The asymmetric/symmetric activation of GPCR dimers as a possible mechanistic rationale for multiple signalling pathways

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G protein-coupled receptors (GPCRs) represent the major target for drug development. Although these receptors can activate their cognate G-proteins in a monomeric form, it is now recognized that they can assemble into dimers, or larger oligomers. However, the functional consequences of such receptor assembly remain elusive. Recent data revealed an ‘asymmetric’ organization of some dimers when activating heterotrimeric G-proteins, while a symmetric organization may be required for the activation of other signalling pathways. Here we describe a mathematical model taking these latest ideas into consideration, and report on the expected consequences of the activation of different signalling pathways. This model helps clarify data already published, and will certainly be helpful to further explain the functional significance of GPCR oligomerization. It may help develop more specific drugs targeting a specific signalling cascade.

Introduction

G protein-coupled receptors (GPCRs) are key components of the signal transduction machinery. They regulate the function of most cells in the body, and account currently for about three per cent of the genes present in a human genome. These receptors respond to a wide variety of structurally diverse ligands, ranging from small molecules, such as biogenic amines, nucleotides and ions, to lipids, peptides, proteins, and even light. Ligands acting on GPCRs are commonly used in drug therapy for numerous diseases. It is estimated that these receptors are targets for approximately half of clinically used drugs [1].

GPCRs, as indicated by their name, signal through their interaction and subsequent activation of G proteins [2]. However, the functioning of these receptors appears more complex than was initially thought and additional accessory proteins play a role in the signal transduction concert. Proteins other than G proteins reported to interact with GPCRs and potentially responsible for G protein-independent GPCR signalling include β-arrestins, tyrosine kinases and PDZ-domain containing proteins (see [3] for review).

β-arrestin proteins act not only by binding to phosphorylated receptors, inhibiting G protein coupling and leading to receptor desensitization, but also by mediating G protein-independent GPCR signalling through various effector pathways such as MAP kinases. Recently, a differential kinetic pattern of β-arrestin and G protein mediated activation was found for the angiotensin II (AngII) receptor [4]. The results were consistent with a slow and prolonged β-arrestin2-mediated ERK1/2 activation stimulated by AngII as compared with the immediate, but transient, ERK1/2 G protein-dependent activation. The authors proposed that β-arrestin2 functions both as a signal terminator and transducer. Binding of β-arrestin2 to the activated receptor finishes G protein-dependent signalling and initiates β-arrestin2-mediated signalling [4].

A dosage-dependent switch from G protein-coupled to G protein-independent signalling was found for β2-adrenoceptors (β2-ARs) [5]. At low agonist concentrations, β2-ARs signal through Ga13 to activate the mitogen-activated

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**Glossary**

- **Dose-dependent switch**: This term refers to the ability of a receptor to signal through two pathways with agonist dosage acting as a switch.
- **Functional selectivity and Biased agonism**: Both terms are related and define the ability of some ligands differentially to activate the various signalling pathways associated with one receptor type.
- **Inverse agonist**: A ligand that reduces the receptor constitutive activity upon binding.
- **Full and partial agonist**: A ligand that upon binding to the receptor fully or partially allows the receptor to elicit its maximum response.
- **Receptor dimerization/oligomerization**: Association of two or more receptor molecules, respectively. Each receptor unit in the dimer/oligomer ensemble is called a protomer.
- **Asymmetric and symmetric active dimer state**: Structural arrangement of a receptor dimer in which either only one or both protomers are activated, respectively.
- **Binding cooperativity**: The increase or decrease in the affinity of a ligand for a receptor dimer binding site given that the other is occupied. Depending on whether the affinity increases or decreases, cooperativity is termed positive or negative, respectively.
- **Functional or induction cooperativity**: The increase or decrease in the propensity of a protomer in a receptor dimer to become active given that the other is already active. Depending on whether the propensity for activation increases or decreases, cooperativity is termed positive or negative.

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protein kinase pathway, whereas at high agonist concentrations signals are also transduced through β2-ARs via an additional pathway that is G protein-independent but tyrosine kinase Src-dependent [5]. The authors speculated that receptor dimerization or a conformational change associated to receptor dimerization could provide the structural mechanism features necessary for the direct activation of Src by β2-AR by bringing two molecules of Src into proximity and allowing them intermolecular autophosphorylation and activation [5].

Although some controversy is present in the literature about the monomeric/dimeric nature of GPCRs [6–8] especially when considering recent results confirming that a monomeric receptor is sufficient to activate G proteins [9–12], there is growing evidence to indicate that GPCRs form dimers or even higher oligomers [13–17]. A number of arguments has been used to explain the reasons for or the processes involved in receptor oligomerization, among them the correct addressing of the receptor to the membrane, the interaction with the G protein, and receptor internalization (for review see [18]). In this regard, it has been proved recently that high-order oligomerization of the α1B-adrenoceptor is required for receptor maturation, surface expression, and function [19] and that homodimerization occurs early in the biosynthetic process in the endoplasmic reticulum as shown by site-directed mutagenesis on β1-adrenoceptors [20].

Mathematical models of GPCR function in which oligomerization is a condition can be found elsewhere [21–27]. In these models, the oligomeric receptor contains either one or two (one inactive and the other active) states. A single state for the oligomeric receptor is suitable for binding studies whereas a double state allows for functional studies by assigning the functional response to the active state. More recently, a three-state dimer receptor model containing one inactive and two active receptor states, one associated to inositol phosphate accumulation and the other to arachidonic acid release pathways, was proposed for the 5-HT2A receptor in order to explain the selectivity shown by some antagonists [28]. In all these models, however, the receptor was considered as a global entity and no differences between the protomers within a particular state were proposed. For instance, the two protomers within each of the active dimer states, designated as (RR)* and (RR)**, in the three-state dimer receptor model [28] were considered as identical units.

The equivalence between protomers for GPCR dimer models needs to be revised since it appears that, at least in some receptors, some complexity within the active receptor dimer is present. Thus, recent data on the BLT1 receptor suggest that the two subunits of the receptor in the G protein-coupled state differ in their conformation [29]. The authors speculated that whereas activation of the G protein is associated with an asymmetry of the receptor dimer, a symmetric dimer might be associated with arrestin in subsequent signalling steps. Further evidence for the concept of an asymmetric active state was obtained from nanodiscs having two rhodopsins, where it was found that only one of the receptors can interact with G protein [11]. An asymmetric geometry of the two protomers constituting a dimeric 5-HT2C receptor has also been proposed for the receptor interaction with its cognate Gα protein [30]. Asymmetric protomer arrangement for the dimer active state has also been observed for class C GPCRs both for the heterodimeric GABAB [31] and T1R taste [32] receptors and for the homodimeric metabotropic glutamate receptors (mGluRs). For the latter receptors it has been observed that, although two homologous heptahelical domains (HDs) are present in the homodimer, a single HD is turned on upon activation [33]. This asymmetric arrangement for mGluR active state was confirmed by the action of positive allosteric modulators (PAMs), for which it was found that one PAM per dimer was sufficient to enhance receptor activity [34].

A recent article on dopamine class A dimers [35] has provided new insights on the crosstalk between protomers for GPCR G protein-dependent signalling. The authors found that maximal functional response was achieved by agonist binding to a single protomer, consistent with the hypothesis of an asymmetrically activated dimer. Interestingly, results other than those expected from traditional receptor theory resulted from agonist and inverse agonist binding to the second protomer; whereas agonist binding to the second protomer blunted signalling, inverse agonist binding enhanced signalling.

With the aim of describing quantitatively the apparent asymmetry of the G protein-bound state of some GPCRs when present in a dimeric form, a three-state dimer receptor model was developed in the present article. The model includes one inactive (RR) and two active

\[
\begin{align*}
R & \xrightarrow{2X_1} Y_2 \\
K_1 & \xrightarrow{2} R'R \\
Y_2 & \xrightarrow{2K_2} R_AR_A \\
Y_5 & \xrightarrow{2Y_6} R_AR_A \\
Y_6 & \xrightarrow{2} R_AR_A \\
\end{align*}
\]

Figure 1. The asymmetric/symmetric three-state dimer model. The model contains one inactive state (RR) and two active states, one defining the G protein-mediated pathway (the asymmetric R* state) and the other the G protein-independent pathway (the symmetric R*R state). The equilibrium constants are defined as

- \( K = \frac{[R'R]}{[R]_2} \times 2 \\
- \( K_1 = \frac{[R']_2}{[R]_2} \times 2 \\
- \( K_2 = \frac{[R_AR_A]}{[R]_2[AR]} \times 2 \\
- \( K_3 = \frac{[Y_4]}{[Y_2][Y_2]} \times 2 \\
- \( K_4 = \frac{[Y_3]}{[Y_2][Y_2]} \times 2 \\
- \( K_5 = \frac{[Y_6]}{[Y_6][Y_6]} \times 2 \\
- \( Y_4 = \frac{[Y_4]}{[Y_2][Y_2]} \times 2 \\
- \( Y_5 = \frac{[Y_5]}{[Y_2][Y_2]} \times 2 \\
- \( Y_6 = \frac{[Y_6]}{[Y_6][Y_6]} \times 2 \\

The activation of a single protomer by one ligand can be done either by a cis- or a trans-activation mechanism, regulated by \( Y_2 \) and \( Y_1 \) equilibrium constants, respectively. It is worth noting that this model is formally equivalent to the VFT domain dimer model, with RR corresponding to VFTon and R* to VFToff [36].
(the asymmetric R*R and the symmetric R*R*) receptor states. The model examined the possible signalling properties of such dimers when assuming that the asymmetric dimer R*R signals through a G protein-dependent pathway while the symmetric R*R signals through a β-arrestin-, a Src- or any other accessory protein-dependent pathway. However, the model can well accommodate any pathways as soon as one is activated when a single subunit is in the active form in a dimer, while the other is activated when both subunits are active.

The asymmetry of the HD active state was incorporated recently in an mGluR model [36] with the purpose of accounting for the function of allosteric modulators in subsequent studies. Here, the model includes both the asymmetric and the symmetric states to analyze the mutual influence between the G protein-dependent and the G protein-independent states, as possible examples of what could be the consequences of two signalling pathways differently activated whether one or both subunits in GPCR dimer are activated.

The asymmetric/symmetric three-state dimer model

Figure 1 shows the asymmetric/symmetric three-state dimer model, in which three states, one inactive and two active, are considered. The active states differentiate themselves by the asymmetric or symmetric array of the protomers within the dimer, with either one (R*R) or both (R*R) of the protomers being active. In agreement with experimental results [11,29–35], the asymmetric active state was assigned to a G protein-mediated signalling pathway and here it is assumed that the symmetric active state is responsible for the β-arrestin, the Src- or any other accessory protein-dependent pathway present in the system. The model was constructed under an induction-based mechanism, in which the binding of the agonist to the inactive RR state induces the activation of either one or both of the protomers within the dimer.

It is worth noting that the asymmetric/symmetric three-state dimer model is formally equivalent to the recently calculated as the left and right asymptotes of fR,R, respectively (Equation 2 and 3).

\[ f_{R,R} = \frac{[R'R] + [R'AR] + [R'AR'] + [RR]RA}{[R]} = 2 \left( c_1 + c_2 |A| + c_3 |A|^2 \right) \]

where

\[ c_1 = K_{1}K_{X}X_{1} \]
\[ c_2 = K_{2}(Y_{1} + Y_{2}) \]
\[ c_3 = Y_{S} \]
\[ c_4 = K_{3}(1 + 2X_{1} + X_{1}X_{2}) \]
\[ c_5 = 2K_{2}(1 + Y_{1} + Y_{2} + Y_{2}Y_{3}) \]
\[ c_6 = 1 + 2Y_{S} + Y_{S}Y_{6} \]
\[ a_{1} = c_{1}, \text{ for } i = 1 \text{ to } 6 \]

using the equilibrium constants depicted in Figure 1. It is noteworthy that Equation 1, expressed as a function of the empirical \( a_{i} \) parameters, is identical to that obtained in the mGluR model [36] and in the two-state dimer receptor model [21,22]. However, the mechanistic interpretation of the parameter values differs.

**Box 1. The fractional functional response through the G protein-mediated signalling pathway**

The fractional functional response through the G protein-mediated signalling pathway results from considering as active those receptor species in which only one of the protomers is activated.

\[ f_{R,R} = \frac{[R'R] + [R'AR] + [R'AR'] + [RR]RA}{[R]} = 2 \left( c_1 + c_2 |A| + c_3 |A|^2 \right) \]

where

\[ c_1 = K_{1}K_{X}X_{1} \]
\[ c_2 = K_{2}(Y_{1} + Y_{2}) \]
\[ c_3 = Y_{S} \]
\[ c_4 = K_{3}(1 + 2X_{1} + X_{1}X_{2}) \]
\[ c_5 = 2K_{2}(1 + Y_{1} + Y_{2} + Y_{2}Y_{3}) \]
\[ c_6 = 1 + 2Y_{S} + Y_{S}Y_{6} \]
\[ a_{1} = c_{1}, \text{ for } i = 1 \text{ to } 6 \]

The potentality (\( A_{50} \)) of the agonist is calculated as \( [A] \) for Response = \( L + \frac{A_{50}}{2a} \) (Equation 4).

\[ A_{50} = \frac{b + \sqrt{b^2 - 4ac}}{2a} \]

where \( a = a_1 - a_2a_6, b = a_3a_6a_5 - 2a_2a_4 + a_1a_4, \) and \( c = -a_1a_2a_3, \) and the ± sign in Equation 4 results for the possibility of \( A \) being either a positive or an inverse agonist.

The sensitivity of the receptor to an increment in the agonist concentration is measured by the first derivative of the receptor function (Equation 5).

\[ \frac{df_{R,R}}{dx} = \frac{-2(a_4 + a_5a_6 + 2a_1a_2a_3^2)}{(a_4 + a_5a_6 + 2a_1a_2a_3^2)^2} \]

The Hill coefficient at the midpoint

\[ n_{H} = \frac{4}{(R-1)(\frac{df_{R,R}}{dx})_{x=0}} \]

can be calculated from Equation 5 [47,48].
proposed model for the Venus Flytrap (VFT) domain dimer of mGluRs [36], with RR = open-open (OO), R*R = closed-open (CO), and R*R* = closed-closed (CC) states. The equations for the binding through both models are the same; however, marked differences appear for the function. In the VFT domain model, the functional response results from the sum of both the CO and CC states, with the former providing partial and the latter full agonism; the asymmetric/symmetric three-state dimer model proposes that each of the two active states corresponds to a specific signalling pathway. Because the binding to the VFT domain was thoroughly analyzed in our previous work [36], it will be omitted here, and we will focus our attention on the functional features of the new model.

The fractional functional response: two pathways in competition
The calculation of the fractional functional response for a receptor system depends on the receptor species assumed to be active. The model depicted in Figure 1 was designed on the basis of maximal simplicity, and only two active species, one for each of two signalling pathways, were included. Interestingly, the model incorporates some structural characteristics linked to the mechanism of receptor activation making the active states of the model more than mere receptor conformations; that is, for the G protein-dependent pathway, one single protomer of the dimer array is activated, whereas for the G protein-independent pathway both protomers are activated. Although the model is based on thermodynamic premises (only equilibrium processes are included and the system considered has reached chemical equilibrium), it contains particular time-dependent molecular characteristics, as the G protein-dependent receptor conformation must be attained prior to the G protein-independent receptor conformation, if we accept that the two protomers within the dimer cannot be activated simultaneously. It is worth noting that this would be true for a system in which the active (R*R) cannot be activated simultaneously. It is worth noting that this would be true for a system in which the active (R*R) cannot be activated simultaneously.

Under these conditions, a bell-shaped curve is obtained for the R*R-mediated G protein-dependent pathway, whereas a sigmoid curve with a maximum value of unity is yielded for the R*R*-mediated G protein-independent pathway. The graphs also show that the G protein response appears prior to the G protein-independent function although it vanishes rapidly as the concentration of the agonist increases, because in the simulation the agonist was assigned a greater preference for the doubly than for the singly activated states. This proposal is consistent with results on β2-AR showing an agonist dosage-dependent switch from G protein-coupled to G protein-independent signalling [5].

Figure 3 shows the functional profiles (G protein-dependent: panel A and G protein-independent: panel B) for a set of agonists characterized by their different propensity for partial and full agonism; the former providing partial and the latter full agonism; the asymmetric/symmetric three-state dimer model proposes that each of the two active states corresponds to a specific signalling pathway. Because the binding to the VFT domain was thoroughly analyzed in our previous work [36], it will be omitted here, and we will focus our attention on the functional features of the new model.

Box 2. The fractional functional response through the G protein-independent signalling pathway

The fractional functional response through the G protein-independent signalling pathway was considered by including as active those receptor species in which both of the protomers are activated.

\[
f_{R*R*} = \frac{[R*R*]}{[R_1] + [R_2] + [R*R*] + [R_1R_2] + [R_1R_2]} = \frac{d_1 + d_2[A] + d_3[A]^2}{d_4 + d_5[A] + d_6[A]^2}
\]

where

\[
[R_1] = [RR] + [R*R] + [R*R*] + [R_1R_2] + [R_1R_2] + [R_1R_2] + [R_2R_2]
\]

\[
d_1 = K_1K_2X_1X_2
\]

\[
d_2 = 2K_1Y_1Y_2
\]

\[
d_3 = Y_6
\]

\[
d_4 = 4K_1(1 + Y_1 + Y_2 + Y_1Y_2)
\]

\[
d_5 = 2(1 + 2Y_5 + Y_6)
\]

\[
d_6 = \frac{d_1}{d_6}
\]

using the equilibrium constants depicted in Figure 1. Analogously to the G protein-dependent pathway, and using the transformation \( x = \log[A] \), the theoretical basal and maximum or minimum responses were calculated (Equation 7). The activation of a protomer facilitates the activation of the second protomer if the latter is occupied by an agonist (\( Y_6 > Y_9 \) and \( Y_6 > Y_9 \)); this proposal resembles the finding in the VFT domain of mGluRs, for which the closure of a subunit facilitates the closure of the second subunit if the latter is occupied by an agonist [38].

(d) The activation of a protomer facilitates the activation of the second protomer if the latter is occupied by an agonist (\( Y_6 > Y_9 \) and \( Y_6 > Y_9 \)); this proposal resembles the finding in the VFT domain of mGluRs, for which the closure of a subunit facilitates the closure of the second subunit if the latter is occupied by an agonist [38].

As the empirical equation for \( f_{R*R*} \) is the same as that for \( f_{R*R} \), the potency (\( A_{50} \)) and the sensitivity of the receptor to an increment in the agonist concentration are also the same as those in Equations 4 and 5. The same occurs for the Hill coefficient at the mid-point (Box 1).
In this study, the asymmetric/symmetric three state dimer model was presented. The model consists of three states, one inactive (RR) and two active states, one with an asymmetric arrangement of the protomers (R*R) and the other with a symmetric disposition (R*R*). Experimental results suggest that the asymmetric geometry of the activated dimer could be associated with a G protein-dependent pathway. Here, the symmetric organization of the protomers is proposed for β-arrestin-, Src- or any other accessory protein-dependent pathway. More generally, the model can accommodate two major signalling pathways arising from either an asymmetric or symmetric arrangement of the protomers within the activated dimer. Because each of these constructions can contain multiple conformational states, multiple secondary pathways could be incorporated into the model. To avoid further complexity in the equations for functional response, the G protein component was not included explicitly in the model. Because of this, the protomer R* in the asymmetric R*R dimer model represents the G protein-bound high affinity state of the receptor [39]. The question arises as to whether the R* conformation of the active protomer in the asymmetric R*R and of either of the protomers in the symmetric R*R* is the same. If this is the case, an additional argument such as steric hindrance within the R*R* molecular structure would be needed in order to explain why the G protein can bind to R*R and not to R*R* since, as has been shown by Sunahara et al. in different studies, a monomeric receptor can activate G proteins [40–42].

The model was shown to be competent for explaining some relevant experimental results. For instance, the dosage-dependent switch from G protein-coupled to G protein-independent signalling [5] can be described by our model under an induction approach in which the symmetric (R*R*) active dimer - able to bind two agonist molecules and associated with the G protein-independent pathway - is formed from the asymmetric (R*R) active dimer - able to bind a single agonist molecule and associated with the G protein-dependent pathway. Also, it might be worth mentioning that, in many instances, bell-shaped curves have been reported for G protein-mediated events. Although this might not be the unique explanation, at least this model proposes one possible explanation (the competing pathway associated to the double-activated dimer) for this phenomenon. The novel proposal that inclusion of inverse agonist favours whereas addition of additional agonist disfavours G protein-dependent dopamine D2 signal can be accounted for by our model, by assuming that the inverse agonist increases the signal (supposed to arise from the asymmetric R*R active state) by binding to the R protomer of the R*-agonist occupied dimer, whereas addition of higher agonist concentration decreases the R*R signal by inducing the formation of the symmetric R*R*, which yields no signal on the former G protein-dependent pathway.

The model can explain the signalling selectivity observed with different ligands even when used at high concentrations because, as it is shown in the lower row of Figure 1, both the G protein-dependent and the G protein-independent pathways can be activated from fully occupied receptor dimers, R*A and R*A, respectively. In general, both pathways can be activated simultaneously with the relative contribution of each being dependent on the full set of ligand-receptor equilibrium constants. The case of ligands that specifically trigger G protein-dependent pathways is explained by the model assuming that these
ligands have the ability to promote the activation of one of the protomers of the dimer but show negative functional cooperativity for the induction of the activation of the second protomer. Within the model (Figure 1) we distinguish between binding cooperativity for the inactive RR state (the binding of a ligand to one of the protomers of RR facilitates or hampers the binding of a second ligand to the same receptor state; left column) and induction or functional cooperativity (the activation of a protomer facilitates or hampers the activation of the second; the connection between the middle and right columns), the latter cooperativity being responsible for the pre-eminence of one signalling pathway over the other (see [36] for a discussion on binding and functional cooperativity effects). Nevertheless, a word of caution is needed, and although there are a number of examples where an asymmetric GPCR is involved in G protein-mediated effects, this cannot be taken as a generalized trait and the possibility that a symmetrically activated dimer may also activate G proteins should be borne in mind. Indeed, GPCR functioning is much more complicated than our model proposes and multiple conformational states are probably included in each of the major asymmetric and symmetric signalling pathways. However, our goal is to offer the simplest model possible that can take into consideration the relatively new notion that symmetry and asymmetry in GPCR dimers is probably responsible for some of the observed effects.

The model used allows also for mechanistic speculations concerning the reasons why nature uses dimeric receptors when it has been shown that monomeric receptors are equally capable of binding and function through a G protein-dependent pathway [9–12]. A plausible reason may be that, as the model has shown, a dimer receptor contains, despite its simplicity, key structural features for initiating a G protein-dependent pathway, and either terminating it when there is an excess concentration of agonist or providing alternative signalling pathways.

It is worth noting that the model presented was designed within a signalling framework. As a proposal, G protein-independent GPCR signalling involving β-arrestins and other accessory proteins was associated with the symmetrically activated receptor dimer. However, some receptors such as the vasopressins have been observed to demonstrate that activation of only one protomer in a receptor heterodimer is sufficient to promote arrestin-mediated internalization of the complex [43]. In this regard, the model does not exclude the possibility that arrestin recruitment for trafficking and signalling follows different trends in terms of symmetry of receptor activation and, thus, does not rule out the possibility that arrestin recruitment for trafficking depends on the asymmetric dimer.

Finally, a comment on the relationship between receptor dimerization, functional selectivity and drug discovery. It has been shown for the B2-AR, and this is probably also true for other GPCRs, that, although most agonists display similar efficacies for both G protein-dependent and β-arrestin-dependent pathways, there are some ligands exhibiting a β-arrestin-biased agonism [44]. The concept of ligand bias agonism (see [45] for discussion) has also been used for the β-arrestin-independent Src phosphorylation of μ-receptor [46]. Including β arrestin- and Src-biased agonists in the drug discovery synthetic efforts opens new avenues for the therapeutic potential of GPCR targets enlarging the structure-activity ligand space. Moreover, the asymmetric nature of the G protein activating-R*R receptor state is particularly attractive to medicinal chemists as it could be triggered both by agonists (binding to the R* protomer) and inverse agonists (binding to the R protomer); this is a complex process that depends on the relative affinity of the ligands for the collection of receptor states (R protomers are present in the inactive RR dimer and in the G protein-dependent R*R dimer whereas R* protomers are present in the G protein-dependent R*R dimer and form the G protein-independent R*R dimer), the concentration of the ligands, and the relative abundance of the receptor states, where the last-mentioned parameter value can be altered in pathological conditions. In addition, there is a growing interest in the design of dimeric ligands. The model developed here can account for this issue by implicitly suggesting three types of dimeric ligands, namely, antagonist-antagonist, agonist-antagonist and agonist-agonist, depending on the receptor state (RR, R*R and R*R*, respectively) for which the ligands are designed. To this end and more generally, the model may provide not only qualitative but also quantitative relationships to help in the discovery of new drugs and the characterization of their interactions with the receptor.

Conflicts of interests
The authors declare there are no conflicts of interest.

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