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Quantifying the allosteric interactions within a G-protein-coupled receptor heterodimer

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G-protein-coupled receptors are central to signal transduction and cell communication. The possibility that cells use receptor heteromerization to modulate individual receptor pathways is a surmise that cannot be precluded. Given the complexity of these processes, mathematical models contribute to understanding how receptors and their respective ligands regulate signaling. Here, a mathematical model is presented that quantifies the allosteric interactions within a receptor heterodimer. The model is based on the operational model of allosterism including constitutive receptor activity, which provides the pharmacological analysis of heteromerization with well-established and widely used modeling and fitting procedures.

Introduction

G-protein-coupled receptors (GPCRs) are a superfamily of membrane receptors that mediate multiple signaling pathways in living organisms. They exist in the cell membrane and connect the signals outside the cell with the change in biological processes inside the cell. Owing to the involvement of these proteins in many diseases, there has been much research on the mechanisms underlying GPCR function and on drugs targeting GPCRs [1]. However, it remains unclear how drugs impact the receptors and thus cause their functional effects.

It was traditionally thought that GPCRs act as monomers, but now increasing evidence shows that they can interact with each other to form dimers and higher-order oligomers [2]. Heteromerization (i.e., the physical combination of different receptor proteins into a new receptor entity) establishes the foundation for direct crosstalk between signaling pathways respectively mediated by these proteins. In this manner, one single ligand can induce alterations in various cellular processes. GPCR heteromerization has been postulated for a wide range of receptors [3–6] and is thought to be related to various neurologic and neuropsychiatric disorders [2,7] including schizophrenia [8], tardive dyskinesia [9] and opioid use disorders [10], among others. Therefore, developing new treatments for these conditions would require a thorough understanding of heteromerization. Moreover, heteromerization has the potential to be exploited for the development of more potent therapies with fewer side effects by utilizing synergistic drug combinations. Finally, although heteromerization enables the cell to make full use of GPCR signaling, the complexity in data analysis poses a great challenge to the scientific research into GPCR function and drug development.

Mathematical modeling is more than just an alternative approach to understanding GPCR signaling and drug effects. Mathematical models quantify the GPCR system by offering a platform for numerical simulation of the interaction between receptors and ligands. In doing so, they can provide a quantitative description of binding and function, as well as cooperativity factors between ligands. Existing mathematical models focus on the simulation of a single GPCR or its homomers, but scarcely address the issue of heteromerization. Therefore, a new model for GPCR heteromers is needed to quantitatively describe the influence of heteromerization on drug effects. Given that there are some features shared by GPCR monomers, homomers and

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heteromers (such as allosteric interactions), it can be helpful to learn from previous mathematical models when constructing a new heteromer model.

There are several mathematical models available to formulate how functional effects change with drug concentration [11–13]. In regard to this work, the operational models of agonism and allosterism [14–17] are of particular interest. Previously, our group has significantly contributed to the development of mathematical models for homomers [18–21] and the analysis of operational models of agonism [22–24]. Here, taking advantage of previous models, especially the operational models of receptor activation including constitutive receptor activity formulated by David Hall [16,17], we develop a model for receptor heteromerization. The translation of the operational parameters for allosteric cooperativity in a monomer to the crosstalk between protomers in a heterodimer brings the utility of the pharmacological concepts present in the operational models to the heterodimer model and facilitates the application of widely used modeling and fitting procedures.

The model we present here is restricted to receptor heterodimers. Thus, it perfectly fits mGlu class C GPCRs, which are known to form strict dimers [25,26]. For class A GPCRs, a higher level of complexity is found in a number of cases and different equilibria between oligomers of varying degree of oligomerization have been described [2]. We view the present work as a first step in the mathematical modeling of receptor heteromerization by analyzing the simplest situation: a strict receptor heterodimer. This analysis sets up the basis for future work, which will include higher order hetero-oligomerization.

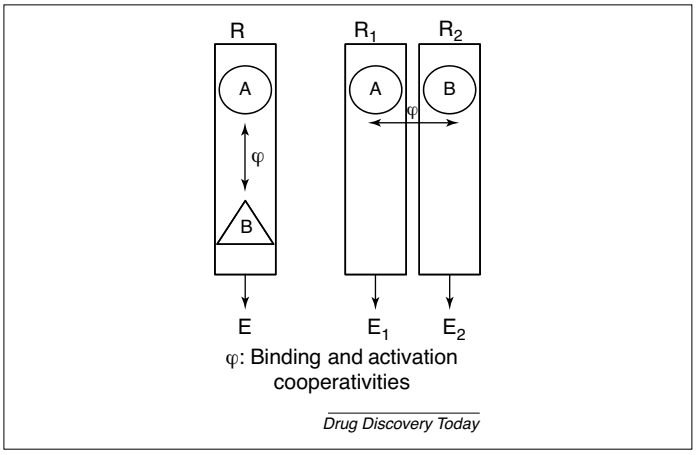

FIGURE 1

Qualitative scheme showing the correspondence of binding and activation cooperativities (φ) between the orthosteric and allosteric sites in a monomer, R (left), and the orthosteric sites of the protomers in a heterodimer, R_1R_2 (right). In the monomer, ligands A and B modulate each other to yield a receptor effect, E. In the heteromer, two receptor effects (E_1 and E_2) associated to their respective protomers (R_1 and R_2) are separately produced.

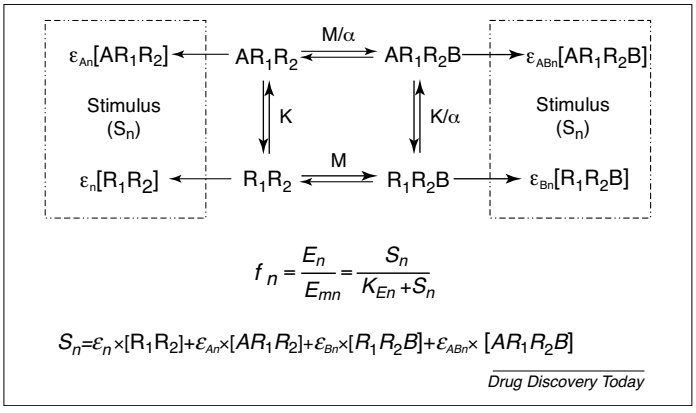

FIGURE 2

An operational model for a receptor heterodimer. Four receptor species (free, singly bound and doubly bound receptor molecules) are at equilibrium. Each of these receptor species has the ability to contribute to pathway stimulation. A rectangular hyperbolic function for the fractional effect on pathway n ($f_n = E_n/E_{mn}$) is proposed, with E_n being the effect, E_{mn} the maximum possible effect in the system, S_n the stimulus, and K_{En} the value of S_n for $E_n/E_{mn} = 1/2$. See main text and Table 1 for further description of the parameters.

An operational model for the crosstalk between protomers in a receptor heterodimer

Figure 1 visualizes how the allosteric interactions between the orthosteric and allosteric sites in a monomer can qualitatively correspond to those between the orthosteric sites in a heterodimer. The quantitative formulation of this suggested correspondence is outlined in Fig. 2. Figure 2 presents a mathematical model for a heterodimer consisting of two different receptors R_1 and R_2. R_1 and R_2 separately mediate pathway 1 and pathway 2. A and B are the ligands for R_1 and R_2, respectively. Owing to the conceptual correspondence between allosteric interactions in a monomer and in a heteromer shown in Fig. 1, the rationale used in [17,22] for the development of an operational model of allosterism for a receptor with constitutive activity was used here for a heterodimer.

The parameters present in Fig. 2 have the following definitions (Table 1): K and M are the dissociation constants for the binding of ligands A and B to protomers R_1 and R_2, respectively. α represents the binding cooperativity between the two ligands in their corresponding receptors. n (1 or 2) is used to distinguish between the two pathways. f_n denotes the fractional response of pathway n. S_n is the stimulus for functional response of pathway n. A rectangular hyperbolic function transduces stimulus S_n into fractional response f_n. E_n represents the produced effect for pathway n and E_{mn} denotes the maximum possible effect of the system for pathway n. K_{En} is the value of S_n for half of E_{mn}, therefore it measures the efficiency of transducing stimulus into fractional response. There is a difference in the number of parameters between the present model and its parent formulation [17,22], originally designed to account for the allosteric interactions between two ligands in a single receptor (Fig. 1, left). The term ε_n, with n equal to 1 (for pathway 1) or 2 (for pathway 2), is used here to define the ability of the free R_1R_2 receptor to generate the functional response. In the original formulation a value of 1 was assumed for ε. Here, because of the possibility of two pathways, the term ε_n needs to be included.

TABLE 1

Definitions of parameters included in Eq. (1)

K	Dissociation equilibrium constant for the binding of ligand A
M	Dissociation equilibrium constant for the binding of ligand B
α	Binding cooperativity between ligands A and B
ε_n	Ability of R_1R_2 molecular entity to activate pathway n
ε_{An}	Ability of AR_1R_2 molecular entity to activate pathway n
ε_{Bn}	Ability of R_1R_2B molecular entity to activate pathway n
δ_n	A measure of the functional interactions between ligands A and B for pathway n. δ_n is included in the definition of $\varepsilon_{ABn} = \varepsilon_{An}\varepsilon_{Bn}\delta_n$, where ε_{ABn} is the ability of AR_1R_2B molecular entity to activate pathway n
χ_n	$\chi_n = [R_1R_2]_T/K_{En}$, with K_{En} defined in $f_n = \frac{E_n}{E_{mn}} = \frac{S_n}{K_{En}+S_n}$, where E_n is the effect, E_{mn} is the maximum possible effect and S_n is the stimulus for pathway n

For pathway n: $\varepsilon_{An}/\varepsilon_n$ defines the intrinsic efficacy of ligand A; $\varepsilon_{Bn}/\varepsilon_n$ defines the intrinsic efficacy of ligand B; and $\delta_n{*}\varepsilon_n$ defines the activation cooperativity between ligands A and B. Intrinsic efficacies and cooperativities are considered positive, null and negative when they are greater than, equal to and lower than 1, respectively.

$K = [A] * [R_1R_2]/[AR_1R_2]$

$M = [B] * [R_1R_2]/[R_1R_2B]$

$M/\alpha = [B] * [AR_1R_2]/[AR_1R_2B]$

$K/\alpha = [A] * [R_1R_2B]/[AR_1R_2B]$

ε_{ABn}, ε_{An} and ε_{Bn} denote the ability of AR_1R_2B, AR_1R_2 and R_1R_2B to activate pathway n, respectively.

Because ε_n is not necessarily 1, the intrinsic efficacies of A–B combination, A and B for pathway n are the ratios $\varepsilon_{ABn}/\varepsilon_n$, $\varepsilon_{An}/\varepsilon_n$ and $\varepsilon_{Bn}/\varepsilon_n$, respectively.

δ_n is introduced to measure the functional interaction: $\varepsilon_{ABn} = \varepsilon_{An} * \varepsilon_{Bn} * \delta_n$.

The activation cooperativity between A and B in the AR_1R_2B complex for pathway n is $(\varepsilon_{ABn}/\varepsilon_n)/((\varepsilon_{An}/\varepsilon_n) * (\varepsilon_{Bn}/\varepsilon_n)) = \delta_n * \varepsilon_n$.

χ_n is a parameter used to account for the basal response of pathway n.

$\chi_n = [R_1R_2]_T/K_{En}$, with $[R_1R_2]_T = [R_1R_2] + [AR_1R_2] + [R_1R_2B] + [AR_1R_2B]$

Values greater than, equal to and lower than 1 for the subsequent parameters or parameter combinations mean the following: (i) for α – positive, null and negative binding cooperativities, respectively; (ii) for $\varepsilon_{An}/\varepsilon_n$ and $\varepsilon_{Bn}/\varepsilon_n$ – positive, null and negative intrinsic efficacies of ligands A and B, respectively; (iii) for $\delta_n * \varepsilon_n$ – positive, null and negative activation cooperativities, respectively. Based on the aforementioned relationships, Eq. (1) for the fractional effect f_n can be obtained (see Appendix 4b in [22] for the rationale followed).

$$f_n = \frac{\chi_n([A][B]\alpha\delta_n\varepsilon_{An}\varepsilon_{Bn} + [A]M\varepsilon_{An} + [B]K\varepsilon_{Bn} + KM\varepsilon_n)}{[A][B]\alpha(\chi_n\delta_n\varepsilon_{An}\varepsilon_{Bn} + 1) + [A]M(\chi_n\varepsilon_{An} + 1) + [B]K(\chi_n\varepsilon_{Bn} + 1) + KM(\chi_n\varepsilon_n + 1)} \quad (1)$$

The basal fractional response without ligands is $f_n = \frac{\varepsilon_n\chi_n}{\varepsilon_n\chi_n+1}$. It is worth noting that, in basal conditions, the total receptor concentration is equal to the free receptor concentration ($[R_1R_2]_T = [R_1R_2]$).

By changing the values of the parameters in Eq. (1), the model can be used to simulate different situations and test various hypotheses about the impact of ligand–receptor interactions on the signaling system. An example follows.

Ligands can substantially alter the relative activity of a receptor heterodimer: changing dominance

To explore how two ligands regulate the functional responses elicited by R_1 or R_2, we used two imaginary ligands with the parameter values set in Fig. 3. A value of 10 for α shows that there is a positive binding cooperativity between the two ligands. Values for $\delta_1 * \varepsilon_1$ of 5 and $\delta_2 * \varepsilon_2$ of 0.5 indicate the positive activation cooperativity between A and B for pathway 1 and the negative activation cooperativity between A and B for pathway 2. The comparison between ε_1 and ε_2 shows the dominance of R_1 and pathway 1 over R_2 and pathway 2 when no ligands are bound to the heterodimer.

The state of dominance can be modulated by the ligands. According to the values of ε_1, ε_{A1} and ε_{B1}, ligand A promotes the activation of pathway 1, whereas B inhibits it. By contrast, the values of ε_2, ε_{A2} and ε_{B2} reveal that A hampers the activation of pathway 2 but B facilitates it. Here, ligand B changes the dominant protomer, increasing the efficacy of R_2 with respect to R_1.

Using these parameter values, the fractional effects of two pathways can be obtained (Fig. 3). Figure 3 displays how two different ligands with positive binding cooperativity and positive or negative activation cooperativity interact to affect the functional responses of pathways 1 and 2. From Fig. 3a and b it is shown that ligand A always increases the functional response of pathway 1 but the influence of ligand B on the pathway depends on the concentration of A. Figure 3c and d show that ligand A constantly decreases the functional response of pathway 2, whereas ligand B has the opposite effect.

It is worth noting that the functional responses obtained in Fig. 3 result from the particular set of parameters we have chosen. A different set of parameters would lead to different plots and many combinations of positive and negative intrinsic efficacies and cooperativities are possible. In this regard, we have chosen a positive binding cooperativity ($\alpha = 10$). However, for many GPCR dimers a negative cooperativity for ligand binding has been reported [2]. Decreasing the binding cooperativity has, as the main effects, a reduction in the apparent affinity and potency of the compounds, resulting in a displacement of the concentration–effect (E/[A]) curves to the right. It is also interesting to comment on the intrinsic efficacy values. As an example, for pathway 2, we have $\varepsilon_{B2}/\varepsilon_2 = 10/0.1 = 100$ as the intrinsic efficacy value of ligand B. As $\varepsilon_2 = 0.1$, lowering ε_{B2} to 1 still results in an agonist ligand: $\varepsilon_{B2}/\varepsilon_2 = 1/0.1 = 10$. However, making $\varepsilon_{B2} = 0.1$ would make ligand B a neutral antagonist. Finally, making $\varepsilon_{B2} < 0.1$ would convert ligand B into an inverse agonist.

Concluding remarks

Proteins usually act together to regulate biological activities [27]. As proteins, GPCRs often form homomers and heteromers under physiological conditions, providing new opportunities for drug design based on allosteric interactions between different receptors. Life is a quantitatively observable process in principle. In this era of quantitative biology, mathematical modeling can greatly enhance our understanding of life and its processes. Efforts in this direction include not only genome-scale con-

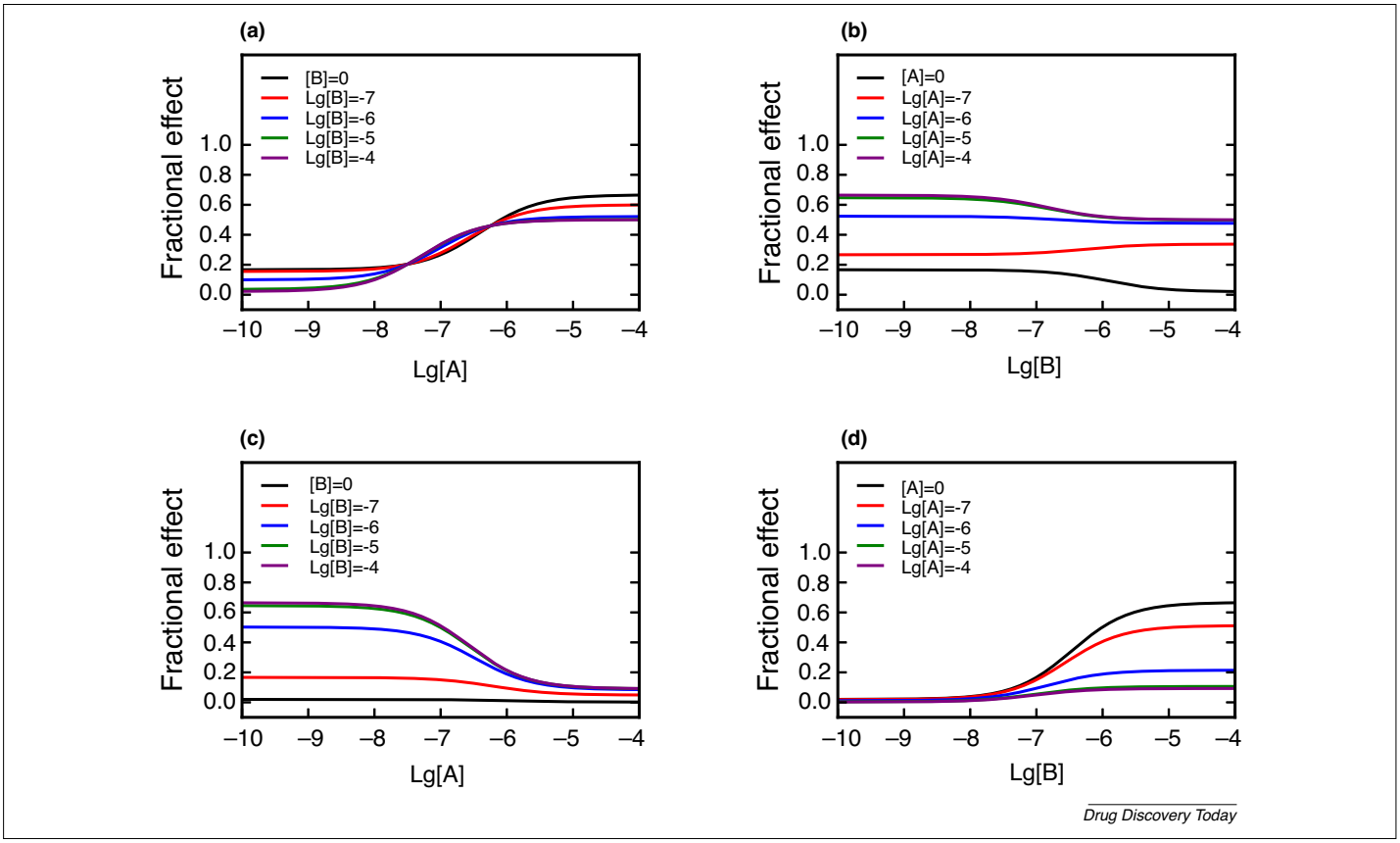

FIGURE 3

(a) and **(b)** Fractional effect for R_1-mediated pathway varies with the concentrations of ligands A and B. **(c)** and **(d)** Fractional effect for R_2-mediated pathway varies with the concentrations of ligands A and B.

Parameter setting:

Binding parameters			Functional parameters: pathway 1					Functional parameters: pathway 2				
K	M	α	χ_1	δ_1	ε_1	ε_{A1}	ε_{B1}	χ_2	δ_2	ε_2	ε_{A2}	ε_{B2}
10^{-6}	10^{-6}	10	0.2	5	1	10	0.1	0.2	5	0.1	0.01	10

structions but also the models of particular pathways or ligand–receptor systems. GPCR monomers and homomers have been modeled but the heteromers have been scarcely addressed. In this study, we propose a mathematical model for the allosteric interactions within a GPCR heterodimer. This model quantifies the functional effects of ligands with different properties on GPCR-mediated signaling pathways. Our model for a receptor heterodimer is based on a previous operational model for the allosteric effects between two binding sites in a single monomeric receptor. The resulting E/[A] equation can be used for simulation and fitting purposes. For the former case, an example has been given. For the latter case, it must be said that it is known that operational models cannot fit a single E/[A] curve [28]. Because of this, some conditions, such as those included in the irreversible inactivation method [29], need to be established. These conditions keep some of the parameters constant, thus enabling fitting [28]. With proper experimental data, the applicability of the present model of a receptor heterodimer for fitting purposes can be tested.

An example is given to illustrate the dominance of one protomer over the other within the heterodimer. In this example, the dominance appears because the two receptors differ in the ability to activate signaling pathways. However, the dominance can also be caused by their discrepancy in ligand binding. Provided that one receptor prevents another from binding to the agonist, the former becomes the dominant protomer by inhibiting the activation of the latter. This phenomenon occurs in the heterodimer consisting of serotonin 2C and one of serotonin 2A and 2B [30]. This level of dominance can also be described using our model by adjusting the dissociation constants of ligand–receptor pairs. Overall, our receptor heterodimer model can be employed to quantify the ligand–receptor system. With more GPCR heterodimers being discovered and their functionality assessed, our model can only be of greater utility. Finally, functional data of complex receptor composition might be attributed to heterodimers if their related experimental results agree with the present model.

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References

1 Santos, R. *et al.* (2017) A comprehensive map of molecular drug targets. *Nat. Rev. Drug Discov.* 16, 19–34

2 Ferre, S. *et al.* (2014) G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. *Pharmacol. Rev.* 66, 413–434

3 Armando, S. *et al.* (2014) The chemokine CXC4 and CC2 receptors form homo- and heterooligomers that can engage their signaling G-protein effectors and betaarrestin. *FASEB J.* 28, 4509–4523

4 Bonaventura, J. *et al.* (2015) Allosteric interactions between agonists and antagonists within the adenosine A2A receptor-dopamine D2 receptor heterotetramer. *Proc. Natl. Acad. Sci. U. S. A.* 112, E3609–E3618

5 Franco, R. *et al.* (2008) G-protein-coupled receptor heteromers: function and ligand pharmacology. *Br. J. Pharmacol.* 153, 590–598

6 Vinals, X. *et al.* (2015) Cognitive impairment induced by delta9-tetrahydrocannabinol occurs through heteromers between cannabinoid CB1 and serotonin 5-HT2A receptors. *PLoS Biol.* 13, e1002194

7 Moreno, J.L. *et al.* (2013) G protein-coupled receptor heterocomplexes in neuropsychiatric disorders. *Prog. Mol. Biol. Transl. Sci.* 117, 187–205

8 Moreno, J.L. *et al.* (2016) Allosteric signaling through an mGlu2 and 5-HT2A heteromeric receptor complex and its potential contribution to schizophrenia. *Sci. Signal.* 9 ra5

9 Oliveira, P.A. *et al.* (2017) Angiotensin II type 1/ adenosine A 2A receptor oligomers: a novel target for tardive dyskinesia. *Sci. Rep.* 7, 1857

10 Moreno, E. *et al.* (2017) Functional mu-opioid-galanin receptor heteromers in the ventral tegmental area. *J. Neurosci.* 37, 1176–1186

11 Hall, D.A. (2000) Modeling the functional effects of allosteric modulators at pharmacological receptors: an extension of the two-state model of receptor activation. *Mol. Pharmacol.* 58, 1412–1423

12 Ehlert, F.J. (2015) Functional studies cast light on receptor states. *Trends Pharmacol. Sci.* 36, 596–604

13 Kenakin, T. (2017) Theoretical aspects of GPCR–ligand complex pharmacology. *Chem. Rev.* 117, 4–20

14 Black, J.W. and Leff, P. (1983) Operational models of pharmacological agonism. *Proc. R. Soc. Lond. B. Biol. Sci.* 220, 141–162

15 Leach, K. *et al.* (2007) Allosteric GPCR modulators: taking advantage of permissive receptor pharmacology. *Trends Pharmacol. Sci.* 28, 382–389

16 Slack, R. and Hall, D. (2012) Development of operational models of receptor activation including constitutive receptor activity and their use to determine the efficacy of the chemokine CCL17 at the CC chemokine receptor CCR4. *Br. J. Pharmacol.* 166, 1774–1792

17 Hall, D.A. (2013) Application of receptor theory to allosteric modulation of receptors. *Prog. Mol. Biol. Transl. Sci.* 115, 217–290

18 Rovira, X. *et al.* (2008) Modeling the binding and function of metabotropic glutamate receptors. *J. Pharmacol. Exp. Ther.* 325, 443–456

19 Rovira, X. *et al.* (2009) Modelling the interdependence between the stoichiometry of receptor oligomerization and ligand binding for a coexisting dimer/tetramer receptor system. *Br. J. Pharmacol.* 156, 28–35

20 Rovira, X. *et al.* (2010) The asymmetric/symmetric activation of GPCR dimers as a possible mechanistic rationale for multiple signalling pathways. *Trends Pharmacol. Sci.* 31, 15–21

21 Brea, J. *et al.* (2009) Evidence for distinct antagonist-revealed functional states of 5-hydroxytryptamine(2A) receptor homodimers. *Mol. Pharmacol.* 75, 1380–1391

22 Roche, D. *et al.* (2013) Mechanistic analysis of the function of agonists and allosteric modulators: reconciling two-state and operational models. *Br. J. Pharmacol.* 169, 1189–1202

23 Roche, D. *et al.* (2013) Multiple active receptor conformation, agonist efficacy and maximum effect of the system: the conformation-based operational model of agonism. *Drug Discov. Today* 18, 365–371

24 Roche, D. *et al.* (2016) Have many estimates of efficacy and affinity been misled? Revisiting the operational model of agonism. *Drug Discov. Today* 21, 1735–1739

25 Doumazane, E. *et al.* (2011) A new approach to analyze cell surface protein complexes reveals specific heterodimeric metabotropic glutamate receptors. *FASEB J.* 25, 66–77

26 Levitz, J. *et al.* (2016) Mechanism of assembly and cooperativity of homomeric and heteromeric metabotropic glutamate receptors. *Neuron* 92, 143–159

27 Changeux, J.P. and Christopoulos, A. (2016) Allosteric modulation as a unifying mechanism for receptor function and regulation. *Cell* 166, 1084–1102

28 Kenakin, T. *et al.* (2012) A simple method for quantifying functional selectivity and agonist bias. *ACS Chem. Neurosci.* 3, 193–203

29 Furchgott, R.F. (1966) The use of β–haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. In *Advances in Drug Research* (3rd Edn.) (Harper, N.J. and Simmonds, A.B., eds), pp. 21–55, Academic Press

30 Moutkine, I. *et al.* (2017) Heterodimers of serotonin receptor subtypes 2 are driven by 5-HT2C protomers. *J. Biol. Chem.* 292, 6352–6368

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