The Binding Site

SCAM studies, experimental approaches such as studies of chimeric receptors and point mutants, as well as molecular modeling allowed for the identification of amino acids that line the putative binding pocket of the D2R [48, 78–96] (Fig. 3.1). The findings for D2R are in agreement with results for other catecholamine-binding receptors [36]. The binding crevice has two polar regions common to all these

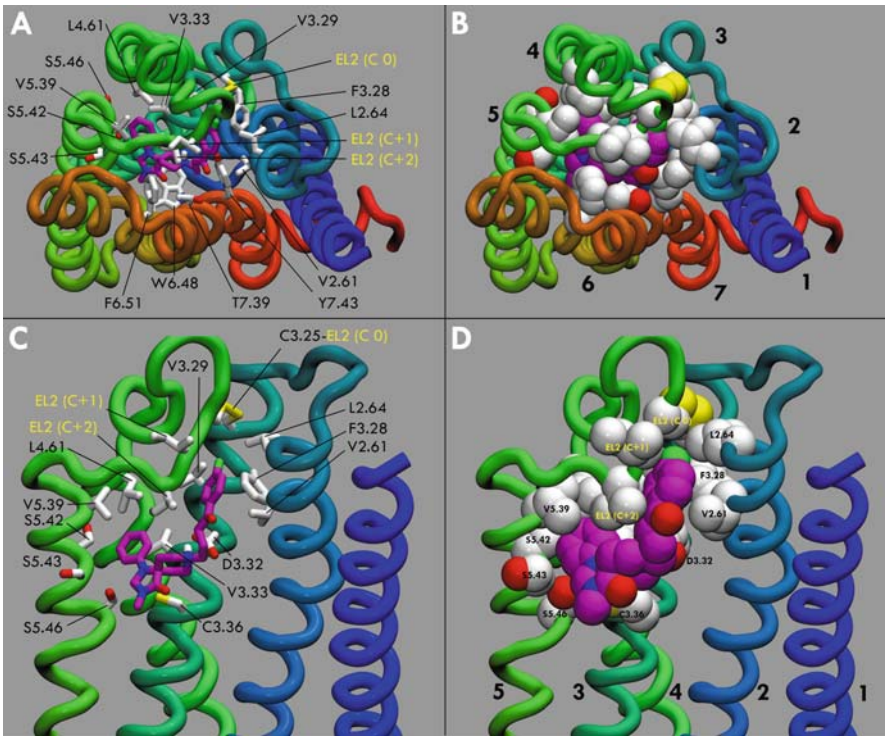

Fig. 3.1 *N*-Methylspiperone (MSP) docked into the binding site crevice of the dopamine D_2 receptor (D2R). Panel (**A**): From an extracellular perspective, TMs 1–7 are colored from *blue* to *red*. MSP (carbon *magenta*, stick) is nestled in the binding site and capped by EL2. D2R side chains within 4 Å of MSP are shown (carbon *white*, stick) and labeled according to the Ballesteros and Weinstein indexing system. Residues in EL2, $Ile183^{EL2(C+1)}$, and especially $Ile184^{EL2(C+2)}$ (labeled in *yellow*), provide substantial hydrophobic contacts to the ligand. Other residues within 4 Å of MSP are $Val91^{2.61}$, $Leu94^{2.64}$, $Phe110^{3.28}$, $Val111^{3.29}$, $Asp114^{3.32}$, $Val115^{3.33}$, $Cys118^{3.36}$, $Leu171^{4.61}$, $Cys182^{EL2(C\,0)}$, $Val190^{5.39}$, $Ser193^{5.42}$, $Ser194^{5.43}$, $Ser197^{5.46}$, $Trp357^{6.48}$, $Phe360^{6.51}$, $Thr383^{7.39}$, and $Tyr387^{7.43}$ [D2R (*short*) UNIPROT sequence P14416-2]. Panel (**B**): To emphasize the hydrophobic packing of the EL2 and crevice residues with bound MSP, the contact side chains from **A** are rendered as van der Waals spheres. TMs are numbered. Panel (**C**): Peering into the crevice from a side view (TMs 6–7 are removed) reveals some key interactions between MSP and the D2R binding site crevice and EL2. Key interactions include a hydrogen bond-reinforced ionic interaction between $Asp114^{3.32}$ and the ligand's amine moiety, deep occupancy of the MSP's phenyl-imidazolidinone group in the primary binding cleft centered between TMs 3 and 5–6, and contacts between the fluorophenyl group with residues in EL2 and TMs 2,3, and 7 (not shown). Missing from this view are the aromatic contacts from TM6 to the ligand's piperazine and phenyl-imidazolidinone moieties and potential hydrogen bonding between $Thr383^{7.39}$ and $Tyr387^{7.43}$ and the butyrophenonyl keto group. Panel (**D**): Same view as Panel **C** with side chains rendered as van der Waals spheres. EL2 contact residues are labeled in *yellow*. TMs are numbered

receptors: $Asp^{3.32}$, which forms ionic interactions with the protonated amine of biogenic amines, and $Ser^{5.42}$, $Ser^{5.46}$, and $Ser^{5.43}$ of TM5, which interact by hydrogen bonding with the *meta*-OH and *para*-OH of the catecholamine. The β-hydroxyl group of (nor)epinephrine, which is not found in dopamine, interacts with $Asn^{6.55}$. $Phe^{5.47}$, $Trp^{6.48}$, $Phe^{6.51}$, and $Phe^{6.52}$ are also expected to interact with the aromatic ring of the ligands [36]. In an exhaustive computational study, Xhaard et al. [36] demonstrated that the docked ligand tends to be in an extended conformation because $Asp^{3.32}$ and TM5 residues are distant from each other, at the opposite ends of the binding pocket.

Prior to the determination of the crystal structure of rhodopsin, Simpson et al. [91] used data from SCAM studies to guide an exploration of the structural basis of the pharmacological specificity of D2R and D4R. Combined substitution of four to six of the residues that faced the binding site crevice in the D2R but were not conserved in the D4R switched the affinity of the receptors for several chemically distinct D4-selective antagonists by three orders of magnitude in both directions (D2- to D4-like and D4- to D2-like). The mutated residues were in TM2, TM3, and TM7 and were predicted to form a divergent cluster that differentiated D2R and D4R binding, which has been supported as well by subsequent studies [94, 95, 97]. Ortore et al. [98] have docked different ligands to both D2R and D4R and have proposed that another difference between the two receptors seems to be due to the extracellular loop 2 (ECL2) region (see below), which is differentially situated in the receptor models, although it should be noted that loop modeling is a complex and developing science [99, 100]. Ligand binding to many members of the GPCR family is regulated allosterically by cations. For example, Na^+ is important for the D2R, and Zn^{2+} was shown to interact with D1, D2, and D4 receptors [101].

3.4 Extracellular Loop 2

It is widely accepted that the extracellular loops (especially ECL2) are of great importance for accommodating high molecular weight GPCR ligands (peptides and proteins). In the rhodopsin structure ECL2 forms a lid-like structure over retinal, but the precise role of ECL2 in binding other lower molecular weight, drug-like ligands is less clear [102]. In more than 800 GPCRs encoded in the human genome, the average size of ECL2 is 27 residues, with a deviation of 13 residues [103]. For nearly all rhodopsin-like GPCRs, the disulfide bond between $Cys^{3.25}$ (Cys-107 in D2R) and the conserved Cys in E2 (Cys_e2, Cys-182 in D2R) connects ECL2 with the extracellular end of TM3, and this disulfide bond (SS-E2) is crucial to the structural integrity and function of many GPCRs. This disulfide bridge is found in more than 90% of GPCRs [14, 103]. The removal of SS-E2 by mutagenesis severely disrupts ligand binding to muscarinic acetylcholine receptors [104, 105] and destabilizes the high-affinity state of the B2AR [106]. Moreover, antagonist protected the B2AR from the effects of reduction by dithiothreitol [107]. Thus, SS-E2 is protected by a conformational change or steric block within the binding site.

In rhodopsin, ECL2 forms a twisted, buried β-hairpin structure that folds deeply into the TM domain with one strand contacting retinal and forming interactions with other extracellular loops [102]. It forms a lid-like structure that shields the retinal in a hydrophobic pocket [51]. The orientation of ECL2 is maintained by the SS-E2 described above [29, 108].

Several reports have implicated ECL2 in ligand specificity in aminergic and other small molecule ligand GPCRs. Zhao et al. [109] found that substitution of three consecutive residues in ECL2 interconverted the ligand specificity for particular antagonists between that of α_{1B}AR and α_{1A}AR. Substitution of a single residue in ECL2 interconverted the pharmacological specificities of canine 5-HT_{1D} and human 5-HT_{1D} receptor [110]. Similarly, substitution of ECL2 and TM5 changed the subtype specificity of the 5-HT_{1D} receptor to that of the 5-HT_{1B} receptor and vice versa [111]. Thus, although it has been argued that the presence of ECL2 within the TMD may be a feature unique to rhodopsin [112, 113], it has also been proposed that ECL2 contributes directly to forming the binding site of aminergic and certain other small molecule ligand GPCRs [114]. To address this issue, SCAM studies were carried out in the short ECL2 of D2R [115]. The reaction of five of these mutants with sulfhydryl reagents inhibited antagonist binding, and bound antagonist protected two, I184C and N186C, the second and fourth residues after the highly conserved Cys_e2 (C+2, C+4). The pattern of accessibility in ECL2 was consistent with a structure similar to that of bovine rhodopsin, in which E2b, the part of ECL2 C-terminal to the conserved disulfide bond, is deeper in the binding site crevice than is E2a, the N-terminal part of ECL2, and E2b was inferred to contribute directly to the binding site in the D2R and probably in other aminergic GPCRs as well (Fig. 3.1).

More recently, the effects of ECL2 mutations on agonist and antagonist binding have been studied in the V1a vasopressin receptor (Class A) ECL2 by a systematic alanine-scanning mutagenesis technique that identified four aromatic amino acids, located in the middle of the ECL2 near the conserved disulfide bond and conserved throughout this subfamily of peptide GPCRs, as important for agonist binding and receptor activation [116]. $Trp206^{(C+1)}$ and $Phe209^{(C+4)}$ were hypothesized to be important for ligand binding and $Tyr218^{(C+13)}$ and $Phe189^{(C-16)}$ appear to be important for orientation/stability of ECL2 over the binding pocket [116]. Furthermore, Klco et al. showed that disruption of ECL2 of the complement C5a receptor (C5aR) by random mutagenesis generated many receptors able to activate G proteins even in the absence of ligands [14]. The authors postulated that ECL2 acts as a negative regulator of C5aR activation possibly by making multiple contacts with the TM domain to stabilize the inactive state.

Through their studies of the serotonin 5-$HT_{4(a)}$ receptor Baneres et al. have suggested the existence of different arrangements of ECL2 depending whether the bound ligand was an agonist (partial or full) or an inverse agonist [117]. In contrast, antagonist binding was inferred not to induce any structural changes of ECL2. Therefore, as in the case of D2R, ECL2 appears to participate in the binding site and rearranges upon activation. Despite the constraint provided by the conserved disulfide bond between ECL2 and the top of TM3, Avlani et al. showed that the

flexibility in ECL2 of the muscarinic acetylcholine M_2 receptor (M_2 mAChR) and its capacity to achieve an open conformation is necessary for the binding of both allosteric and orthosteric ligands [4]. They postulated ECL2 as a gatekeeper with respect to entrance into the orthosteric binding site crevice.

Other studies stress ECL2's importance for ligand binding as in the M_3 muscarinic acetylcholine receptor (M3R) and the thyroid-stimulating hormone receptor (TSHR). ECL2 of the M3R was subjected to random mutagenesis. In contrast to the model proposed by Klco et al. [14], the results of this study suggested that specific ECL2 residues stabilize the active state of the M3R, and are required for efficient agonist-induced M3R activation [108]. The authors also proposed a mechanism in which conformational flexibility in the ECL2 loop is required for efficient receptor activation [108]. Kleinau et al. suggested an activation mechanism in which TM6 glides along ECL2 according to the diverse receptor activation states. Disruption of this critical interface by introduction of mutations in the TSHR alters its basal activity [118].

The crystal structure of B2AR provided the first non-rhodopsin ECL2 structure with which to address these hypothesized functional roles. The conformation of B2AR ECL2 and its orientation to the TMD are significantly different from that in rhodopsin, rendering the binding site crevice of B2AR directly exposed to the water phase [31–33]. Strikingly, however, the ligand-binding residue positions in E2b of rhodopsin and B2AR are remarkably consistent, if counted from CysE2, namely the C+2 and C+4 positions, even though rhodopsin has eight extra residues between CysE2 and the start of TM5 at position 5.36. This is in remarkable agreement with the SCAM studies in D2R [115], in which protection by antagonist suggests that the same two positions, Ile184 and Asn186, face the binding site crevice. In this study several residues in D2R were also found to be accessible to MTS reagent but not protected by ligand, and these were proposed to line the ligand entry pathway. This is most obvious in the C+1 position, because E2b can easily be aligned between D2R and B2AR, whereas E2a varies significantly. In the B2AR structure, C+1 is in the vestibule through which the extracellular milieu gains access to the bound carazolol [31–33]. Given the consistency between the D2R SCAM experimental data for ECL2 with both the rhodopsin and B2AR structures, it is likely that C+2 and C+4 play an important role in ligand binding in the other catecholamine receptors as well, and play an important role in ligand specificity (see Fig. 3.1).

3.5 GPCR Oligomerization

Class C GPCRs, including the metabotropic glutamate receptors and γ-aminobutyric acid type B ($GABA_B$) receptors, have been shown to form homo- and heterodimers in the plasma membrane, with important consequences for trafficking of receptors to the cell surface and for ligand-induced activation and G protein coupling [119]. Class C GPCRs have unique characteristics with dimerization potential: an N-terminal Venus flytrap (VTF) module with structural and functional homology to bacterial periplasmic proteins [120], and cysteine-rich domains (CRDs) [121].

Although the formation of dimers for Class C GPCRs is clear, there is still some controversy regarding the existence of dimers in Class A receptors [122, 123]. Nonetheless, there is increasing agreement that Class A GPCRs can interact to form homo- or heterodimers/oligomers [40, 124–149]. Evidence for dopamine receptor homo- and heteromerization is reviewed extensively in Chapter 10; here we focus on structural aspects of oligomerization and the relationship of oligomerization to receptor activation and signaling.

For rhodopsin, dimers and higher-order oligomers have been visualized in disc membranes by atomic force microscopy [150], and an oligomeric arrangement has been inferred in its native environment [12, 151]. Oligomerization also has been inferred from ligand-binding studies [132, 152–155]. Guo et al. recently demonstrated using biophysical and biochemical approaches that the D2R forms higher-order oligomers in living cells at physiological levels of expression [147].

3.5.1 GPCR Oligomerization and Signaling

What is physiologically most relevant is understanding the role of the dimeric or oligomeric organization of GPCRs in signaling [156, 157]. Indeed, one of the great challenges in GPCR biology today is strengthening the weak mechanistic link between the physical interactions of receptors in the membrane and signaling cross talk of presumed heterodimers or hetero-oligomers. There is a great deal of evidence from many laboratories that many GPCRs interact as heterodimers (reviewed in [158, 159]). As indicated above, a number of findings support the existence of higher-order homo-oligomers as well [150, 155, 160, 161]. This raises the possibility that GPCR heteromers may interact not as heterodimers per se but rather as higher-order hetero-oligomers composed of homodimer subunits.

A large number of studies have demonstrated signaling cross talk between coexpressed GPCRs [162]. In almost all cases, however, the mechanistic link between heteromerization and signaling is tenuous. Although activation of two coexpressed receptors may be essential, signaling cross talk could nonetheless take place downstream of parallel homomeric receptor-mediated G protein activation and in such a case would not be a direct result of heteromeric signaling. Such a downstream cross talk mechanism, while often ignored, is very difficult to rule out. One example of this complexity is a recent fascinating study of a putative D1–D2R heterodimer that has been carried out both in heterologous cells [163] and in the brain [164]. These receptors appear to be coexpressed in some neurons in vivo [164]. In heterologous cells they have been inferred to physically interact based on fluorescence resonance energy transfer (FRET) [165, 166] as well as co-internalization [167, 168] and co-retention of mutants [169]. Activating both D1 and D2Rs leads to Gq-mediated signaling [163, 164], whereas D_1 signaling is normally Gs/olf mediated and D_2 signaling is normally Go/i mediated. These findings are intriguing and open exciting avenues of drug design targeted selectively to specific heteromers [170]. However, the plot appears thicker, as D1R-mediated Gq signaling has been observed in the brain [171, 172] where in some studies it has been shown to be insensitive to D2R

blockade [173], suggesting a role for other cellular factors in the coupling of D1R to the Gq pathway. Evidence for a priming effect for D1R-mediated Gq signaling is an example of such a potential mechanism [174, 175].

D2R has also been reported to interact with the dopamine D_3 receptor (D3R), and coexpression of D_2 and D_3 receptors has been reported to modulate the function of both receptors [176, 177]. More recently the D2R has been shown to modulate and to physically associate with the dopamine transporter as well [178, 179].

In addition to its reported interactions with receptors from the dopamine subfamily, there is a substantial literature on heteromerization of D2R with multiple other Class A receptors. There is evidence for direct physical interaction between D2R and the SST5 somatostatin receptor [180], D2R and adenosine A2A receptor [181, 182], and D2R and CB1 cannabinoid receptor [183]. In each of these cases, changes in signaling were observed upon receptor coexpression, with either altered D2R pharmacology by the partner protomer and/or an alteration in the properties of the partner in response to drugs acting at the D2R. In the case of the D2R–CB1 heteromer, dual-agonist mediated activation of Gs was reported, although neither receptor alone is able to activate this Gα subunit [183]. These results are intriguing and suggest the possibility of an untapped level of pharmacological diversity for new compound development, as well as a host of potential roles for in vivo signaling specificity for these putative heteromers. However, in none of these studies is it possible to rule out downstream signaling cross talk and thus to establish incontrovertibly that direct signaling by the D2R heteromer is responsible for the cross talk.

Such a mechanistic interrogation of heteromeric signaling in Class A GPCRs has been difficult. Our mechanistic understanding of the functional role of GPCR dimerization is more advanced in the Class C receptors, due in part to the availability of a clever adaptation of the endoplasmic reticulum (ER) retention signal from the $GABA_B$ receptor to enable controlled cell surface expression and signaling by defined metabotropic glutamate receptor (mGluR) heterodimers [184]. These studies have shown evidence for asymmetric activation of the heterodimer [185, 186]. Furthermore, one agonist can activate the dimer, but two agonists are required for full activation [187]. In addition, within the same Class C, T1R3 taste receptors are known to form functional heterodimers with either T1R1 or T1R2 in order to respond to a large panel of ligands and to trigger umami and sweet taste sensations, respectively (reviewed in [188]).

Unfortunately, related approaches with ER retention signals have been unsuccessful in Class A receptors, and it has not been possible to differentiate clearly the role of each subunit in homomeric and heteromeric signaling with coexpressed receptors. However, multiple lines of study do suggest interaction between Class A receptors in a heteromeric functional unit. Thus, for example, ligand-binding dissociation kinetics have recently been linked to the GPCR dimerization process (reviewed in [189]). In chemokine receptor heteromers, a CCR2-selective drug accelerates the dissociation of a CCR5- or CXCR4-selective drug when the receptors are coexpressed in heterologous cells and in native lymphocytes [190–192]. Moreover, although it remains to be proven conclusively, it seems reasonable to

infer that bivalent drugs engaging two different receptors, i.e., heteromer-selective compounds, might act simultaneously on two protomers in a heteromer and thereby directly activate downstream heteromer-specific signaling machinery [193–195] raising the possibility of their selective therapeutic potential [196]. Although there is evidence of G protein signaling by coexpressed nonfunctional receptor chimeras, this was proposed to occur by transmembrane domain swapping [197], which is unlikely to be universal [198]. Curiously, coexpression of two loss of function glycoprotein hormone receptors (receptors with either agonist binding or the ability to activate G proteins compromised) [199–201] led to function, but among Class A receptors such rescue seems to be limited to glycoprotein hormone receptors, which have very large extracellular N-terminal binding sites. This is similar to the transactivation seen in the Class C $GABA_B$ receptor, in which agonist binding to one protomer signals to G protein through the second protomer [184].

Another major question facing the field is the relationship between findings in heterologous cells and in ex vivo or in vivo cell systems. Most studies have focused on heterologous cells, but new approaches are being developed, including heteromer-specific antibodies (L. Devi, personal communication) as well as transgenic approaches with modified receptors.

Recent studies of purified B2AR and rhodopsin reconstituted into nanodiscs [202, 203] or in detergent solution [204] have demonstrated clearly that these receptors as monomers *can* activate G proteins. If, however, these receptors are indeed organized as dimers (or higher-order units) in native membranes, these elegant biophysical studies beg the physiologically relevant question. That is, if the receptors are capable of functioning as monomers but are closely associated as dimers or oligomers in the membrane, then what functional role does the second protomer play in drug binding and G protein activation? For example, in the $GABA_BB$ receptor the GB2 subunit is necessary for high-affinity binding of agonist to GB1 [205]. Studies in the D2R indicate that conformational change at the TM4 dimer interface is part of the receptor activation mechanism [145], although we cannot as yet establish whether this is achieved by changes in one or both protomers. Similarly, in the LBT4 Baneres and colleagues have shown evidence receptor for conformational changes in protomer B upon agonist binding to protomer A [185], again consistent with a role for the dimer interface in activation.

3.5.2 GPCR Oligomers – Structural Considerations

Dimer interface has been the subject of various studies over the years because of its crucial value in elucidating the structural mechanism(s) for cross talk between receptors within an oligomeric arrangement [145]. Guo et al. have shown that in the D2R TM4 forms a symmetrical dimer interface and that a conformational change at this interface is part of the receptor activation mechanism [145, 206]. Based on atomic force microscopy (AFM) maps of rhodopsin, Liang et al. proposed an oligomeric model in which TM4, TM5, and ECL2 form a dimeric interface, whereas contacts between TM1, TM2, and the cytoplasmic loop connecting TM5 and TM6

facilitate the formation of oligomers [151, 160, 207, 208]. TM1 and TM4 were postulated to be the most common interfaces of oligomerization by a correlated mutation analysis-based method [143, 144, 209].

It is unclear if other dimer orientations are also permissible [119]. For example, besides TM4 and TM5 of rhodopsin [160], other TMs have been implicated in dimer interfaces [210]: TM6 of the β_2-adrenergic, cholecystokinin, and leukotriene B_4 receptors [211, 212], TM5 and TM6 of the adrenergic–muscarinic chimera [213–216], TM1 and TM4 of the D2R [145, 147, 206], TM1 and TM7 of the α-adrenergic receptor [217], TM1 and TM4 of the chemokine receptor [218], TM4, TM1, and TM5/6 in the β1-adrenoceptor [130], TM1, TM2, and TM4 in the complement C5a [219], and TM5 in the adenosine A2A receptor [220].

Bouvier et al. showed that a peptide derived from the TM6 of the B2AR inhibits dimerization of these receptors and proposed a helix–helix interaction involving a conserved GxxxG motif on TM6 [221]. Although TM1 and TM4 can form simultaneous symmetric interfaces in an oligomeric structure [147], TM6 cannot form a symmetrical interface in this oligomer, although it might contribute to an asymmetrical interface.

3.5.3 Oligomer Rearrangements upon Activation

Even though GPCR monomers appear sufficient to activate a G protein [122, 202, 203, 222], their dimerization may modulate this activation through changes at the dimerization interface or a larger-scale reorientation of the two subunits [145, 147, 162, 223, 224]. Cross-linking in the D2R homodimer suggested a conformational rearrangement at the TM4 dimer interface upon receptor activation, passing from a conformation consistent with the 1N3M pdb file to an alternative TM4 interface [145, 147]. Consequently, the D2R inactive state is consistent with the AFM model while the active state is consistent with a squid rhodopsin electron cryomicroscopy (ECM) model [145, 147]. Consistent with this proposal, cross-links of the TM4 interface activated D2R, even in the absence of agonist [145, 147]. This idea was substantiated by recent studies. Brock et al. showed that an agonist-induced rearrangement may indeed occur in the activation of the dimeric metabotropic glutamate receptors [225]. Similarly, a possible dimeric rearrangement was also observed in the mGluR1α receptor. Upon ligand binding, although the distance between ICL1 and ICL2 in each protomer is unchanged, the distance between the ICL1s becomes larger, whereas that between ICL2s becomes smaller [226]. Damian et al. have also shown that in the leukotriene B_4 (LTB_4) receptor, conformational changes take place in one of the protomers upon activation of the other [185].

These observations suggest that in addition to the activation-related conformational changes within a GPCR protomer after activation (mainly a conformational change in TM6 and an associated opening of a binding cleft for G protein between TM6 and TM3) [227], it seems that a rearrangement of the interface of the two protomers is also vital for activation [145, 147]. Mechanisms that might account for this conformational rearrangement include a rigid body clockwise rotation of

contacting TM4s upon activation, protomer displacement involving a large movement and reorganization, or partner change among protomer partners [145, 147], although Niv et al. found using computational methods that rigid body rotation of interacting TM4s is an unlikely mechanism [228].

3.5.4 GPCR Oligomerization and GPCR–G Protein Interactions

MGlu receptor heteromers have been inferred to activate with individual protomers in an asymmetrical relationship [186, 229]. For the BLT1 receptor, the active form of the receptor dimer also is nonsymmetric with only one subunit reaching the fully active state [230]. A single agonist per dimer appears to be sufficient for activation of heterodimeric receptors such as $GABA_B$ [205, 231] and T1R receptors [232]. Similar findings have been reported for the mGlu receptor [187], but other findings in mGlu receptors suggest that activation by agonist binding to both protomers produces greater activation [187]. G protein-specific interactions have been proposed to account for such asymmetric behavior [185, 233, 234]. Jastrzebska et al. speculated that activation of a GPCR dimer could be achieved by a single protomer and that the combination of interactions including the regions of specific trimeric G proteins and two protomers facilitates more efficient coupling [235].

In the classic view, supported by innumerable mutagenesis studies of GPCRs, a monomeric GPCR interacts through ICL2, ICL3, and/or proximal carboxyl-terminal regions with a single heterotrimeric G protein. Structural studies of the receptor–G protein interface have led to the identification of several points of contact between the G protein and the receptor on both α- and β/γ-subunits [236]. When the first crystal structure of a heterotrimeric G protein was solved, it was argued that the surface area of a GPCR monomer was too small to account for the simultaneous interaction with both α- and β/γ-subunits of a G protein [29, 60, 236–238]. A single G protein molecule might instead interact with a GPCR dimer [19, 29, 160, 236, 239–241]. Consistent with this, Baneres and Parello have shown that activated leukotriene B_4 (LTB_4) receptor BLT1 dimer and $G_{\alpha 12\beta 1\gamma 2}$ form an assembly containing one G protein heterotrimer and one receptor dimer (242). If the signaling unit is a GPCR dimer complexed with a heterotrimeric G protein, then both *cis*- and *trans*-activation between two protomers may occur [145].

In recent years, increasing attention has been placed on developing an improved understanding of the interaction between G proteins and the D2R. Senogles et al. have demonstrated that random point mutations in the ICL3 of $D2R_s$ modify G_i protein coupling specificity. Specifically, ICL3 mutations R233G and A234T alter the predicted helical character of ICL3 and disrupt the $D2R_s$/G protein interface [243]. Moreover, a receptor-mimetic peptide derived from the N terminus of D2R ICL3 (D_2N) directly activates G_i/G_o proteins [244, 245]. The crystallographic structure of D_2N with $G\alpha_{i1}$ has further elucidated D_2N/G protein interactions, suggesting that the α4/β6 region of Gα (residues Q304/E308 and T321) is connected to a short basic cluster of D_2N and ^{11}RRRK^{14} (corresponding to ^{216}RRRK^{219} in human D2R) [246].

3.5.5 Consequences of GPCR Oligomerization

One of the most fundamental aspects of oligomerization is its importance for GPCR pharmacology. Ligand binding to GPCRs may result in changes in the binding characteristics of additional ligands targeting the same GPCR, creating a cooperative effect on the binding of another GPCR through an allosteric mechanism [247–249]. Stabilization of a particular conformation of the dimer by a bifunctional agonist might lead to an increase of specificity and efficacy of the signaling [250]. A number of different functionalities and pharmacologic characteristics have been reported and attributed to the generation of GPCR heterodimer/oligomer complexes. Nevertheless, it is crucial to keep in mind that these effects can not only be attributed to direct protein–protein interactions but also to indirect effects produced via downstream signaling and feedback control [251].

The capacity of a GPCR to alter the binding affinity of its binding partner may ultimately be applied clinically in future drug development. More than 50% of all drugs with annual worldwide sales of more than $50 billion regulate the function and activity of many GPCRs in attempts to treat various diseases and disorders [131, 252]. As previously mentioned, GPCR dimerization is important prior to plasma membrane delivery, and incorrect folding may interfere with dimerization and can lead to alteration in cell surface delivery and function [253–255]. In designing potential drugs that may take advantage of our growing knowledge of GPCR structure and function, taking into account oligomerization and heterodimer formation may be critical. Moreover, since receptor heterodimers can generate distinct signals from their corresponding homodimers, understanding the structural basis for higher-order receptor structure may offer a means to improve tissue selectivity and improve drug therapeutic function [251].

References

1. Soyer O, Dimmic MW, Neubig RR, Goldstein RA. Using evolutionary methods to study G-protein coupled receptors. Pac Symp Biocomput 2002;7:625–36.
2. Soyer OS, Dimmic MW, Neubig RR, Goldstein RA. Dimerization in aminergic G-protein-coupled receptors: application of a hidden-site class model of evolution. Biochemistry 2003;42:14522–31.
3. Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: an evolutionary success. EMBO J 1999;18:1723–9.
4. Avlani VA, Gregory KJ, Morton CJ, Parker MW, Sexton PM, Christopoulos A. Critical role for the second extracellular loop in the binding of both orthosteric and allosteric G protein-coupled receptor ligands. J Biol Chem 2007.
5. Weinstein H. Protein interactions in GPCR signaling: a very moving story. Abstr Pap Am Chem Soc 2006;231:102.
6. Niv MY, Skrabanek L, Filizola M, Weinstein H. Modeling activated states of GPCRs: the rhodopsin template. J Comput Aided Mol Des 2006;20:437–48.
7. Ballesteros J, Weinstein H. Integrated methods for the construction of three-dimensional models of structure-function relations in G protein-coupled receptors. Methods Neurosci 1995;25:366.

8. Ballesteros JA, Shi L, Javitch JA. Structural mimicry in G-protein-coupled receptors: implications of the high-resolution structure of rhodopsin for structure-function analysis of rhodopsin-like receptors. Mol Pharmacol 2001;60:1.
9. Thompson, Menard, Pombal, Grillner. Forebrain dopamine depletion impairs motor behavior in lamprey. Eur J Neurosci 2008;27:1452–60.
10. Chan WY, McKinzie DL, Bose S, et al. Allosteric modulation of the muscarinic M-4 receptor as an approach to treating schizophrenia. Proc Natl Acad Sci U S A 2008;105:10978–83.
11. Thompson MD, Burnham WM, Cole DE. The G protein-coupled receptors: pharmacogenetics and disease. Crit Rev Clin Lab Sci 2005;42:311–92.
12. Palczewski K, Hofmann KP, Baehr W. Rhodopsin – advances and perspectives. Vision Res 2006;46:4425–6.
13. Bouchard C, Ribeiro P, Dube F, Anctil M. A new G protein-coupled receptor from a primitive metazoan shows homology with vertebrate aminergic receptors and displays constitutive activity in mammalian cells. J Neurochem 2003;86:1149–61.
14. Klco JM, Wiegand CB, Narzinski K, Baranski TJ. Essential role for the second extracellular loop in C5a receptor activation. Nat Struct Mol Biol 2005;12:320–6.
15. Minneman K. Heterodimerization and surface localization of G protein coupled receptors. Special Issue: In Memory of Art Hancock 2007;73:1043–50.
16. Lefkowitz RJ. The superfamily of heptahelical receptors. Nat Cell Biol 2000;2:E133–36.
17. Lefkowitz RJ. Historical review: a brief history and personal retrospective of seven-transmembrane receptors. Trends Pharmacol Sci 2004;25:413–22.
18. Lefkowitz RJ. Seven transmembrane receptors: a brief personal retrospective. Biochim Biophys Acta 2007;1768:748–55.
19. Maggio R., Innamorati G., Parenti M. G protein-coupled receptor oligomerization provides the framework for signal discrimination. J Neurochem 2007;103:1741–52.
20. Han DS, Wang SX, Weinstein H. Active state-like conformational elements in the beta(2)-AR and a photoactivated intermediate of rhodopsin identified by dynamic properties of GPCRs. Biochemistry 2008;47:7317–21.
21. Huang P, Li J, Chen CG, Visiers I, Weinstein H, Liu-Chen LY. Functional role of a conserved motif in TM6 of the rat mu opioid receptor: constitutively active and inactive receptors result from substitutions of Thr6.34(279) with Lys and Asp. Biochemistry 2001;40:13501–9.
22. Prioleau C, Visiers I, Ebersole BJ, Weinstein H, Sealfon SC. Conserved helix 7 tyrosine acts as a multistate conformational switch in the 5HT2C receptor – Identification of a novel "locked-on" phenotype and double revertant mutations. J Biol Chem 2002;277:36577–84.
23. Visiers I, Ballesteros JA, Weinstein H. Three-dimensional representations of G protein-coupled receptor structures and mechanisms. G Protein Pathways, Pt a, Receptors 2002;343:329–71.
24. Ballesteros JA, Jensen AD, Liapakis G, et al. Activation of the beta(2)-adrenergic receptor involves disruption of an ionic lock between the cytoplasmic ends of transmembrane segments 3 and 6. J Biol Chem 2001;276:29171–7.
25. Konvicka K, Guarnieri F, Ballesteros JA, Weinstein H. Proposed structure for the NPxxY sequence motif in transmembrane segment 7 of G-protein coupled receptors. Biophys J 1997;72:A74.
26. Konvicka K, Guarnieri F, Ballesteros JA, Weinstein H. A proposed structure for transmembrane segment 7 of G protein-coupled receptors incorporating an Asn-Pro/Asp-Pro motif. Biophys J 1998;75:601–11.
27. Bhattacharya M, Babwah AV, Ferguson SS. Small GTP-binding protein-coupled receptors. Biochem Soc Trans 2004;32:1040–4.
28. Bhattacharya S, Hall S, Vaidehi N. Agonist-induced conformational changes in Bovine rhodopsin: insight into activation of G-protein-coupled receptors. J Mol Biol 2008;382:539–55.
29. Palczewski K, Kumasaka T, Hori T, et al. Crystal structure of rhodopsin: A G protein-coupled receptor. Science 2000;289:739–45.

30. Day PW, Rasmussen SGF, Parnot C, et al. A monoclonal antibody for G protein-coupled receptor crystallography. Nature Methods 2007;4:927–9.
31. Rasmussen SGF, Choi HJ, Rosenbaum DM, et al. Crystal structure of the human beta(2) adrenergic G-protein-coupled receptor. Nature 2007;450:383–U4.
32. Cherezov V, Rosenbaum DM, Hanson MA, et al. High-resolution crystal structure of an engineered human beta(2)-adrenergic G protein-coupled receptor. Science 2007;318: 1258–65.
33. Rosenbaum DM, Cherezov V, Hanson MA, et al. GPCR engineering yields high-resolution structural insights into beta(2)-adrenergic receptor function. Science 2007;318: 1266–73.
34. Warne T, Serrano-Vega MJ, Baker JG, et al. Structure of a beta(1)-adrenergic G-protein-coupled receptor. Nature 2008;454:486–491.
35. Jaakola VP, Griffith MT, Hanson MA, et al. The 2.6 angstrom crystal structure of a human A(2A) adenosine receptor bound to an antagonist. Science 2008;322:1211–7.
36. Xhaard H, Rantanen VV, Nyronen T, Johnson MS. Molecular evolution of adrenoceptors and dopamine receptors: Implications for the binding of catecholamines. J Med Chem 2006;49:1706–19.
37. Missale C, Nash R, Robinson S, Jaber M, Caron M. Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.
38. Pivonello R, Ferone D, de Herder WW, et al. Dopamine receptor expression and function in corticotroph ectopic tumors. J Clin Endocrinol Metab 2007;92:65–9.
39. Thevenin D, Lazarova T. Stable interactions between the transmembrane domains of the adenosine A2A receptor. Protein Sci 2008;17:1188–99.
40. Javitch JA. The ants go marching two by two: oligomeric structure of G-protein-coupled receptors. Mol Pharmacol 2004;66:1077–82.
41. Ballesteros J, Palczewski K. G protein-coupled receptor drug discovery: implications from the crystal structure of rhodopsin. Curr Opin Drug Discov Devel 2001;4:561–74.
42. Lefkowitz RJ. Seven transmembrane receptors: something old, something new. Acta Physiol 2007;190:9–19.
43. Sansom MSP, Weinstein H. Hinges, swivels and switches: the role of prolines in signalling via transmembrane alpha-helices. Trends Pharmacol Sci 2000;21:445–51.
44. Visiers I, Ballesteros J, Weinstein H. A spatially ordered sequence of intramolecular rearrangements observed from simulations of agonist-related activation of 5HT(2C) receptors. Biophys J 2000;78:394Pos.
45. Ceruso MA, Weinstein H. Structural mimicry of proline kinks: tertiary packing interactions support local structural distortions. J Mol Biol 2002;318:1237–49.
46. Shi L, Liapakis G, Xu R, Guarnieri F, Ballesteros JA, Javitch JA. Beta2 adrenergic receptor activation. Modulation of the proline kink in transmembrane 6 by a rotamer toggle switch. J Biol Chem 2002;277:40989–96.
47. Ballesteros JA, Deupi X, Olivella M, Haaksma EEJ, Pardo L. Serine and threonine residues bend alpha-helices in the chi(1) = g(–) conformation. Biophys J 2000;79:2754–60.
48. Shi L, Simpson MM, Ballesteros JA, Javitch JA. The first transmembrane segment of the dopamine D2 receptor: accessibility in the binding-site crevice and position in the transmembrane bundle. Biochemistry 2001;40:12339–48.
49. Liapakis G, Simpson MM, Javitch JA. The substituted-cysteine accessibility method (SCAM) to elucidate membrane protein structure. Curr Protoc Neurosci 2001;Chapter 4:Unit 4.15.
50. Hubbell WL, Altenbach C, Hubbell CM, Khorana HG, Douglas CR. Rhodopsin structure, dynamics, and activation: a perspective from crystallography, site-directed spin labeling, sulfhydryl reactivity, and disulfide cross-linking. Adv Protein Chem 2003;63: 243–90.
51. Shukla AK, Sun J-P, Lefkowitz RJ. Crystallizing thinking about the {beta}2-adrenergic receptor. Mol Pharmacol 2008;73:1333–8.

52. Kobilka BK, Deupi X. Conformational complexity of G-protein-coupled receptors. Trends Pharmacol Sci 2007;28:397–406.
53. Schwartz TU, Schmidt D, Brohawn SG, Blobel G. Homodimerization of the G protein SRbeta in the nucleotide-free state involves proline cis/trans isomerization in the switch II region. Proc Natl Acad Sci U S A 2006;103:6823–8.
54. Schwartz TW, Frimurer TM, Holst B, Rosenkilde MM, Elling CE. Molecular mechanism of 7TM receptor activation – a global toggle switch model. Annu Rev Pharmacol Toxicol 2006;46:481–519.
55. Sheikh SP, Vilardarga JP, Baranski TJ, et al. Similar structures and shared switch mechanisms of the beta2-adrenoceptor and the parathyroid hormone receptor. Zn(II) bridges between helices III and VI block activation. The J Biol Chem 1999;274: 17033–41.
56. Binet V, Duthey BA, Lecaillon J, et al. Common structural requirements for heptahelical domain function in class A and class C GPCRS. J Biol Chem 2007.
57. Park PS, Lodowski DT, Palczewski K. Activation of G protein-coupled receptors: beyond two-state models and tertiary conformational changes. Ann Rev Pharmacol Toxicol 2008;48:107–41.
58. Xu W, Li J, Chen CG, et al. Comparison of the amino acid residues in the sixth transmembrane domains accessible in the binding-site crevices of mu, delta, and kappa opioid receptors. Biochemistry 2001;40:8018–29.
59. Javitch JA, Fu DY, Liapakis G, Chen JY. Constitutive activation of the beta(2) adrenergic receptor alters the orientation of its sixth membrane-spanning segment. J Biol Chem 1997;272:18546–9.
60. Bourne H. How receptors talk to trimeric G proteins. Curr Opin Cell Biol 1997;9: 134–42.
61. Elling CE, Frimurer TM, Gerlach LO, Jorgensen R, Holst B, Schwartz TW. Metal ion site engineering indicates a global toggle switch model for seven-transmembrane receptor activation. J Biol Chem 2006;281:17337–46.
62. Gether U, Ballesteros JA, Seifert R, SandersBush E, Weinstein H, Kobilka BK. Structural instability of a constitutively active G protein-coupled receptor – agonist-independent activation due to conformational flexibility. J Biol Chem 1997;272: 2587–90.
63. Li J, Huang P, Chen CG, de Riel JK, Weinstein H, Liu-Chen LY. Constitutive activation of the mu opioid receptor by mutation of D3.49(164), but not D3.32(147): D3.49(164) is critical for stabilization of the inactive form of the receptor and for its expression. Biochemistry 2001;40:12039–50.
64. Rasmussen SGF, Jensen AD, Liapakis G, Ghanouni P, Javitch JA, Gether U. Mutation of a highly conserved aspartic acid in the beta(2) adrenergic receptor: constitutive activation, structural instability, and conformational rearrangement of transmembrane segment 6. Mol Pharmacol 1999;56:175–84.
65. Haydock K, Weinstein H. Molecular dynamics simulations to model the properties of constitutively active GPCR mutants. Biophys J 1995;68:A255.
66. Cotecchia S. Constitutive activity and inverse agonism at the alpha1adrenoceptors. Biochem Pharmacol 2007;73:1076–83.
67. Cotecchia S, Exum S, Caron MG, Lefkowitz RJ. Regions of the alpha 1-adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function. Proc Natl Acad Sci U S A 1990;87:2896–900.
68. Gether U, Lin S, Ghanouni P, Ballesteros JA, Weinstein H, Kobilka BK. Agonists induce conformational changes in transmembrane domains III and VI of the beta(2) adrenoceptor. Embo J 1997;16:6737–47.
69. Parnot C. Systematic identification of mutations that constitutively activate the angiotensin II type 1A receptor by screening a randomly mutated cDNA library with an original pharmacological bioassay. Proc Natl Acad Sci USA 2000;97:7615–20.

70. Parnot C, Miserey-Lenkei S, Bardin S, Corvol P, Clauser E. Lessons from constitutively active mutants of G protein-coupled receptors. Trends Endocrinol Metab 2002;13:336–43.
71. Ballesteros J, Kitanovic S, Guarnieri F, et al. Functional microdomains in g-protein-coupled receptors – the conserved Arginine-cage motif in the gonadotropin-releasing hormone receptor. J Biol Chem 1998;273:10445–53.
72. Ballesteros JA, Kitanovic S, Guarnieri F et al. Functional microdomains in G protein-coupled receptors: the conserved arginine cage motif in the gonadotropin-releasing hormone receptor. J Biol Chem 1998;273:10445–53.
73. Shapiro DA, Kristiansen K, Weiner DM, Kroeze WK, Roth BL. Evidence for a model of agonist-induced activation of 5-hydroxytryptamine 2A serotonin receptors that involves the disruption of a strong ionic interaction between helices 3 and 6. J Biol Chem 2002;277:11441–9.
74. Huang P, Visiers I, Weinstein H, Liu-Chen LY. The local environment at the cytoplasmic end of TM6 of the mu opioid receptor differs from those of rhodopsin and monoamine receptors: Introduction of an ionic lock between the cytoplasmic ends of helices 3 and 6 by a L6.30(275)E mutation inactivates the mu opioid receptor and reduces the constitutive activity of its T6.34(279)K mutant. Biochemistry 2002;41:11972–80.
75. Dror RO, Arlow DH, Borhani DW, Jensen Mo, Piana S, Shaw DE. Identification of two distinct inactive conformations of the (beta)2-adrenergic receptor reconciles structural and biochemical observations. Proc Natl Acad Sci U S A 2009 – Available Online.
76. Kobilka BK. G protein coupled receptor structure and activation. Biochim Biophys Acta 2007;1768:794–807.
77. Kobilka B, Schertler GF. New G-protein-coupled receptor crystal structures: insights and limitations. Trends Pharmacol Sci 2008;29:79–83.
78. Neve KA, Cox BA, Henningsen RA, Spanoyannis A, Neve RL. Pivotal role for aspartate-80 in the regulation of dopamine D2 receptor affinity for drugs and inhibition of adenylyl cyclase. Mol Pharmacol 1991;39:733–9.
79. Neve KA, Cumbay MG, Thompson KR, Yang R, Buck DC. Modeling and mutational analysis of a putative sodium-binding pocket on the dopamine D2 receptor. Mol Pharmacol 2001;60:373.
80. Mansour A, Meng F, Meador-Woodruff JH, Taylor LP, Civelli O, Akil H. Site-directed mutagenesis of the human dopamine D2 receptor. Eur J Pharmacol 1992;227:205–14.
81. Javitch JA. Mapping the binding-site crevice of the D2 receptor. Adv Pharmacol 1998;42:412–5.
82. Javitch JA. Probing structure of neurotransmitter transporters by substituted-cysteine accessibility method. Neurotransmitter Transporters 1998;296:331–46.
83. Javitch JA, Ballesteros JA, Chen JY, Chiappa V, Simpson MM. Electrostatic and aromatic microdomains within the binding-site crevice of the D2 receptor: contributions of the second membrane-spanning segment. Biochemistry 1999;38:7961–8.
84. Javitch JA, Ballesteros JA, Weinstein H, Chen J. A cluster of aromatic residues in the sixth membrane-spanning segment of the dopamine D2 receptor is accessible in the binding-site crevice. Biochemistry 1998;37:998–1006.
85. Javitch JA, Fu D, Chen J. Residues in the fifth membrane-spanning segment of the dopamine D2 receptor exposed in the binding-site crevice. Biochemistry 1995;34:16433–9.
86. Javitch JA, Fu D, Chen J, Karlin A. Mapping the binding-site crevice of the dopamine D2 receptor by the substituted-cysteine accessibility method. Neuron 1995;14:825–31.
87. Javitch JA, Fu DY, Chen JY. Differentiating dopamine D-2 ligands by their sensitivities to modification of the cysteine exposed in the binding-site crevice. Mol Pharmacol 1996;49:692–8.
88. Javitch JA, Li X, Kaback J, Karlin A. A cysteine residue in the third membrane-spanning segment of the human D2 dopamine receptor is exposed in the binding-site crevice. Proc Natl Acad Sci U S A 1994;91:10355–9.

89. Javitch JA, Shi L, Simpson MM, Chen J, Chiappa V. The fourth transmembrane segment of the dopamine D2 receptor: accessibility in the binding-site crevice and position in the transmembrane bundle. Biochemistry 2000;39:12,190.
90. Fu DY, Ballesteros JA, Weinstein H, Chen JY, Javitch JA. Residues in the seventh membrane-spanning segment of the dopamine D2 receptor accessible in the binding-site crevice. Biochemistry 1996;35:11278–85.
91. Simpson MM, Ballesteros JA, Chiappa V, et al. Dopamine D4/D2 receptor selectivity is determined by a divergent aromatic microdomain contained within the second, third, and seventh membrane-spanning segments. Mol Pharmacol 1999;56:1116–26.
92. Coley C, Woodward R, Johansson AM, Strange PG, Naylor LH. Effect of multiple serine/alanine mutations in the transmembrane spanning region V of the D2 dopamine receptor on ligand binding. Journal of neurochemistry 2000;74:358–66.
93. Ericksen SS, Cummings DF, Weinstein H, Schetz JA. Ligand selectivity of D-2 dopamine receptors is modulated by changes in local dynamics produced by sodium binding. J Pharmacol Exp Thers 2009;328:40–54.
94. Schetz JA, Benjamin PS, Sibley DR. Nonconserved residues in the second transmembrane-spanning domain of the D4 dopamine receptor are molecular determinants of D4-selective pharmacology. Mol Pharmacol 2000;57:144–52.
95. Schetz JA, Sibley DR. Tandem sulfur-containing amino acids are epicritical determinants of dopamine D(2) receptor pharmacology. Eur J Pharmacol 2000;388:R5–R7.
96. Neve KA. Regulation of dopamine D2 receptors by sodium and pH. Mol Pharmacol 1991;39:570.
97. Kortagere S, Gmeiner P, Weinstein H, Schetz JA. Certain 1,4-disubstituted aromatic piperidines and piperazines with extreme selectivity for the dopamine D4 receptor interact with a common receptor microdomain. Mol Pharmacol 2004;66:1491–9.
98. Ortore G, Tuccinardi T, Bertini S, Martinelli A. A theoretical study to investigate D2DAR/D4DAR selectivity; receptor modeling and molecular docking of dopaminergic ligands. J Med Chem 2006;49:1397–407.
99. Mehler EL, Hassan SA, Kortagere S, Weinstein H. Ab initio computational modeling of loops in G-protein-coupled receptors: lessons from the crystal structure of rhodopsin. Proteins 2006;64:673–90.
100. Mehler EL, Periole X, Hassan SA, Weinstein H. Key issues in the computational simulation of GPCR function: representation of loop domains. J Comput Aided Mol Des 2002;15:13.
101. Christopoulos A, Kenakin T. G protein-coupled receptor allosterism and complexing. Pharmacol Rev 2002;54:323–74.
102. de Graaf C, Foata N, Engkvist O, Rognan D. Molecular modeling of the second extracellular loop of G-protein coupled receptors and its implication on structure-based virtual screening. Proteins 2008;71:599–620.
103. Karnik S, Gogonea C, Patil S, Saad Y, Takezako T. Activation of G-protein-coupled receptors: a common molecular mechanism. Trends Endocrinol Metabol 2003;14:431–7.
104. Savarese TM, Wang CD, Fraser CM. Site-directed mutagenesis of the rat m1 muscarinic acetylcholine receptor. Role of conserved cysteines in receptor function. J Biol Chem 1992;267:11439–48.
105. Zeng FY, Soldner A, Schoneberg T, Wess J. Conserved extracellular cysteine pair in the M3 muscarinic acetylcholine receptor is essential for proper receptor cell surface localization but not for G protein coupling. J Neurochem 1999;72:2404–14.
106. Noda K, Saad Y, Graham RM, Karnik SS. The high affinity state of the beta 2-adrenergic receptor requires unique interaction between conserved and non-conserved extracellular loop cysteines. J Biol Chem 1994;269:6743.
107. Lin S, Gether U, Kobilka BK. Ligand stabilization of the beta 2 adrenergic receptor: effect of DTT on receptor conformation monitored by circular dichroism and fluorescence spectroscopy. Biochemistry 1996;35:14,445.

108. Scarselli M, Li B, Kim SK, Wess J. Multiple residues in the second extracellular loop are critical for M3 muscarinic acetylcholine receptor activation. J Biol Chem 2007;282:7385–96.
109. Zhao MM, Hwa J, Perez DM. Identification of critical extracellular loop residues involved in alpha 1-adrenergic receptor subtype-selective antagonist binding. Mol Pharmacol 1996;50:1118.
110. Wurch T, Pauwels PJ. Coupling of canine serotonin 5-HT(1B) and 5-HT(1D) receptor subtypes to the formation of inositol phosphates by dual interactions with endogenous G(i/o) and recombinant G(alpha15) proteins. J Neurochem 2000;75:1180.
111. Wurch T, Colpaert FC, Pauwels PJ. Chimeric receptor analysis of the ketanserin binding site in the human 5-hydroxytryptamine1D receptor: importance of the second extracellular loop and fifth transmembrane domain in antagonist binding. Mol Pharmacol 1998;54:1088.
112. Menon S, Han M, Sakmar T. Rhodopsin: Structural basis of molecular physiology. Physiol Rev 2001;81:1659–88.
113. Bourne HR, Meng EC. Structure. Rhodopsin sees the light. Science 2000;289:733.
114. Shi L, Javitch JA. The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop. Annu Rev Pharmacol Toxicol 2002;42:437–67.
115. Shi L, Javitch JA. The second extracellular loop of the dopamine D2 receptor lines the binding-site crevice. Proc Natl Acad Sci U S A 2004;101:440–5.
116. Conner M, Hawtin SR, Simms J, et al. Systematic analysis of the entire second extracellular loop of the V1a vasopressin receptor: key residues, conserved throughout a G-protein-coupled receptor family, identified. J Biol Chem 2007;282:17405–12 .
117. Baneres JL, Mesnier D, Martin A, Joubert L, Dumuis A, Bockaert J. Molecular characterization of a purified 5-HT4 receptor: a structural basis for drug efficacy. J Biol Chem 2005;280:20253–60.
118. Kleinau G, Jaeschke H, Mueller S, Worth CL, Paschke R, Krause G. Molecular and structural effects of inverse agonistic mutations on signaling of the thyrotropin receptor – a basally active GPCR. Cell Mol Life Sci 2008;65:3664–76.
119. White J, Grodnitzky J, Louis J, et al. Dimerization of the class A G protein-coupled neurotensin receptor NTS1 alters G protein interaction. Proc Natl Acad Sci U S A 2007;104:12199–204.
120. Mowbray SL, Petsko GA. The x-ray structure of the periplasmic galactose binding protein from Salmonella typhimurium at 3.0-A resolution. J Biol Chem 1983;258:7991–7.
121. Okamoto T, Sekiyama N, Otsu M, et al. Expression and purification of the extracellular ligand binding region of metabotropic glutamate receptor subtype 1. J Biol Chem 1998;273:13089–96.
122. Chabre M, Lemaire M. Monomeric G-protein-coupled receptor as a functional unit. Biochemistry 2005;44:9395–403.
123. Meyer B, Segura J-M, Martinez K, et al. FRET imaging reveals that functional neurokinin-1 receptors are monomeric and reside in membrane microdomains of live cells. Proc Natl Acad Sci U S A 2006;103:2138–43.
124. Overton M, Blumer K. Use of fluorescence resonance energy transfer to analyze oligomerization of G-protein-coupled receptors expressed in yeast. Methods 2002;27:324–32.
125. Overton MC, Blumer KJ. G-protein-coupled receptors function as oligomers in vivo. Curr Biol 2000;10:341–4.
126. Overton MC, Blumer KJ. The extracellular N-terminal domain and transmembrane domains 1 and 2 mediate oligomerization of a yeast G protein-coupled receptor. J Biol Chem 2002;277:41463–72.
127. Overton MC, Chinault SL, Blumer KJ. Oligomerization, biogenesis, and signaling is promoted by a glycophorin A-like dimerization motif in transmembrane domain 1 of a yeast G protein-coupled receptor. J Biol Chem 2003;278:49369–77.

128. Overton MC, Chinault SL, Blumer KJ. Oligomerization of G-protein-coupled receptors: lessons from the yeast Saccharomyces cerevisiae. Eukaryot Cell 2005;4:1963–70.
129. Canals M, Lopez-Gimenez JF, Milligan G. Cell surface delivery and structural reorganization by pharmacological chaperones of an oligomerization-defective alpha(1b)-adrenoceptor mutant demonstrates membrane targeting of GPCR oligomers. Biochemical J 2009;417:161–72.
130. Carrillo JJ, Lopez-Gimenez JF, Milligan G. Multiple interactions between transmembrane helices generate the oligomeric {alpha}1b-Adrenoceptor. Mol Pharmacol 2004;66:1123–37.
131. Fredholm BB, Hokfelt T, Milligan G. G-protein-coupled receptors: an update. Acta Physiol 2007;190:3–7.
132. Lopez-Gimenez JF, Canals M, Pediani JD, Milligan G. The {alpha}1b-adrenoceptor exists as a higher-order oligomer: effective oligomerization is required for receptor maturation, surface delivery, and function. Mol Pharmacol 2007;71:1015–29.
133. Milligan G. Oligomerisation of G-protein-coupled receptors. J Cell Sci 2001;114:1265–71.
134. Milligan G. G protein-coupled receptor dimerization: function and ligand pharmacology. Mol Pharmacol 2004;66:1–7.
135. Milligan G. GPCR dimerisation: molecular basis and relevance for function and pharmacology. Biochim Biophys Acta 2007;1768:825–35.
136. Milligan G, Anonymous, Behan D, inventors; The University Court of the University of Glasgow, assignee. Materials and methods relating to G-protein coupled receptor oligomers patent US 07405053. 2008.
137. Milligan G, Ramsay D, Pascal G, Carrillo JJ. GPCR dimerisation. Life sciences 2003;74:181–8.
138. Skrabanek L, Murcia M, Bouvier M, et al. Requirements and ontology for a G protein-coupled receptor oligomerization knowledge base. BMC Bioinformatics 2007;8:177.
139. Filizola M, Guo W, Javitch JA, Weinstein H. Dimerization in G-protein coupled receptors: correlation analysis and electron density maps of rhodopsin from different species suggest subtype-specific interfaces. Biophys J 2003;84:269A-70A.
140. Filizola M, Olmea O, Weinstein H. Using correlated mutation analysis to predict the heterodimerization interface of GPCRs. Biophys J 2002;82:2307.
141. Filizola M, Wang S, Weinstein H. Dynamic models of G-protein coupled receptor dimers: indications of asymmetry in the rhodopsin dimer from molecular dynamics simulations in a POPC bilayer. J Comput Aided Mol Des 2006;20, 405–416.
142. Filizola M, Weinstein H. Structural models for dimerization of G-protein coupled receptors: the opioid receptor homodimers. Biopolymers 2002;66:317–25.
143. Filizola M, Weinstein H. The structure and dynamics of GPCR oligomers: a new focus in models of cell-signaling mechanisms and drug design. Curr Opin Drug Discov Devel 2005;8:577–84.
144. Filizola M, Weinstein H. The study of G-protein coupled receptor oligomerization with computational modeling and bioinformatics. FEBS J 2005;272:2926–38.
145. Guo W, Shi L, Filizola M, Weinstein H, Javitch J. From the cover: Crosstalk in G protein-coupled receptors: changes at the transmembrane homodimer interface determine activation. Proc Natl Acad Sci U S A 2005;102:17495–500.
146. Wang XS, Filizola M, Ceruso M, Weinstein H. Rhodopsin dimers: molecular dynamics simulations using discrete representations of the membrane and water environment. Biophys J 2005;88:81A-A.
147. Guo W, Urizar E, Kralikova M, et al. Dopamine D2 receptors form higher order oligomers at physiological expression levels. Embo J 2008;27:2293–304.
148. Hastrup H, Karlin A, Javitch JA. Symmetrical dimer of the human dopamine transporter revealed by cross-linking Cys-306 at the extracellular end of the sixth transmembrane segment. Proc Natl Acad Sci U S A 2001;98:10055–60.

149. McVey M, Ramsay D, Kellett E, et al. Monitoring receptor oligomerization using time-resolved fluorescence resonance energy transfer and bioluminescence resonance energy transfer – The human delta-opioid receptor displays constitutive oligomerization at the cell surface, which is not regulated by receptor occupancy. J Biol Chem 2001;276: 14092–9.
150. Fotiadis D, Liang Y, Filipek S, Saperstein DA, Engel A, Palczewski K. Atomic-force microscopy: rhodopsin dimers in native disc membranes. Nature 2003;421:127–8.
151. Fotiadis D, Jastrzebska B, Philippsen A, Muller D, Palczewski K, Engel A. Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. Theory and simulation/Macromolecular assemblages – Joel Janin and Michael Levitt/Edward H Egelman and Andrew GW Leslie 2006;16:252–9.
152. Strange P. Oligomers of D2 dopamine receptors: evidence from ligand binding. J Mol Neurosci2005;26:155–60.
153. Park P, Sum CS, Hampson DR, Van Tol HHM, Wells JW. Nature of the oligomers formed by muscarinic m2 acetylcholine receptors in Sf9 cells. Eur J Pharmacol 2001;421: 11–22.
154. Park PSH, Filipek S, Wells JW, Palczewski K. Oligomerization of G protein-coupled receptors: Past, present, and future. Biochemistry 2004;43:15643–56.
155. Paul S.-H. Park JWW. Oligomeric potential of the M2 muscarinic cholinergic receptor. J Neurochem 2004;90:537–48.
156. Pin JP, Neubig R, Bouvier M, et al. International Union of Basic and Clinical Pharmacology. LXVII. Recommendations for the recognition and nomenclature of G protein-coupled receptor heteromultimers. Pharmacol Rev 2007;59:5–13.
157. Bartfai T, Benovic JL, Bockaert J, et al. The state of GPCR research in 2004. Nat Rev Drug Discov 2004;3:574–626.
158. Maggio R, Novi F, Scarselli M, Corsini GU. The impact of G-protein-coupled receptor hetero-oligomerization on function and pharmacology. FEBS J 2005;272:2939–46.
159. Prinster S, Hague C, Hall R. Heterodimerization of G protein-coupled receptors: specificity and functional significance. Pharmacol Rev 2005;57:289–98.
160. Liang Y, Fotiadis D, Filipek S, Saperstein DA, Palczewski K, Engel A. Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes. The J Biol Chem 2003;278:21655–62.
161. Philip F, Sengupta P, Scarlata S. Signaling through a G protein-coupled receptor and its corresponding G protein follows a stoichiometrically limited model. J Biol Chem 2007;282:19203–16.
162. Breitwieser GE. G protein-coupled receptor oligomerization: implications for G protein activation and cell signaling. Circ Res 2004;94:17–27.
163. Lee SP, So CH, Rashid AJ, et al. Dopamine D1 and D2 receptor co-activation generates a novel phospholipase C-mediated calcium signal. The J Biol Chem 2004;279:35671–8.
164. Rashid AJ, So CH, Kong MM, et al. D1-D2 dopamine receptor heterooligomers with unique pharmacology are coupled to rapid activation of Gq/11 in the striatum. Proc Natl Acad Sci U S A 2007;104:654–9.
165. O'Dowd BF, Ji X, Alijaniaram M, et al. Dopamine receptor oligomerization visualized in living cells. J Biol Chem 2005;280:37225–35.
166. Dziedzicka-Wasylewska M, Faron-Gorecka A, Andrecka J, Polit A, Kusmider M, Wasylewski Z. Fluorescence studies reveal heterodimerization of dopamine D1 and D2 receptors in the plasma membrane. Biochemistry 2006;45:8751–9.
167. So CH, Varghese G, Curley KJ, et al. D1 and D2 dopamine receptors form heterooligomers and cointernalize after selective activation of either receptor. Mol Pharmacol 2005;68: 568–78.
168. So CH, Verma V, O'Dowd BF, George SR. Desensitization of the dopamine D1 and D2 receptor hetero-oligomer mediated calcium signal by agonist occupancy of either receptor. Mol Pharmacol 2007;72:450–62.

169. Kong MMC, Fan T, Varghese G, O'Dowd BF, George SR. Agonist-induced cell surface trafficking of an intracellularly sequestered D1 dopamine receptor homo-oligomer. Mol Pharmacol 2006;70:78–89.
170. Milligan G. G-protein-coupled receptor heterodimers: pharmacology, function and relevance to drug discovery. Drug Discov Today 2006;11:541–9.
171. Mannoury la Cour C, Vidal S, Pasteau V, Cussac D, Millan MJ. Dopamine D1 receptor coupling to Gs/olf and Gq in rat striatum and cortex: A scintillation proximity assay (SPA)/antibody-capture characterization of benzazepine agonists. Neuropharmacology 2007;52:1003–14.
172. Jin LQ, Wang HY, Friedman E. Stimulated D-1 dopamine receptors couple to multiple G alpha proteins in different brain regions. J Neurochem 2001;78:981–90.
173. Ming YL, Zhang H, Long LH, Wang F, Chen JG, Zhen XC. Modulation of Ca2+ signals by phosphatidylinositol-linked novel D1 dopamine receptor in hippocampal neurons. J Neurochem 2006;98:1316–23.
174. Ali MK, Bergson C. Elevated intracellular calcium triggers recruitment of the receptor cross-talk accessory protein calcyon to the plasma membrane. J Biol Chem 2003;278:51654–63.
175. Lidow MS, Roberts A, Zhang L, Koh PO, Lezcano N, Bergson C. Receptor crosstalk protein, calcyon, regulates affinity state of dopamine D1 receptors. Eur J Pharmacol 2001;427:187–93.
176. Novi F, Millan MJ, Corsini GU, Maggio R. Partial agonist actions of aripiprazole and the candidate antipsychotics S33592, bifeprunox, N-desmethylclozapine and preclamol at dopamine D-2L receptors are modified by co-transfection of D-3 receptors: potential role of heterodimer fort-nation. J Neurochem 2007;102:1410–24.
177. Scarselli M, Novi F, Schallmach E, et al. D2/D3 dopamine receptor heterodimers exhibit unique functional properties. J Biol Chem 2001;276:30308–14.
178. Bolan EA, Kivell B, Jaligam V, et al. D2 receptors regulate dopamine transporter function via an ERK 1/2-dependent and PI3 kinase-independent mechanism. Mol Pharmacol 2007;71:1222–32.
179. Lee F, Pei L, Moszczynska A, Vukusic B, Fletcher P, Liu F. Dopamine transporter cell surface localization facilitated by a direct interaction with the dopamine D2 receptor. The Embo J 2007;26:10.
180. Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC, Patel YC. Receptors for dopamine and somatostatin: formation of hetero-oligomers with enhanced functional activity. Science 2000;288:154–7.
181. Hillion J, Canals M, Torvinen M, et al. Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. J Biol Chem 2002;277:18091–7.
182. Kamiya T, Saitoh O, Yoshioka K, Nakata H. Oligomerization of adenosine A(2A) and dopamine D-2 receptors in living cells. Biochem Biophys Res Commun 2003;306:544–9.
183. Kearn CS, Blake-Palmer K, Daniel E, Mackie K, Glass M. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? Mol Pharmacol 2005;67:1697–704.
184. Pin JP, Galvez T, Prezeau L. Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. Pharmacol Ther 2003;98:325–54.
185. Damian M, Martin A, Mesnier D, Pin JP, Baneres JL. Asymmetric conformational changes in a GPCR dimer controlled by G-proteins. Embo J 2006;25:5693–702.
186. Hlavackova V, Goudet C, Kniazeff J, et al. Evidence for a single heptahelical domain being turned on upon activation of a dimeric GPCR. Embo J 2005;24:499–509.
187. Kniazeff J, Bessis AS, Maurel D, Ansanay H, Prezeau L, Pin JP. Closed state of both binding domains of homodimeric mGlu receptors is required for full activity. Nature Struct Mol Biol2004;11:706–13.

188. Chandrashekar J, Hoon MA, Ryba NJP, Zuker CS. The receptors and cells for mammalian taste. Nature 2006;444:288–94.
189. Springael JY, Urizar E, Costagliola S, Vassart G, Parmentier M. Allosteric properties of G protein-coupled receptor oligomers. Pharmacol Ther 2007;115:410–8.
190. El Asmar L, Springael JY, Ballet S, Andrieu EU, Vassart G, Parmentier M. Evidence for negative binding cooperativity within CCR5-CCR2b heterodimers. Mol Pharmacol 2005;67:460–9.
191. Sohy D, Parmentier M, Springael JY. Allosteric transinhibition by specific antagonists in CCR2/CXCR4 heterodimers. J Biol Chem 2007;282:30062–9.
192. Springael JY, Le Minh PN, Urizar E, Costagliola S, Vassart G, Parmentier M. Allosteric modulation of binding properties between units of chemokine receptor homo- and hetero-oligomers. Mol Pharmacol 2006;69:1652–61.
193. Waldhoer M, Fong J, Jones RM, et al. A heterodimer-selective agonist shows in vivo relevance of G protein-coupled receptor dimers. Proc Natl Acad Sci U S A 2005;102:9050–5.
194. Daniels DJ, Lenard NR, Etienne CL, Law PY, Roerig SC, Portoghese PS. Opioid-induced tolerance and dependence in mice is modulated by the distance between pharmacophores in a bivalent ligand series. Proc Natl Acad Sci U S A 2005;102:19208–13.
195. Breit A, Gagnidze K, Devi LA, Lagace M, Bouvier M. Simultaneous activation of the {delta} Opioid Receptor ({delta}OR)/Sensory Neuron-Specific Receptor-4 (SNSR-4) Hetero-Oligomer by the mixed bivalent agonist bovine adrenal medulla peptide 22 activates SNSR-4 but inhibits {delta}OR signaling. Mol Pharmacol 2006;70:686–96.
196. Park PSH, Palczewski K. Diversifying the repertoire of G protein-coupled receptors through oligomerization. Proc Natl Acad Sci U S A 2005;102:8793–4.
197. Maggio R, Vogel Z, Wess J. Coexpression studies with mutant muscarinic adrenergic-receptors provide evidence for intermolecular cross-talk between G-protein-linked receptors. Proc Natl Acad Sci U S A 1993;90:3103–7.
198. Hadac EM, Ji ZS, Pinon DI, Henne RM, Lybrand TP, Miller LJ. A peptide agonist acts by occupation of a monomeric G protein-coupled receptor: Dual sites of covalent attachment to domains near TM1 and TM7 of the same molecule make biologically significant domain-swapped dimerization unlikely. J Med Chem 1999;42:2105–11.
199. Osuga Y, Hayashi M, Kudo M, Conti M, Kobilka B, Hsueh AJW. Co-expression of defective luteinizing hormone receptor fragments partially reconstitutes ligand-induced signal generation. J Biol Chem 1997;272:25006–12.
200. Urizar E, Montanelli L, Loy T, et al. Glycoprotein hormone receptors: link between receptor homodimerization and negative cooperativity. Embo J 2005;24:1954–64.
201. Ji I, Lee C, Jeoung M, Koo Y, Sievert GA, Ji TH. Trans-activation of mutant follicle-stimulating hormone receptors selectively generates only one of two hormone signals. Mol Endocrinol 2004;18:968–78.
202. Whorton MR, Bokoch MP, Rasmussen SGF, et al. A monomeric G protein-coupled receptor isolated in a high-density lipoprotein particle efficiently activates its G protein. Proc Natl Acad Sci U S A 2007;104:7682–7.
203. Bayburt TH, Leitz AJ, Xie G, Oprian DD, Sligar SG. Transducin activation by nanoscale lipid bilayers containing one and two rhodopsins. J Biol Chem 2007;282:14875–81.
204. Ernst OP, Gramse V, Kolbe M, Hofmann KP, Heck M. Monomeric G protein-coupled receptor rhodopsin in solution activates its G protein transducin at the diffusion limit. Proc Natl Acad Sci U S A 2007;104:10859–64.
205. Galvez T. Allosteric interactions between GB1 and GB2 subunits are required for optimal GABAB receptor function. EMBO J 2001;20:2152–9.
206. Guo W, Shi L, Javitch JA. The fourth transmembrane segment forms the interface of the dopamine D2 receptor homodimer. J Biol Chem 2003;278:4385–8.
207. Salom D, Le Trong I, Pohl E, et al. Improvements in G protein-coupled receptor purification yield light stable rhodopsin crystals. J Struct Biol 2006;156:497–504.

208. Salom D, Lodowski DT, Stenkamp RE, et al. Crystal structure of a photoactivated deprotonated intermediate of rhodopsin. Proc Natl Acad Sci U S A 2006;103(44):16123–8.
209. Taylor Ds, Fung HK, Rajgaria R, et al. Mutations affecting the oligomerization interface of G–protein–coupled receptors revealed by a novel denovo protein design framework. Biophys J 2008;94:2470–81.
210. Vohra S, Chintapalli SV, Illingworth CJR, et al. Computational studies of family a and family B GPCRs. Biochem Soc Trans 2007;35:749–54.
211. Hebert TE, Moffett S, Morello JP, et al. A peptide derived from a beta(2)-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. J Biol Chem 1996;271:16384–92.
212. Harikumar K, Pinon D, Miller L. Transmembrane segment IV contributes a functionally important interface for oligomerization of the Class II G protein-coupled secretin receptor. J Biol Chem 2007;282:30363–72.
213. Gouldson PR, Reynolds CA. Simulations on dimeric peptides: evidence for domain swapping in G-protein-coupled receptors? Biochem Soc Trans 1997;25:1066–71.
214. Gouldson PR, Snell CR, Bywater RP, Higgs C, Reynolds CA. Domain swapping in G-protein coupled receptor dimers. Protein Eng 1998;11:1181–93.
215. Gouldson PR, Snell CR, Reynolds CA. A new approach to docking in the beta(2)-adrenergic receptor that exploits the domain structure of G-protein-coupled receptors. J Med Chem 1997;40:3871–86.
216. Maggio R, Vogel Z, Wess J. Reconstitution of functional muscarinic receptors by coexpression of amino-terminal and carboxyl-terminal receptor fragments. FEBS Lett 1993;319:195–200.
217. Stanasila L, Perez J-B, Vogel H, Cotecchia S. Oligomerization of the {alpha}1a- and {alpha}1b-Adrenergic Receptor Subtypes: potential implications in receptor internalization. J Biol Chem 2003;278:40239–51.
218. Hernanz-Falcon P, Rodriguez-Frade JM, Serrano A, et al. Identification of amino acid residues crucial for chemokine receptor dimerization. Nat Immunol 2004;5:216–23.
219. Klco JM, Lassere TB, Baranski TJ. C5a receptor oligomerization – I. Disulfide trapping reveals oligomers and potential contact surfaces in a G protein-coupled receptor. J Biol Chem 2003;278:35345–53.
220. Damien T, Tzvetana LMF, R. RCR. Oligomerization of the fifth transmembrane domain from the adenosine A2A receptor. Protein Sci 2005;14:2177–86.
221. Bouvier M. Oligomerization of G-protein-coupled transmitter receptors. Nat Rev Neurosci 2001;2:274–86.
222. Whorton MR, Jastrzebska B, Park PSH, et al. Efficient coupling of transducin to monomeric rhodopsin in a phospholipid bilayer. J Biol Chem 2007;282:9297–9301.
223. Ridge KD, Marino JP, Ngo T, Ramon E, Brabazon DM, Abdulaev NG. NMR analysis of rhodopsin-transducin interactions. Vision Res 2006;46:4482–92.
224. Ridge KD, Palczewski K. Visual rhodopsin sees the light: Structure and mechanism of G protein signaling. J Biol Chem 2007;282:9297–9301.
225. Brock C, Oueslati N, Soler S, Boudier L, Rondard P, Pin JP. Activation of a dimeric metabotropic glutamate receptor by intersubunit rearrangement. J Biol Chem 2007;282:33000–8.
226. Tateyama M, Abe H, Nakata H, Saito O, Kubo Y. Ligand-induced rearrangement of the dimeric metabotropic glutamate receptor 1[alpha]. Nat Struct Mol Biol 2004;11:637–42.
227. Farrens DL, Altenbach C, Yang K, Hubbell WL, Khorana HG. Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. Science 1996;274:768–70.
228. Niv MY, Filizola M. Influence of oligomerization on the dynamics of G-protein coupled receptors as assessed by normal mode analysis. Proteins 2008;71:575–86.

229. Goudet C, Kniazeff J, Hlavackova V, et al. Asymmetric functioning of dimeric metabotropic glutamate receptors disclosed by positive allosteric modulators. J Biol Chem 2005;280:24380–5.
230. Damian M, Mary S, Martin A, Pin JP, Baneres JL. G protein activation by the leukotriene B-4 receptor dimer – Evidence for an absence of trans-activation. J Biol Chem 2008;283:21084–92.
231. Kniazeff J, Galvez T, Labesse G, Pin JP. No ligand binding in the GB2 subunit of the GABAB receptor is required for activation and allosteric interaction between the subunits. J Neurosci 2002;22:7352–61.
232. Xu H, Staszewski L, Tang H, Adler E, Zoller M, Li X. Different functional roles of T1R subunits in the heteromeric taste receptors. Proc Natl Acad Sci USA 2004;101:6.
233. Filipek S, Krzysko KA, Fotiadis D, et al. A concept for G protein activation by G protein-coupled receptor dimers: the transducin/rhodopsin interface. Photochem Photobiol Sci 2004;3:628–38.
234. Filipek S, Stenkamp RE, Teller DC, Palczewski K. G protein-coupled receptor rhodopsin: a prospectus. Ann Rev Physiol 2003;65:851–79.
235. Jastrzebska B, Fotiadis D, Jang GF, Stenkamp RE, Engel A, Palczewski K. Functional and structural characterization of rhodopsin oligomers. J Biol Chem 2006;281:11917–22.
236. Hamm HE. How activated receptors couple to G proteins. Proc Natl Acad Sci U S A 2001;98:4819–21.
237. Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB. The 2.0 A crystal structure of a heterotrimeric G protein. Nature 1996;379:311–9.
238. Sondek J, Bohm A, Lambright DG, Hamm HE, Sigler PB. Crystal structure of a G-protein beta gamma dimer at 2.1A resolution. Nature 1996;379:369–74.
239. Willardson BM, Pou B, Yoshida T, Bitensky MW. Cooperative binding of the retinal rod G-protein, transducin, to light-activated rhodopsin. J Biol Chem 1993;268:6371–82.
240. Terrillon S, Durroux T, Mouillac B, et al. Oxytocin and vasopressin V1a and V2 receptors form constitutive homo- and heterodimers during biosynthesis. Mol Endocrinol 2003;17:677–91.
241. Terrillon S, Bouvier M. Roles of G-protein-coupled receptor dimerization. EMBO Rep 2004;5:30–4.
242. Banères J-L, Parello J. Structure-based analysis of GPCR function: Evidence for a novel pentameric assembly between the dimeric leukotriene B4 receptor BLT1 and the G-protein. J Mol Biol 2003;329:815–29.
243. Senogles SE, Heimert TL, Odife ER, Quasney MW. A region of the third intracellular loop of the short form of the D2 dopamine receptor dictates Gi coupling specificity. J Biol Chem 2004;279:1601–6.
244. Voss T, Wallner E, Czernilofsky AP, Freissmuth M. Amphipathic alpha-helical structure does not predict the ability of receptor-derived synthetic peptides to interact with guanine nucleotide-binding regulatory proteins. J Biol Chem 1993;268:4637–42.
245. Nanoff C, Koppensteiner R, Yang Q, Fuerst E, Ahorn H, Freissmuth M. The carboxyl terminus of the Galpha-subunit is the latch for triggered activation of heterotrimeric G proteins. Mol Pharmacol 2006;69:397–405.
246. Johnston CA, Siderovski DP. Structural basis for nucleotide exchange on G alpha i subunits and receptor coupling specificity. Proc Natl Acad Sci U S A 2007;104:2001–6.
247. Franco R, Casado V, Cortes A, et al. Basic concepts in G-protein-coupled receptor homo- and heterodimerization. Sci World J 2007;7:48–57.
248. Franco R, Ciruela F, Casado V, et al. Partners for adenosine A1 receptors. J Mol Neurosci: MN 2005;26:221–32.
249. Springael JY, de Poorter C, Deupi X, Van Durme J, Pardo L, Parmentier M. The activation mechanism of chemokine receptor CCR5 involves common structural changes but a different network of interhelical interactions relative to rhodopsin. Cell Signal 2007;19:1446–56.

250. Kenakin T. Efficacy at G-protein-coupled receptors. Nat Rev Drug Discov 2002;1:103–10.
251. Milligan G, Smith NJ. Allosteric modulation of heterodimeric G-protein-coupled receptors. Trends Pharmacol Sci 2007;28:615–20.
252. Jacoby E, Bouhelal R, Gerspacher M, Seuwen K. The 7Â TM G-protein-coupled receptor target family. Chem Med Chem 2006;1:760–82.
253. Pidasheva S, Grant M, Canaff L, Ercan O, Kumar U, Hendy GN. Calcium-sensing receptor dimerizes in the endoplasmic reticulum: biochemical and biophysical characterization of CASR mutants retained intracellularly. Human Mol Gen 2006;15:2200–9.
254. Calebiro D, de Filippis T, Lucchi S, et al. Intracellular entrapment of wild-type TSH receptor by oligomerization with mutants linked to dominant TSH resistance. Human Mol Gen 2005;14:2991–3002.
255. McElvaine AT, Mayo KE. A dominant-negative human growth hormone-releasing hormone (GHRH) receptor splice variant inhibits GHRH binding. Endocrinology 2006;147:1884–94.