Critical re-evaluation of the slope factor of the operational model of agonism

Short title: Slope factor of the operational model of agonism

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Conflict of interest

The author declares no conflict of interest.

Availability of data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Background and Purpose

Although being a relative term, agonist efficacy is a cornerstone in the proper assessment of agonist selectivity and signalling bias. The operational model of agonism (OMA) has become successful in the determination of agonist efficacies and ranking them. In 1985, Black et al. introduced the slope factor to the OMA to make it more flexible and allow for fitting steep as well as flat concentration-response curves.

Experimental Approach

Functional analysis of OMA demonstrates that the slope factor implemented by Black et al. affects relationships among parameters of the OMA, leading to wrong estimates of operational efficacy and affinity of an agonist. In contrast, functional analysis of OMA with Hill coefficient as a slope factor has not this flaw.

Key Results

Fitting of the OMA with Black et al. slope factor to concentration-response curves of several models (homotropic allosteric modulation, substrate inhibition and non-competitive auto-inhibition) gives wrong estimates of operational efficacy and affinity. In contrast, fitting of the OMA modified by the Hill coefficient to concentration-response curves of these several models gives correct estimates of operational efficacy and affinity.

Conclusion and Implications

The OMA modified by the Hill coefficient can be used for correct ranking of agonism and subsequent analysis, like quantification of signalling bias.

Keywords

Receptor theory; operational model of agonism; operational efficacy; slope factor

Introduction

Since its introduction in 1983, the operational model of agonism (OMA) has become a golden standard in an evaluation of agonism and subsequently also of signalling bias(Black and Leff, 1983). The OMA describes the response of the system as a function of ligand concentration using three parameters: 1) The equilibrium dissociation constant of agonist (K_A) to the receptor initiating functional response; 2) The maximal possible response of the system (E_{MAX}); 3) The operational efficacy of agonist (τ). Original OMA describes the dependence of functional response on the concentration of ligand by rectangular hyperbola (Eq. 2). In practice, positive cooperativity or positive feedback lead to steep and negative cooperativity or negative feedback lead to flat concentration-response curves that the OMA does not fit to. Therefore, an equation intended for the description of non-hyperbolic concentration curves (Eq. 7), particularly for systems where the number of receptors varies, was introduced by Black et al.(Black et al., 1985). Since then, Eq. 7 is commonly used. The presented mathematical analysis of Eq. 7 shows that the slope factor affects the relationship between observed maximal response to agonist and operational efficacy and the relationship between the concentration of agonist for half-maximal response and its equilibrium dissociation constant. The presented case study shows that fitting Eq. 7 results in wrong estimates of model parameters.

The presented case study shows that the use of the Hill coefficient as a slope factor to the original rectangular hyperbola of OMA instead of the slope factor by Black et al. is a viable alternative. The Hill–Langmuir equation was originally formulated to describe the cooperative binding of oxygen molecules to haemoglobin(Gesztelyi et al., 2012). The Hill coefficient reflects the sense and magnitude of cooperativity among concurrently binding ligands. A value of the Hill coefficient lower than 1 indicates negative cooperativity that is manifested as a flat concentration-response curve. The smaller value of the Hill coefficient the flatter curve is. A value of the Hill coefficient greater than 1 indicates positive cooperativity that is manifested as a steep concentration-response curve. The greater value of the Hill coefficient the steeper curve is. In contrast to Black et al. slope factor, the proposed implementation of the Hill coefficient (Eq. 10) does not affect the centre or asymptotes of the hyperbola describing concentration-response curves. Therefore, the Hill coefficient does not affect relationships among three parameters of the OMA, K_A, E_{MAX} and τ. This makes OMA (Eq. 11) more practical in many ways.

The general concept of the operational model of agonism

In general, OMA consist of two functions. One function describes the binding of an agonist to a receptor as the dependence of the concentration of agonist-receptor complexes [AR] on the

concentration of an agonist [A]. The second function describes the dependence of functional response (E) to an agonist on [AR]. OMA expresses the dependence of E on [A].

Rectangular hyperbolic OMA

Definition of OMA

In the simplest case, when both binding and response function are described by rectangular hyperbola (Supplementary Information Figure S1), the resulting function is also rectangular hyperbola. For example, in bi-molecular reaction, the dependence of ligand binding to the receptor [AR] is described by Eq. 1 where [A] is the concentration of ligand and K_A is its equilibrium dissociation constant that represents the concentration of ligand at which half of the total number of receptors, R_T, is bound to the ligand and the other half of the receptors is free.

$$[AR] = \frac{R_T[A]}{[A] + K_A} \qquad \text{Eq. 1}$$

If the bound ligand is an agonist, it activates the receptor and produces functional response E. In the system where its response is proportional to [AR], the dependence of response E on agonist binding [AR] is given by Eq. 2.

$$E = \frac{E_{MAX}[AR]}{[AR] + K_E} \qquad \text{Eq. 2}$$

Where E_{MAX} is the maximum possible response of the system and K_E is the value of [AR] that elicits a half-maximal effect. Various agonists produce a functional response of different strengths. The OMA was postulated to introduce the "transducer ratio" τ that is given by Eq. 3.

$$\tau = \frac{R_T}{K_E} \qquad \text{Eq. 3}$$

The substitution of Eq. 2 with Eq. 1 and Eq. 3 gives Eq. 4.

$$E = \frac{[A]\tau E_{MAX}}{[A](\tau+1) + K_A} \qquad \text{Eq. 4}$$

Analysis of OMA

Eq.4 is the equation of OMA(Black and Leff, 1983). It has three parameters: The equilibrium dissociation constant of agonist (K_A) at the response-inducing state of the receptor that is specific to a combination of ligand and receptor. The maximal possible response of the system (E_{MAX}) is specific to the system. And the "transducer ratio" (τ) that is specific to a combination

of ligand and system. Eq. 4 is a rectangular hyperbola with the horizontal asymptote, the observed maximal response to agonist A (E'_{MAX}), given by Eq. 5.

$$E'_{MAX} = \frac{\tau}{\tau+1} E_{MAX} \qquad \text{Eq. 5}$$

A more efficacious agonist (having a high value of parameter τ) elicits higher E'_{MAX} than less efficacious agonists (having a low value of parameter τ). Thus, τ is actually operational efficacy. The relationship between parameter τ and E'_{MAX} is hyperbolic meaning that two highly efficacious agonists (e.g., τ values 10 and 20) differ in E'_{MAX} values less than two weak agonists (e.g., τ values 0.1 and 0.2).

In Eq. 4, the concentration of agonist A for half-maximal response (EC_{50}), is given by Eq. 6.

$$EC_{50} = \frac{K_A}{\tau+1} \qquad \text{Eq. 6}$$

According to Eq. 6, for $\tau > 0$, the EC_{50} value is always lower than the K_A value. The K_A to EC_{50} ratio is greater for efficacious agonists than for weak agonists. Similarly to E'_{MAX}, the relationship between parameter τ and EC_{50} is hyperbolic. In contrast to E'_{MAX} values, the ratio K_A to EC_{50} ratio is more profound for two highly efficacious agonists (e.g., τ values 10 and 20) than two weak agonists (e.g., τ values 0.1 and 0.2).

Limitations of OMA

The OMA has several weak points. The major drawback of OMA is the lack of physical basis of the agonist equilibrium dissociation constant K_A. Agonist binding [AR] used in the Eq. 2 refers to agonist binding to the conformation from which the functional response is initiated. In the radioligand binding experiments agonists bind to all receptor conformations including the inactive ones. Usually, agonist binding to the functional-response activating conformation cannot be distinguished in the binding experiment and K_A cannot be determined. All three parameters of OMA (E_{MAX}, K_A and τ) are interdependent (Jakubík et al., 2019). To fit Eq. 4 to the experimental data one of the parameters must be fixed. Thus, the maximal response of the system E_{MAX} has to be determined before fitting Eq. 4. Another limitation of the OMA is that the shape of the functional response is a rectangular hyperbola.

Non-hyperbolic OMA

Definition of non-hyperbolic OMA

In practice, concentration-response curves steeper or flatter than the ones described by Eq. 4 are observed. In such cases, Eq. 4 does not fit experimental data. As stated by the authors, Eq. 7

was devised for non-hyperbolic dependence of functional response on concertation of agonist (Black et al., 1985).

$$E = \frac{[A]^n \tau^n E_{MAX}}{[A]^n \tau^n + ([A] + K_A)^n} \qquad \text{Eq. 7}$$

Analysis of non-hyperbolic OMA

Introduced power factor **n** changes slope and shape of functional response curve (Supplementary information Figure S3). Nevertheless, Eq. 7 has rectangular asymptotes: The horizontal asymptote ([A]→±∞) E'_{MAX} is given by Eq. 8 and vertical asymptote (E→±∞) is equal to $-K_A/(\tau+1)$ (Supplementary Information Eq. A2 and Eq. A3). From Eq. 7, the EC_{50} value is given by Eq. 9.

$$E'_{MAX} = \frac{\tau^n E_{MAX}}{1 + \tau^n} \qquad \text{Eq. 8}$$

$$EC_{50} = \frac{K_A}{(2 + \tau^n)^{\frac{1}{n}} - 1} \qquad \text{Eq. 9}$$

Evidently, the introduced slope factor **n** affects both the observed maximal response E'_{MAX} and half-efficient concentration of agonist EC_{50} (Figure 1A and C). The influence of the slope factor on E'_{MAX} is **bidirectional** (Supplementary Information Table S1, Figure S2). For operational efficacies $\tau > 1$, an increase in the value of slope factor increases E'_{MAX}. (Figure 1A and 2A blue lines). For operational efficacies $\tau < 1$, an increase in slope factor decreases E'_{MAX} (Figure 1C and 2A yellow lines). The effect of slope factor on E'_{MAX} is the most eminent for low values of operational efficacy τ, making the estimation of model parameters of weak partial agonists impractical. Imagine full agonist τ=10 and very weak agonist τ=0.1. For n=1: Full agonist E'_{MAX} is 90 % and weak agonist E'_{MAX} is 10 % of system E_{MAX}. For n=2: Full agonist E'_{MAX} is 99 % (one-tenth more) and weak agonist E'_{MAX} is just 1 % (ten times less).

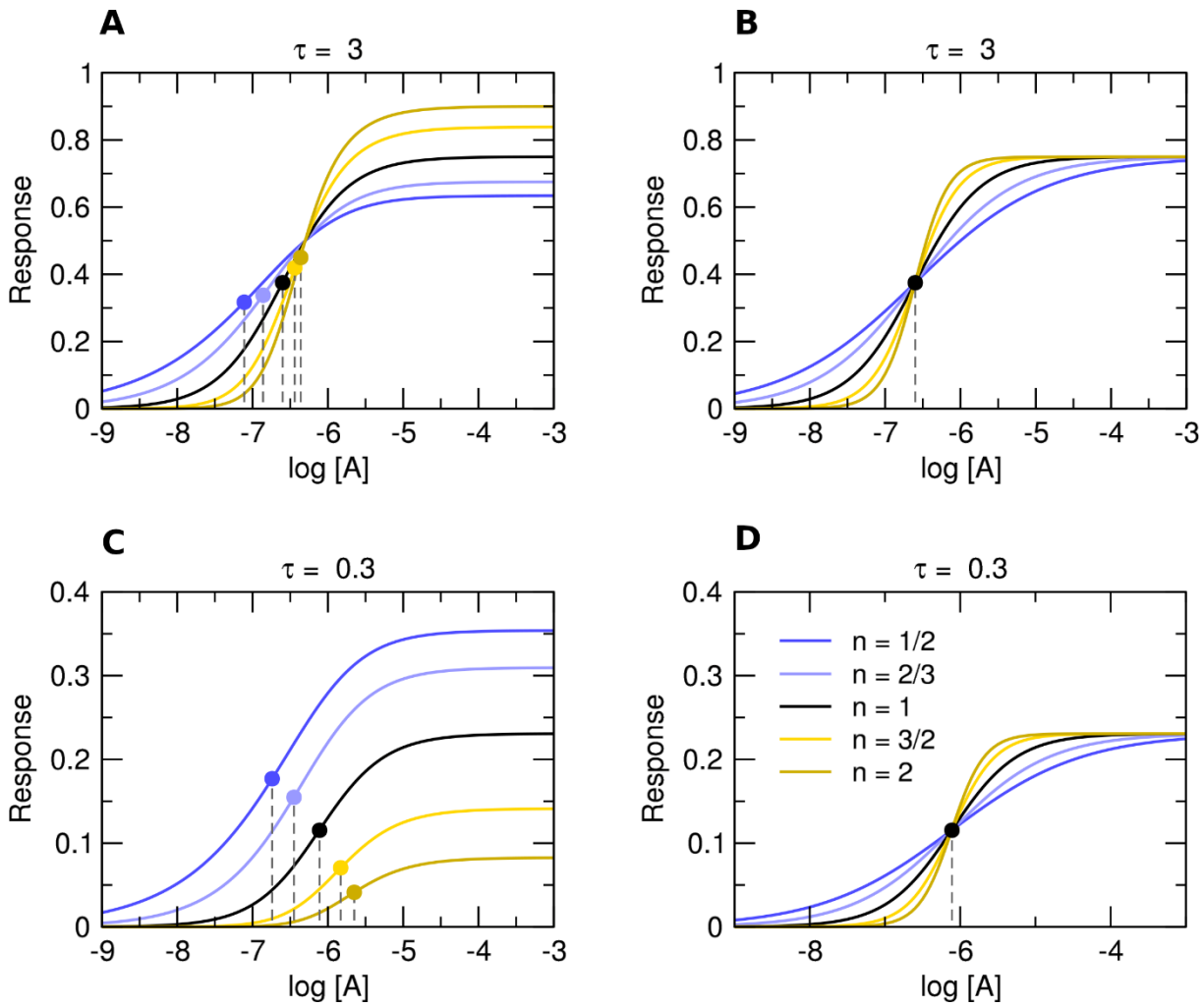

Figure 1 Theoretical concentration curves
Theoretical response curves according to Eq. 7, left and Eq. 11, right. Simulation parameters: $E_{MAX} = 1$; $\tau = 3$ (top) or $\tau = 0.3$ (bottom); $K_A = 10^{-6}$. Values of slope factors are listed in the legend.

An increase in the value of the slope factor increases the EC_{50} value (Figure 2B). Again, the effect of the slope factor on the EC_{50} value is more eminent at low values of operational efficacy τ (red lines). Paradoxically, any combination of operational efficacy τ and slope factor fulfilling the inequality in Figure 2C (blue area) results in EC_{50} values **greater** than K_A (e.g., Figure 1C, yellow lines). For example, $EC_{50} > K_A$ applies if $\tau = 0.5$ and $n > 1.6$, or if $\tau = 1$ and $n > 1.6$, or if $\tau = 1.5$ and $n > 2.15$. The upper asymptote of inequality is 2. Thus, the possibility of $EC_{50} > K_A$ applies to $\tau < 2$.

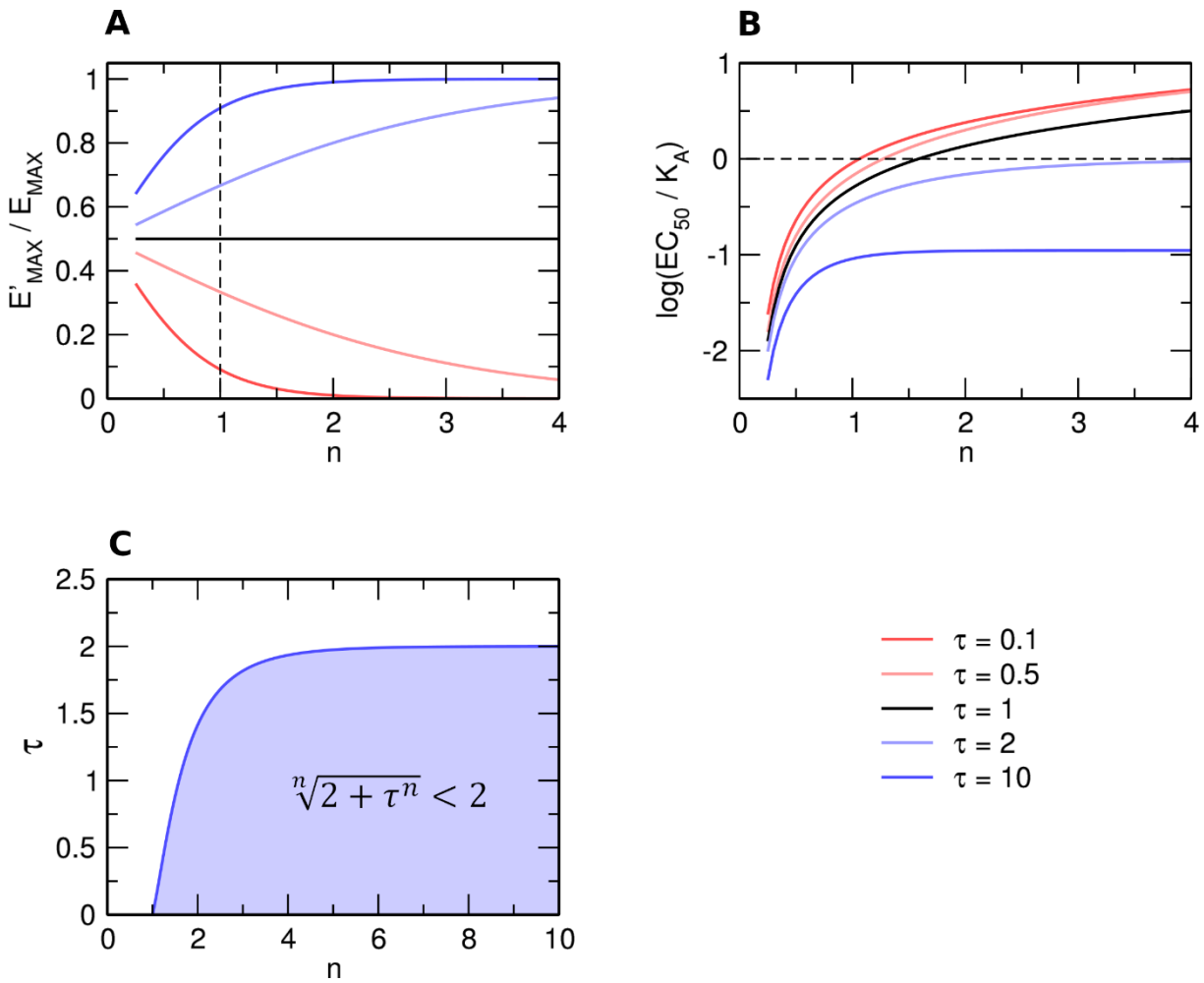

Figure 2 Analysis of Eq. 7
A, Dependency of observed E'_{MAX} to system E_{MAX} ratio (ordinate) on slope factor n (abscissa) and operational efficacy τ (legend). B, Dependency of EC_{50} to K_A ratio (ordinate) on slope factor n (abscissa) and operational efficacy τ (legend). C, Inequality plot of slope factor **n** (abscissa) and operational efficacy τ (ordinate) yielding half-efficient concentration EC_{50} greater than equilibrium dissociation constant K_A.

The operational efficacy τ may be also considered as a measure of "receptor reserve". In the system with a relatively small capacity of a functional response output, the full agonist reaches its maximal response before reaching full receptor occupancy. Thus, the agonist EC_{50} value is lower than its affinity for the receptor. The smaller occupancy fraction is needed for the full response to a given agonist the greater is difference between agonist EC_{50} and K_A values. According to OMA (Eq. 2), the relation between EC_{50} and K_A is described by Eq. 6. The greater value of operational efficacy τ, the smaller EC_{50} value and the greater the difference from K_A. Thus, the value of operational efficacy τ is a measure of receptor reserve of a given agonist at a given system. In a system with a large capacity of functional output, agonists do not have a receptor reserve and must reach full receptor occupancy to elicit a full signal. In such a system,

even full agonists have small operational efficacies. Thus, the parameter τ is specific to a combination of ligand and system.

Nevertheless, for agonists that elicit at least some response in a given system, the parameter τ must be greater than 0. Then according to Eq.6 of the operational model of agonism, the EC_{50} value must be smaller than the K_A value. In principle, the EC_{50} value greater than the K_A can be achieved only by some parallel mechanism that increases the apparent K'_A, provided that a difference K'_A to K_A is greater that EC_{50} to K_A. For example, such mechanism may be negative allosteric modulation of agonist binding or non-competitive inhibiton of functional response.

Limitations of the non-hyperbolic OMA

Besides all limitations of the hyperbolic OMA, the non-hyperbolic version of OMA has additional drawbacks. The most important is the lack of mechanistic background for factor **n**. Exponentiation of agonist concentration [A] to power factor **n** results in S-shaped curves. As it will be shown later, a combination of common binding mechanisms and functional responses does not result in S-shaped curves. Importantly, as shown above, exponentiation of operational efficacy τ to power factor **n** breaks the logical relationship between observed maximal response E'_{MAX} and operational efficacy τ. That, as it will be shown later, impedes correct estimation of τ and K_A values.

OMA with Hill coefficient

Definition of OMA with Hill coefficient

Hill coefficient may serve as an alternative slope factor in the OMA. Hill equation incorporates the Hill coefficient as a slope factor to rectangular hyperbola (Gesztelyi et al., 2012). The major advantage of the Hill coefficient as a slope factor is that it allows for a change in the eccentricity (vertices) of the hyperbola-like curves without changing centre (EC_{50}) and asymptotes (E'_{MAX}) (Supplementary Information Figure S3). The Hill–Langmuir equation published in 1910 can be formulated as Eq. 10.

$$E = \frac{[A]^{nH} E'_{MAX}}{[A]^{nH} + EC_{50}{}^{nH}} \qquad \text{Eq. 10}$$

Where nH is the Hill coefficient. Substitution of E'_{MAX} by Eq. 5 and EC_{50} by Eq. 6 gives Eq. 11.

$$E = \frac{[A]^{nH} \frac{\tau}{\tau+1} E_{MAX}}{[A]^{nH} + (\frac{K_A}{\tau+1})^{nH}} \qquad \text{Eq. 11}$$

As expected, the Hill coefficient does not influence maximal observed response E'_{MAX} either half-efficient concentration of agonist EC_{50} (Figure 1B and D).

Implications of OMA with Hill coefficient

Analysis of the OMA with slope factor by Black et al. (Eq. 7) have shown that the slope factor **n** has a bidirectional effect on the relationship between the parameters E'_{MAX} and τ. and that the slope factor **n** affects the relationship between the parameters EC_{50} and K_A. In contrast, in Eq. 11 neither value of E'_{MAX} nor the value of EC_{50} is affected by the Hill coefficient (Figure 1B and D). The parameters E'_{MAX} and EC'_{50} can be readily obtained by fitting Eq. 10 to the single concentration-response data.

Limitations of OMA with Hill coefficient

The major criticism of the Hill equation is its parsimonious character. It is relatively simple and its parameters are easy to estimate. However, as a model, it is just an approximation. In an experiment, the slope of the concentration-response curve different from unity may be a result of the parallel signaling mechanism providing feedback or allosteric cooperativity. E.g., positive cooperativity results in steep concentration-response curves and negative cooperativity results in flat concentration-response curves.

OMA of homotropic allosteric modulation

OMA of homotropic allosteric of binding

The slope factor **n** was introduced to OMA to fit non-hyperbolic functional response curves with variation in receptor concentration(Black et al., 1985). However mere variation in R_T does not affect the slope of the concentration-response curve (Supplementary Information Figure S4). Neither does signal amplification or dumping (Supplementary Information Figure S5). The simplest mode of interaction that leads to variation in the slope of concentration-response curves is allosteric modulation. Let's take for example homotropic allosteric modulation and suppose that ligand A binds to the orthosteric (AR) and the allosteric binding site (RA) with the same affinity. For the sake of simplicity suppose that a ligand A activates the receptor only from the orthosteric binding site. And that A bound to the orthosteric binding site (AR) has the same operational efficacy as A in the ternary complex (ARA). In such a case, the amount of active complexes AR and ARA is given by Eq. 12.

$$[AR] + [ARA] = \frac{R_T[A]}{[A] + K_A \frac{[A] + K_A}{\alpha[A] + K_A}} \qquad \text{Eq. 12}$$

Where α is the factor of binding cooperativity. Values of α greater than 1 denote positive cooperativity. Values of α smaller than 1 denote negative cooperativity. The substitution of Eq. 12 and Eq. 3 into Eq. 2 gives Eq. 13 (Supplementary Information Eq. A18).

$$E = \frac{E_{MAX}\tau[A](K_A+\alpha[A])}{K_A^2+2[A]K_A+\alpha[A]^2+\tau[A](K_A+\alpha[A])} \qquad \text{Eq. 13}$$

Allosteric modulation does not affect observed maximal response E'_{MAX} (Supplementary Information Eq.A23). For equipotent homotropic allosteric modulation, the value of apparent K_A (K'_A) is given by Eq. 14 (Supplementary information Eq. A29) and the value of half-efficient concentration is given by Eq. 15.

$$K'_A = \frac{K_A}{\sqrt{\alpha}} \qquad \text{Eq. 14}$$

$$EC_{50} = \frac{K'_A}{\tau+1} \qquad \text{Eq. 15}$$

As can be seen in Figure 3, positive cooperativity results in a steep curve and a decrease in the apparent value of K_A. On the other hand, negative cooperativity results in a flat curve and an increase in the apparent value of K_A.

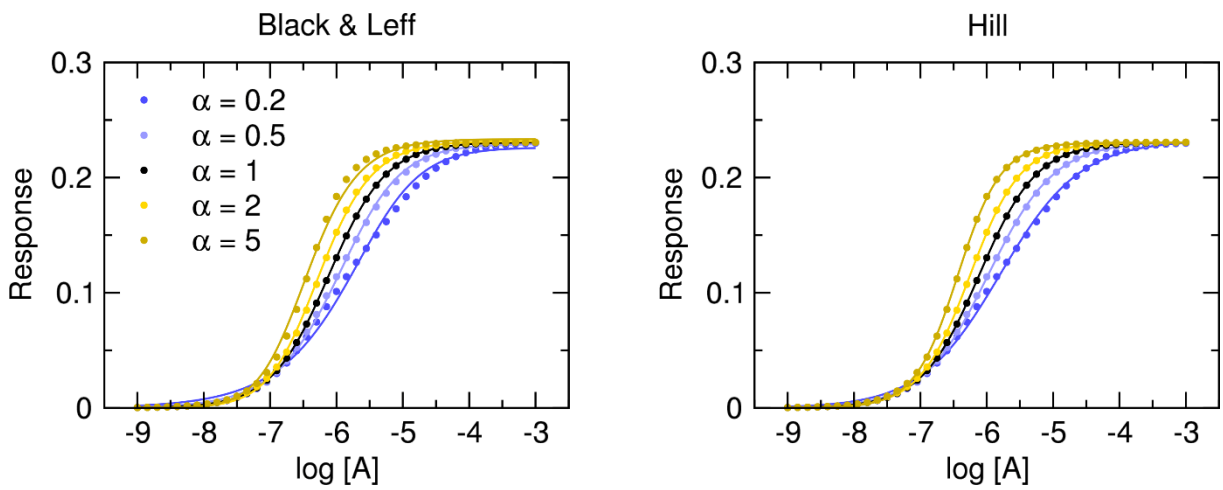

Figure 3 Homotropic allosteric modulation of binding
Dots, functional response to an agonist (τ=0.3, E_{MAX}=1, R_T=1) acting at two sites with the same $K_A = 10^{-6}$ M exerting binding cooperativity α indicated in the legend, according to Eq. 13. Full lines, left, Black & Leff equation (Eq. 7), right, Hill equation (Eq. 10) fitted to the data. Parameter estimates are in Table 1.

As can be seen in Figure 3 by the naked eye, the Hill equation fits the data better than the Black & Leff equation. Importantly, fitting the Hill equation gives correct estimates of apparent maximal response E'_{MAX}, while fitting the Black & Leff equation results in overestimated E'_{MAX}

values in the case of negative cooperativity ($\alpha<1$) and slightly underestimated E'_{MAX} values in the case of positive cooperativity (Table 1). As result, fitting the Hill equation gives correct estimates of operational efficacies τ. It gives also correct estimates of half-efficient concentrations EC_{50} and thus correct values of apparent as well as real equilibrium dissociation constant K_A. In contrast, fitting the Black & Leff equation gives correct parameter estimates only in the case of neutral cooperativity ($\alpha=1$). In the case of negative cooperativity ($\alpha<1$) values of τ and pK_A are underestimated. In the case of positive cooperativity ($\alpha>1$) values of τ and pK_A are overestimated.

Table 1 Results of fitting Eq. 7 and 10 to model Eq. 13
Eq. 7 and 10 were fitted to model data Eq. 13 with E_{MAX} fixed to 1. Apparent pK'_A values were calculated from model Eq. 13 according to Eq. 14 or from EC_{50} values according to Eq. 11.

Model Eq. 13 and 14				Fit Eq. 7			Fit Eq. 10				
τ	pK_A	α	pK'_A	n	τ	pK_A	n_H	EC_{50}	E'_{MAX}	τ	pK'_A
0.3	6.00	0.2	5.65	0.59	0.123	5.30	0.72	5.80	0.23	0.3	5.65
0.3	6.00	0.5	5.85	0.78	0.210	5.69	0.87	5.98	0.23	0.3	5.85
0.3	6.00	1.0	6.00	1.00	0.300	6.00	1.00	6.11	0.23	0.3	6.00
0.3	6.00	2.0	6.15	1.35	0.411	6.32	1.14	6.25	0.23	0.3	6.15
0.3	6.00	5.0	6.35	1.54	0.461	6.58	1.33	6.44	0.23	0.3	6.35

Fitting Eq. 13 with fixed system E_{MAX} to the model of functional response homotropic allosteric modulation of binding yields correct parameter estimates that are associated with the low level of uncertainty (Supplementary Information Figure S6).

OMA of homotropic allosteric modulation of efficacy

Homotropic allosteric modulation of agonist binding may result in the change in operational efficacy of agonist τ. If the operational efficacy of AR is τ and the operational efficacy of ARA is $\beta\tau$ then the activation constant K_E changes with the concentration of an agonist according to Eq. 16.

$$K_E = K_E \frac{[A]+K_A}{\beta[A]+K_A} \qquad \text{Eq. 16}$$

Where β is the cooperativity factor of efficacy. Values of β greater than 1 denote positive cooperativity, a decrease in apparent K'_E. Values of β smaller than 1 denote negative cooperativity, an increase in apparent K'_E.

For the sake of simplicity suppose neutral cooperativity (α = 1) in the binding of A to the orthosteric and allosteric sites. Then agonist binding described by Eq. 12 simplifies to Eq. Substitution of K_E by Eq. 16 and ([AR] + [ARA]) by Eq. 1 in Eq. 2 gives Eq. 17 (Supplementary Information Eq. A34).

$$E = \frac{[A]\tau E_{MAX}(\beta[A]+K_A)}{K_A^2+2[A]K_A+[A]^2+\tau[A](\beta[A]+K_A)} \qquad \text{Eq. 17}$$

The apparent maximal response E'_{MAX} is given by Eq. 18 (Supplementary Information Eq. A39).

$$E'_{MAX} = E_{MAX}\frac{\beta\tau}{1+\beta\tau} \qquad \text{Eq. 18}$$

Thus, apparent operational efficacy τ' is given by Eq. 19.

$$\tau' = \beta\tau \qquad \text{Eq. 19}$$

The EC_{50} value in homotropic allosteric modulation of efficacy is given by Eq. 20 (Supplementary information Eq. A42).

$$EC_{50} = \pm\frac{\sqrt{K_A^2(4\beta^2\tau+\beta^2(\tau^2-4\tau+8)+4\beta(\tau-2)+4)}\pm K_A(\beta(\tau-2)+2)}{2\beta(1+\beta\tau)} \qquad \text{Eq. 20}$$

Both positive (β >1) and negative (β < 1) cooperativity in operational efficacy lead to steep concentration-response curves (Figure 4, Table 2). Obviously, the Hill equation fits the data better than the Black & Leff equation. Strong negative cooperativity (β = 0.2) results in bell-shaped concentration-response curve (Figure 4, dark blue). Except for the bell-shaped curve (β=0.2), fitting of the Hill equation gives correct estimates of apparent maximal response E'_{MAX} and thus correct estimates of apparent operational efficacy τ'. In contrast, fitting the Black & Leff equation results in overestimated τ values. In the case of positive cooperativity (β>1), τ values are close to apparent operational efficacy τ'. Except for β=2, pK_A values are overestimated.

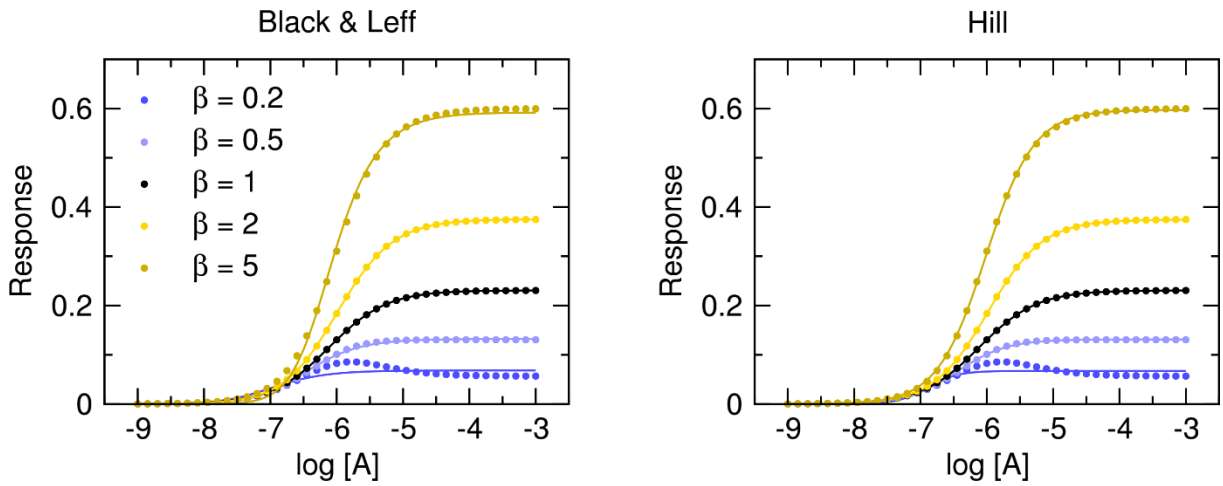

Figure 4 Homotropic allosteric modulation of efficacy
Dots, functional response to an agonist (τ=0.3, E_{MAX}=1, R_T=1) acting at two sites with the same $K_A = 10^{-6}$ M exerting cooperativity in efficacy β indicated in the legend, according to Eq. 17. Full lines, left, Black & Leff equation (Eq. 7), right, Hill equation (Eq. 10) fitted to the data. Parameter estimates are in Table 2.

Table 2 Results of fitting Eq. 7 and 10 to model Eq. 17
Eq. 7 and 10 were fitted to model data Eq. 17 with E_{MAX} fixed to 1

Model Eq. 13 and 14				Fit Eq. 7			Fit Eq. 10			
τ	pK_A	β	τ'	n	τ	pK_A	n_H	EC_{50}	E'_{MAX}	τ'
0.3	6.00	0.2	0.06	1.48	0.171	7.15	1.93	6.89	0.067	0.072
0.3	6.00	0.5	0.15	1.35	0.248	6.57	1.16	6.45	0.131	0.151
0.3	6.00	1.0	0.30	1.00	0.300	6.00	1.00	6.11	0.230	0.300
0.3	6.00	2.0	0.60	1.12	0.636	5.86	1.06	5.98	0.374	0.598
0.3	6.00	5.0	1.50	2.05	1.199	6.14	1.19	6.02	0.597	1.481

Fitting Eq. 17 with fixed system E_{MAX} to the model of functional response homotropic allosteric modulation of efficacy yields correct parameter estimates that are associated with the low level of uncertainty, except for neutral cooperativity (β=1) (Supplementary Information Figure S7). This should not represent a problem as for neutral cooperativity (β=1) Hill coefficient equals one and thus OMA in the form of Eq. 4 can be used.

OMA of substrate inhibition

Another common mechanism affecting the functional response to an agonist is substrate inhibition. In substrate inhibition, substrate concentration-dependently inhibits the reaction. In the case of functional response to receptor activation, agonist-receptor complexes [AR] are the substrate. Functional response is given by Eq. 21.

$$E = E_{MAX} \frac{[AR]}{K_E + [AR](1 + \frac{[AR]}{K_I})} \qquad \text{Eq. 21}$$

Where K_I is the inhibition constant, the concentration of [AR] that causes 50% inhibition of the functional response. After substitution of Eq. 1 for [RA], Eq. 21 becomes Eq. 22 (Supplementary information Eq. A50).

$$E = E_{MAX} \frac{[A]\tau([A]+K_A)}{\sigma\tau[A]^2 + ([A]+K_A)^2 + [A]\tau(A+K_A)} \qquad \text{Eq. 22}$$

Where $\sigma = R_T/K_I$. The apparent maximal response E'_{MAX} is given by Eq. 23 (Supplementary Information Eq. A46).

$$E'_{MAX} = E_{MAX} \frac{\tau}{1+\tau(1+\sigma)} \qquad \text{Eq. 23}$$

Thus, apparent operational efficacy τ' is given by Eq. 24.

$$\tau' = \frac{\tau}{\sigma\tau+1} \qquad \text{Eq. 24}$$

The EC_{50} value for the model of substrate inhibition is given by Eq. 25 (Supplementary information Eq. A53).

$$EC_{50} = \pm \frac{\sqrt{\sigma^2\tau^2+4\sigma\tau^2+8\sigma\tau+\tau^2+16\tau+8} - 2K_A\sigma\tau - K_A\tau}{2(\sigma\tau+2\tau+1)} \qquad \text{Eq. 25}$$

The shape for functional response is similar to homotropic allosteric modulation of efficacy (Figure 5). Again, the Hill equation fits the data better than the Black & Leff equation. For strong inhibition (σ=10) curves of the functional response have a bell shape. Except for the bell-shaped curve, fitting of the Hill equation gives correct estimates of apparent maximal response E'_{MAX} and thus correct estimates of apparent operational efficacy τ'. In contrast, fitting the Black & Leff equation results in underestimated τ values and overestimated pK_A values. Stronger inhibition (greater value of σ) results in the larger overestimation of pK_A.

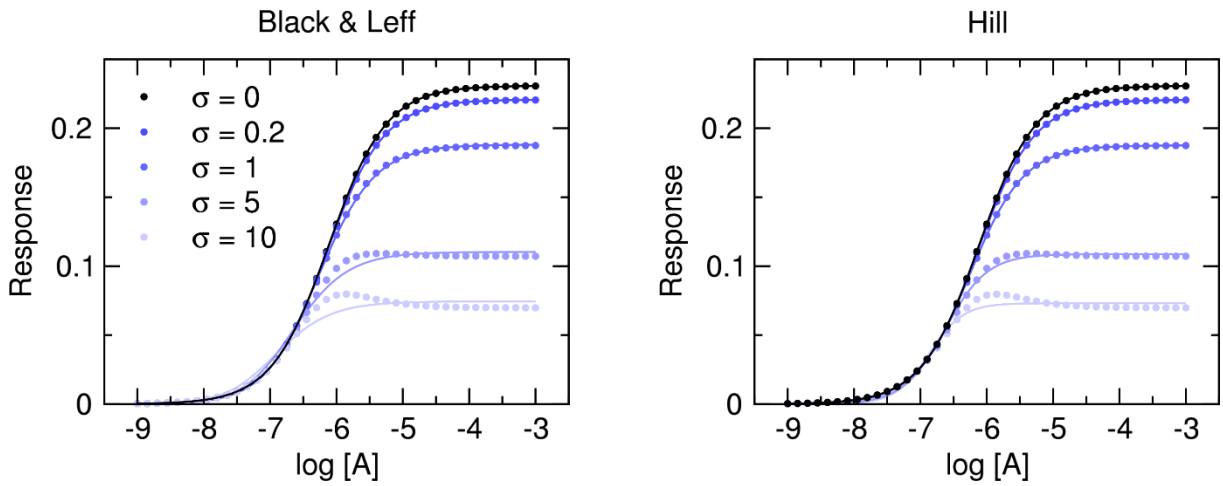

Figure 5 Substrate inhibition of functional response
Dots, functional response to an agonist (τ=0.3, E_{MAX}=1, R_T=1, K_A = 10^{-6} M) in the model of substrate inhibition according to Eq. 22. Values of σ are indicated in the legend. Full lines, left, Black & Leff equation (Eq. 7), right, Hill equation (Eq. 10) fitted to the data. Parameter estimates are in Table 3.

Table 3 Results of fitting Eq. 7 and 10 to model Eq. 17
Eq. 7 and 10 were fitted to model data Eq. 22 with E_{MAX} fixed to 1.

Model Eq. 13 and 14				Fit Eq. 7			Fit Eq. 10			
τ	pK_A	σ	τ'	n	τ	pK_A	n_H	EC_{50}	E'_{MAX}	τ'
0.3	6.00	0	0.300	1.00	0.300	6.00	1.01	6.00	0.226	0.300
0.3	6.00	0.5	0.283	1.02	0.292	6.05	1.01	6.15	0.221	0.283
0.3	6.00	1	0.231	1.14	0.276	6.25	1.06	6.26	0.188	0.231
0.3	6.00	5	0.120	1.37	0.218	6.76	1.37	6.63	0.109	0.121
0.3	6.00	10	0.075	1.37	0.159	7.04	1.72	6.87	0.073	0.079

Fitting Eq. 22 with fixed system E_{MAX} to the model of functional response of substrate inhibition yields correct parameter estimates that are associated with the low level of uncertainty (Supplementary Information Figure S8).

OMA of non-competitive inhibition

As shown in Figure 2, OMA with slope factor **n** allows for EC_{50} values higher than K_A. The simplest mode of interaction that increases observed EC_{50} above K_A is non-competitive autoinhibition. Under non-competitive auto-inhibition, substrate concentration-dependently inhibits the reaction. It results in a concentration-dependent increase in EC_{50} and a decrease in E'_{MAX}. In the case of functional response to receptor activation, agonist-receptor complexes [AR] are the substrate. Functional response is given by Eq. 22.

$$E = \frac{E_{MAX}[AR]}{(K_E+[AR])\frac{[AR]}{K_I+[AR]}} \qquad \text{Eq. 26}$$

For K_I >0, after substitution of Eq. 1 for [RA], Eq. 26 becomes Eq. 27 (Supplementary information Eq. A57).

$$E = E_{MAX}\frac{\tau[A]}{K_A+[A](1+\tau)}\frac{\sigma[A]}{K_A+[A](1+\sigma)} \qquad \text{Eq. 27}$$

Where $\sigma=R_T/K_I$. The apparent maximal response E'_{MAX} is given by Eq. 28 (Supplementary Information Eq. A57).

$$E'_{MAX} = E_{MAX}\frac{\tau}{1+\tau}\frac{\sigma}{1+\sigma} \qquad \text{Eq. 28}$$

Thus, apparent operational efficacy τ' is given by Eq. 29.

$$\tau' = \frac{\sigma\tau}{\sigma+\tau+1} \qquad \text{Eq. 29}$$

The EC_{50} value for the model of non-competitive auto-inhibition is given by Eq. 30 (Supplementary information Eq. A61).

$$EC_{50} = K_A\frac{\sqrt{\sigma^2+6\sigma\tau+8\sigma+\tau^2+8\tau+8}+\sigma+\tau+2}{2(1+\sigma)(1+\tau)} \qquad \text{Eq. 30}$$

Non-competitive auto-inhibition decreases apparent maximal response E'_{MAX}, increases observed half-efficient concentration EC_{50} and results in steep curves (Figure 6). Interestingly, a typical slope factor for non-competitive autoinhibition is 1.2 regardless of values of K_I and K_E. Both models fit well. Fitting Hill equation gives correct estimates of apparent maximal response E'_{MAX} and thus correct estimates of apparent operational efficacy τ' (Table 4). In contrast, for $K_I \geq 1$, fitting the Black & Leff equation results in underestimated values of τ and pK_A.

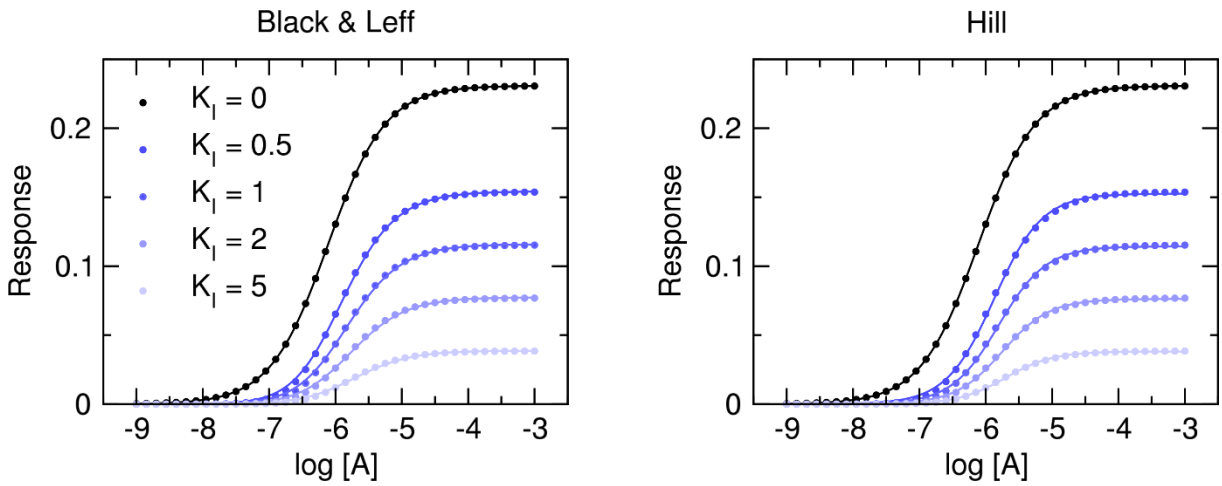

Figure 6 Non-competitive auto-inhibition of functional response
Dots, functional response to an agonist (τ=0.3, E_{MAX}=1, R_T=1, K_A = 10^{-6} M) in the model of non-competitive autoinhibition according to Eq. 27. Values of inhibition constants K_I are indicated in the legend. Full lines, left, Black & Leff equation (Eq. 7), right, Hill equation (Eq. 10) fitted to the data. Parameter estimates are in Table 4.

Table 4 Results of fitting Eq. 7 and 10 to model Eq. 17
Eq. 7 and 10 were fitted to model data Eq. 27 with E_{MAX} fixed to 1.

Model Eq. 13 and 14				Fit Eq. 7			Fit Eq. 10			
τ	pK_A	K_I	τ'	n	τ	pK_A	n_H	EC_{50}	E'_{MAX}	τ'
0.3	6.00	0	0.300	1.00	0.300	6.00	1.00	6.11	0.231	0.300
0.3	6.00	0.5	0.182	1.44	0.307	6.02	1.20	5.87	0.153	0.181
0.3	6.00	1	0.130	1.38	0.230	5.94	1.21	5.81	0.114	0.129
0.3	6.00	2	0.083	1.32	0.154	5.87	1.22	5.75	0.076	0.082
0.3	6.00	5	0.040	1.25	0.077	5.8	1.22	5.70	0.038	0.040

Fitting Eq. 28 with fixed system E_{MAX} to the model of functional response of substrate inhibition yields correct parameter estimates that are associated with the low level of uncertainty only when correct initial estimates of τ and σ are given (Supplementary Information Figure S9 and S10). In the case of K_I=5 (Supplementary Information Figure S9), estimates of operational efficacy τ and inhibition factor σ are swapped pointing to the symmetry of Eq. 28. This symmetry makes calculation of τ and σ impossible as any τ and σ combination resulting in an appropriate apparent efficacy τ' (Eq. 28) fits well a given functional-response data (Supplementary Information Figure S10).

Discussion

The operational model of agonism (OMA) (Black and Leff, 1983) is widely used in an evaluation of agonism. The OMA characterizes a functional response to an agonist by the equilibrium dissociation constant of agonist (K_A), the maximal possible response of the system (E_{MAX}) and

the operational efficacy of agonist (τ) (Eq. 4). To fit non-hyperbolic functional responses slope factor n was introduced to the OMA (Eq. 7) (Black et al., 1985). Analysis of Eq. 7 has shown that the slope factor **n** has a bidirectional effect on the relationship between the parameters E'_{MAX} and τ (Figure 1A versus 1C) and also affects the relationship between the parameters EC_{50} and K_A. Fitting Eq. 7 to the models of homotropic allosteric modulation of affinity (Figure 3), homotropic allosteric modulation of efficacy (Figure 4), substrate inhibition (Figure 5) and non-competitive auto-inhibition (Figure 6) result in wrong estimates of τ and K_A values (Tables 1 to 4). For example, the OMA in the form of Eq. 7 may represent a critical issue in the analysis of the signalling bias. Biased agonists stabilize specific conformations of the receptor leading to non-uniform modulation of individual signalling pathways (Lefkowitz, 2013). To measure an agonist bias, the parameters τ and K_A must be determined and $\log(\tau/K_A)$ values of tested and reference agonist compared at two signalling pathways (Kenakin et al., 2012). It is evident from the analysis of Eq. 7, that as far as the EC_{50} value is dependent on parameters **n** and τ (Figure 1A vs. C), $\log(\tau/K_A)$ values cannot be compared to judge possible signalling bias unless the parameter **n** is equal to 1 for both tested and reference agonist.

Despite dire effects of slope factor **n**, the OMA in the form of Eq. 7 is widely accepted (Christopoulos and El-Fakahany, 1999; Kenakin, 2012, 2017; Kenakin and Christopoulos, 2013; Keov et al., 2014; Luttrell et al., 2015; Stott et al., 2016; Burgueño et al., 2017). The OMA in the form of Eq. 7 entered textbooks (Kenakin, 2014). Very little concern on factor **n** has been risen. For example, Kenakin et al. (Kenakin et al., 2012) analysed in detail the effects of slope factor **n** on EC_{50} and τ to K_A ratio but did not deal with the bi-directional effect of **n** on τ neither proposed an alternative approach to avoid potential pitfalls. To force a proper shape on functional-response curves, Gregory et al. (Gregory et al., 2020) introduced the second slope factor into their OMA and OMAMA analysis making equations even more complex. So far, the greatest criticism of OMA was voiced by Roche et al. (Roche et al., 2016), noting that to accommodate the shape of theoretical curves OMA in the form of Eq. 7 tends to overestimate equilibrium dissociation constant K_A and operational efficacy τ and thus be misleading. They advocated for the use of the Hill equation.

The fitting of the Hill equation (Eq. 10) to the functional response is straightforward and easier than fitting Eq. 7. As shown in Figures 3 to 6, Eq. 10 fits well to various functional-response curves, often better than Eq. 7. In contrast to Eq. 7, Eq. 10 gives correct estimates of maximal response to agonist E'_{MAX} and its half-efficient concentration EC_{50} as documented by Tables 1 to 4. In contrast to Eq. 7, in the case of Eq. 11, neither value of E'_{MAX} nor the value of EC_{50} is

affected by the Hill coefficient (Figure 1B and D). Therefore, biased signalling may be inferred from the comparison of the ratio of intrinsic activity (E'_{MAX}/EC_{50}) of tested agonist to the intrinsic activity of reference agonist at two signalling pathways as in the case of Hill equation the E'_{MAX}/EC_{50} ratio is equivalent to τ / K_A ratio (Ehlert et al., 1999; Griffin et al., 2007). If needed, apparent operational efficacy τ' can be calculated from known E'_{MAX} values and known maximal response of the system E_{MAX}. From the relationship between τ' and EC_{50} (Jakubík et al., 2019, 2020) the mechanism of functional response can be inferred by comparison to explicit models. Four examples of explicit models are given above. Subsequently, equilibrium dissociation constant K_A and operational efficacy τ alongside the rest of model-specific parameters can be obtained by fitting the explicit model to experimental data.

Conclusions

Analysis of the OMA with slope factor **n** by Black et al. (Eq. 7) has shown that i) The slope factor **n** has a bidirectional effect on the relationship between the parameters E'_{MAX} and τ. ii) The slope factor **n** affects the relationship between the parameters EC_{50} and K_A. Fitting Eq. 7 gives wrong estimates of τ and K_A values. Thus, parameters obtained by fitting Eq. 7 cannot be used for the evaluation of signalling bias. Analysis of the OMA with Hill coefficient (Eq. 10) has shown that the Hill coefficient does not affect the relationship between the parameters E'_{MAX} and τ neither between the parameters EC_{50} and K_A. Fitting Hill equation (Eq. 10) to the concentration-response data gives good estimates of EC_{50} and E'_{MAX} values, which are suitable for further analysis of OMA, OMAMA and signalling bias.

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Supplementary Information: Critical re-evaluation of the slope factor of the operational model of agonism

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Hyperbola

A hyperbola is the set of points in a plane whose distances from two fixed points, called foci, has an absolute difference that is equal to a positive constant. It consists of two separate curves, called branches. Hyperbola is completely determined by its centre, vertices, and asymptotes. Points on the separate branches of the graph where the distance is at a minimum are called vertices. The midpoint between vertices is its centre. Hyperbola is asymptotic to certain lines drawn through the centre. Hyperbola which asymptotes form right angle is called rectangular or equilateral. The simplest rectangular hyperbola with coordinate axes as its asymptotes is reciprocal function y = 1/x (Figure S1, black). The reciprocal function can be geometrically translated by coefficients a, b, c, and d (Figure S1, green a=1, b=1, c=1, d=-1).

$$y = \frac{ax+b}{cx+d} \qquad \text{Eq. A1}$$

Where vertical and horizontal asymptotes are given by Eq. A2 and A3, respectively.

$$\lim_{y \to \pm\infty} = -\frac{d}{c} \qquad \text{Eq. A2}$$

$$\lim_{x \to \pm\infty} = \frac{a}{c} \qquad \text{Eq. A3}$$

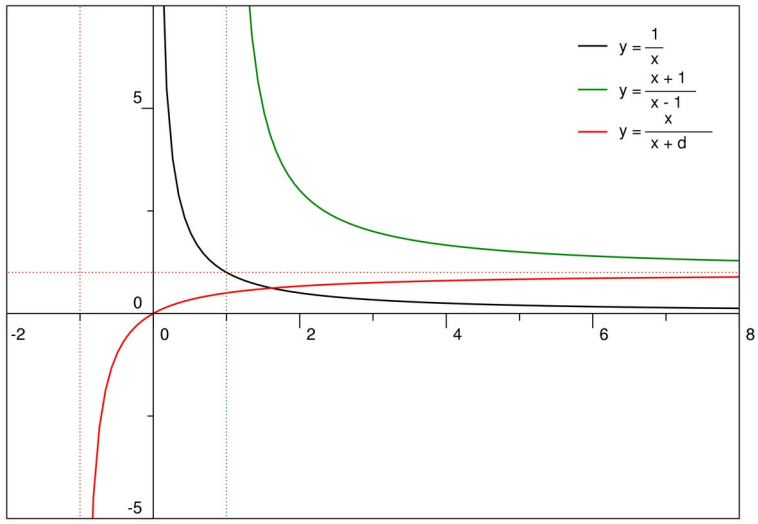

Figure S1 Examples of rectangular hyperbolas
Green, hyperbola with parameters a=1, b=1, c=1 and d=-1. Red, hyperbola with parameters a=1, b=0, c=1, d=1. Dashed lines, vertical and horizontal asymptotes of respective hyperbolas. Only right branches are shown.

A hyperbola with parameters a > 0, b = 0, c = 1 and d > 0 (e.g., Figure S1 red) is mass action equilibrium function.

Power function

Exponentiation is a mathematical operation, written as b^n, involving two numbers, the base b and the exponent n. b ≥ 0 can be risen to any value of n. b < 0 can be risen only to integers or fractions with odd denominator. Table S1 summarizes relationships between b^n and b values ≥ 0. Reciprocal relationships apply for b values < 0 as the power function is symmetric at the origin of axes. Importantly relationship between values of b^n and n are opposite in the range 0 < b < 1 to the range b > 1, resulting in the S-shape of the function (Figure S2).

Table S1 Behaviour of power function, relationship between b^n and b values.

	n = 0	**0 < n < 1**	**n = 1**	**n >1**
b = 0	$b^n = 1$	$b^n = b$	$b^n = b$	$b^n = b$
0 < b <1	$b^n = 1$	$b^n > b$	$b^n = b$	$b^n < b$
b = 1	$b^n = 1$	$b^n = b$	$b^n = b$	$b^n = b$
b > 1	$b^n = 1$	$b^n < b$	$b^n = b$	$b^n > b$

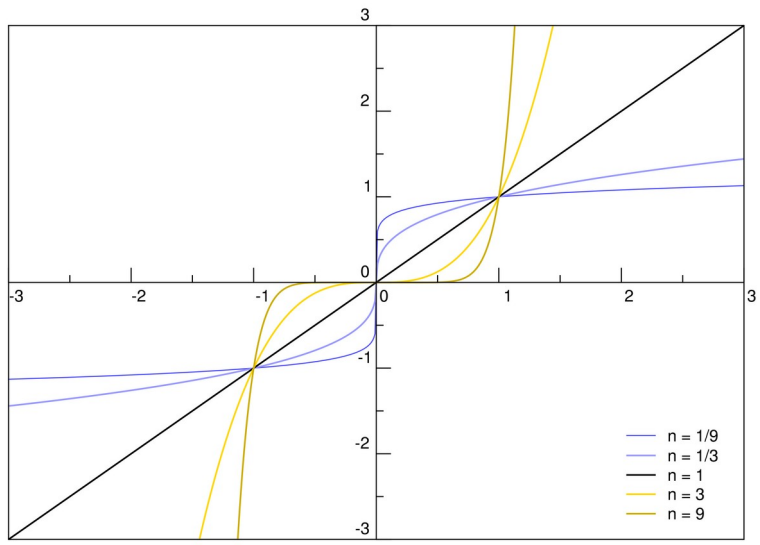

Figure S2 Examples of power functions
Curves of $y = x^n$ for various values of exponent n. Each curve passes through the point (0, 0) because 0 raised to any power is 0 and through the point (1, 1) because number 1 raised to any power is 1. For n = 1, y = x because any number raised to the power of 1 is the number itself.

Hyperbola and power function

Exponentiation of x in the hyperbola (Eq. A1) results in non-hyperbolic function (Eq. A4, Figure S3).

$$y = \frac{ax^n + b}{cx^n + d} \qquad \text{Eq. A4}$$

However, resulting function still possesses asymptotes given by Eq. A2 and A3 (Figure S3).

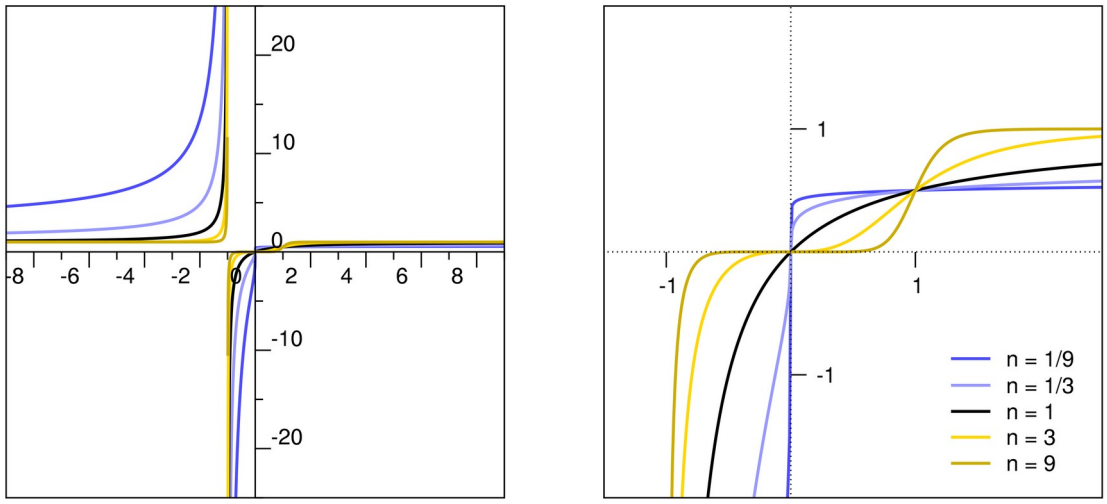

Figure S3 Examples of functions according to Eq. A4
Curves according to Eq. A4 for various values of exponent n. Left, both branches are shown. Right, detail of right branches. Black, for n=1 curve is rectangular hyperbola. Shades of blue, n < 1, and shades of yellow, n > 1, curves are S-shaped.

OMA from scratch

Binding:

$$[AR] = \frac{R_T[A]}{K_A+[A]} \qquad \text{Eq. A5}$$

Functional response:

$$E = E_{MAX}\frac{[AR]}{K_E+[AR]} \qquad \text{Eq. A6}$$

Substitution of [AR] by Eq. A5

$$E = E_{MAX}\frac{\frac{R_T[A]}{K_A+[A]}}{K_E+\frac{R_T[A]}{K_A+[A]}} \qquad \text{Eq. A7}$$

After rearrangement

$$E = E_{MAX}\frac{R_T[A]}{[A](K_E+R_T)+K_E K_A} \qquad \text{Eq. A8}$$

Division by K_E and substitution $\tau=R_T/K_E$:

$$E = E_{MAX}\frac{\tau[A]}{[A](1+\tau)+K_A} \qquad \text{Eq. A9}$$

Apparent E'_{MAX} for $[A] >> K_A$:

$$E'_{MAX} = E_{MAX}\frac{\tau}{1+\tau} \qquad \text{Eq. A10}$$

For half-efficient concentration EC50 Eq. A9 equals half of Eq. A10:

$$E_{MAX}\frac{\tau EC_{50}}{EC_{50}(1+\tau)+K_A} = \frac{E_{MAX}}{2}\ \frac{\tau}{1+\tau} \qquad \text{Eq. A11}$$

$$\frac{EC_{50}}{EC_{50}(1+\tau)+K_A} = \frac{1}{2(1+\tau)} \qquad \text{Eq.A12}$$

$$\frac{EC_{50}2(1+\tau)}{(1+\tau)(EC_{50}+\frac{K_A}{1+\tau})} = 1 \qquad \text{Eq. A13}$$

$$2EC_{50} = EC_{50}+\frac{K_A}{1+\tau} \qquad \text{Eq. A14}$$

$$EC_{50} = \frac{K_A}{1+\tau} \qquad \text{Eq. A15}$$

Effect of changes in receptor number on functional response

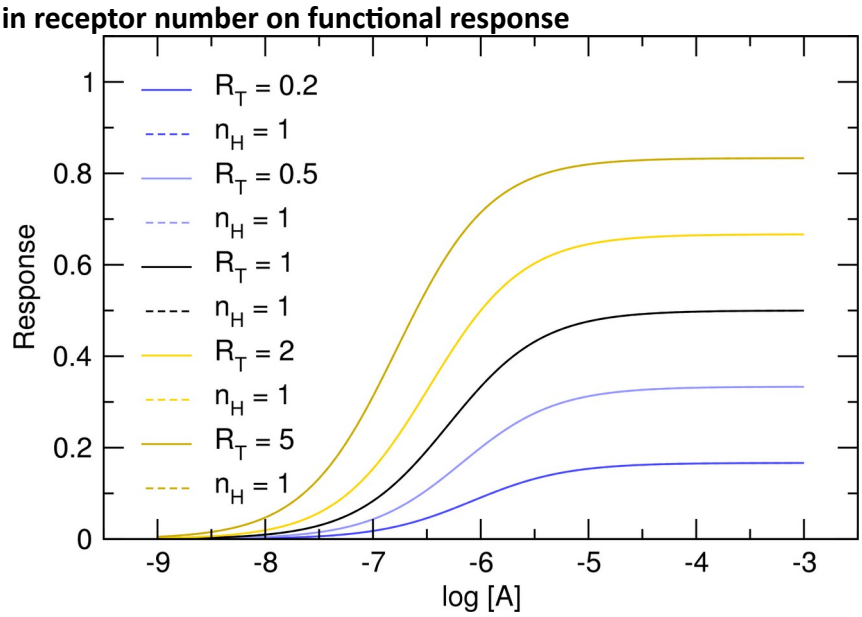

Figure S4 Effect of changes in receptor number on functional response
Curves according to OMA for various number of receptors (R_T). $E_{MAX} = 1$, $K_A = 10^{-6}$M, $\tau = 1$, R_T is in indicated in the legend.

Effect of signal amplification on functional response

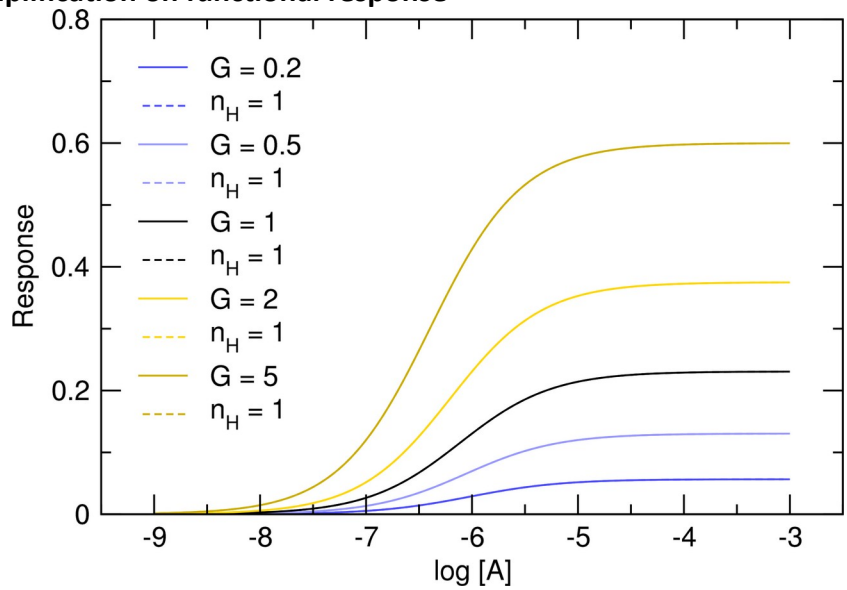

Figure S5 Effect of signal amplification on functional response
Curves according to OMA for various extent of signal amplification, gain factor (G) is indicated in the legend. E_{MAX} = 1, $K_A = 10^{-6}$M, τ = 0.3, R_T is in indicated in the legend.

Homotropic allosteric modulation of binding

In the case equipotent homotropic allosteric modulation of binding, the amount of receptor with an agonist A bound to the orthosteric binding site is given by Eq. A16.

$$[AR]+[ARA] = \frac{R_T[A]}{[A]+K_A\dfrac{[A]+K_A}{\alpha[A]+K_A}} \qquad \text{Eq. A16}$$

Substitution of active complexes of Eq. A6 by Eq. A16:

$$E = E_{MAX}\frac{\dfrac{R_T[A]}{[A]+K_A\dfrac{[A]+K_A}{\alpha[A]+K_A}}}{K_E+\dfrac{R_T[A]}{[A]+K_A\dfrac{[A]+K_A}{\alpha[A]+K_A}}} \qquad \text{Eq. A17}$$

After rearrangement:

$$E = E_{MAX}\frac{R_T[A](K_A+\alpha[A])}{\alpha K_E[A]^2+\alpha R_T[A]^2+K_A^2K_E+2K_AK_E[A]+K_AR_T[A]} \qquad \text{Eq. A18}$$

After division by K_E and substitution $\tau=R_T/K_E$:

$$E = E_{MAX} \frac{\tau[A](K_A+\alpha[A])}{\alpha[A]^2+\alpha\tau[A]^2+K_A^2+2K_A[A]+K_A\tau[A]} \quad \text{Eq. A19}$$

$$E = E_{MAX} \frac{\tau[A](\alpha[A]+K_A)}{\alpha[A]^2+K_A^2+2K_A[A]+\tau[A](K_A+\alpha[A])} \quad \text{Eq. A20}$$

Apparent E'_{MAX} for $[A] >> K_A$:

$$E'_{MAX} = E_{MAX} \frac{\tau\alpha[A]^2}{\alpha[A]^2+\tau\alpha[A]^2} \quad \text{Eq. A21}$$

$$E'_{MAX} = E_{MAX} \frac{\tau\alpha[A]^2}{\alpha[A]^2(1+\tau)} \quad \text{Eq. A22}$$

$$E'_{MAX} = E_{MAX} \frac{\tau}{1+\tau} \quad \text{Eq. A23}$$

To determine apparent K'_A (a concentration of A needed for 50 % of occupancy) let Eq. A16 equal to 0.5.

$$\frac{1}{2} = \frac{K'_A}{K'_A+K_A\frac{K'_A+K_A}{\alpha K'_A+K_A}} \quad \text{Eq. A24}$$

After rearrangement

$$\frac{1}{2} = \frac{K'_A(\alpha K'_A+K_A)}{\alpha K'^2_A+K_A^2+2K_AK'_A} \quad \text{Eq. A25}$$

For positive values of K'_A, K_A and α:

$$\alpha K'^2_A+K_A^2+2K_AK'_A = 2K'_A(\alpha K'_A+K_A) \quad \text{Eq. A26}$$

$$\alpha K'^2_A+K_A^2+2K_AK'_A = 2\alpha K'^2_A+2K'_AK_A \quad \text{Eq. A27}$$

$$\alpha K'^2_A = K_A^2 \quad \text{Eq. A28}$$

$$K'_A = \frac{K_A}{\sqrt{\alpha}} \quad \text{Eq. A29}$$

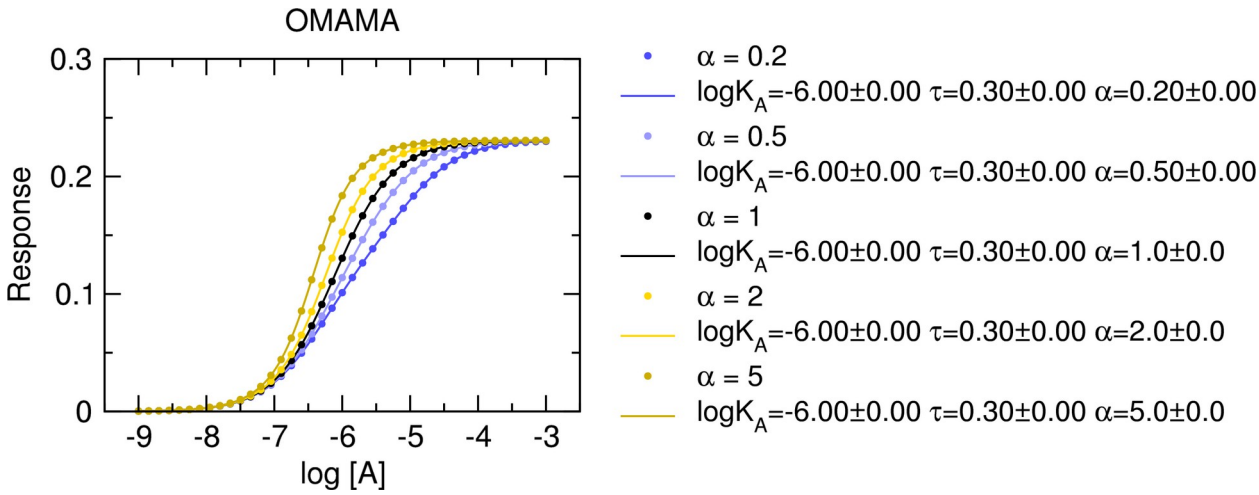

Figure S6 Fitting Eq. A20 to the model of homotropic allosteric modulation of binding
Dots, functional-response data modelled according to Eq. A17. E_{MAX} = 1, K_A = 10^{-6}M, τ = 0.3, R_T =1. Factors of cooperativity α are indicated in the legend. Lines, fits of Eq. A20 to the model data. E_{MAX} was fixed to 1. Parameter estimates of the fits are indicated in the legend.

Parameter estimates are correct, associated with low level of uncertainty.

Homotropic allosteric modulation of efficacy

For neutral binding cooperativity (α=1), binding Eq. A16 becomes Eq. A5. Functional response in the case of homotropic allosteric modulation of efficacy (operational efficacy of AR is τ and operational efficacy of ARA is βτ). Then response is given by Eq. A30

$$E = E_{MAX} \frac{[AR]+[ARA]}{K_E \frac{[A]+K_A}{[A]*\beta+K_A}+[AR]+[ARA]} \qquad \text{Eq. A30}$$

After substitution of active complexes of Eq. A30 by Eq. A5:

$$E = E_{MAX} \frac{\frac{R_T[A]}{K_A+[A]}}{K_E \frac{[A]+K_A}{[A]*\beta+K_A}+\frac{R_T[A]}{K_A+[A]}} \qquad \text{Eq. A31}$$

After rearrangement

$$E = E_{MAX} \frac{R_T[A](\beta K_A+[A])}{K_A^2 K_E+2K_A K_E[A]+K_A R_T[A]+K_E[A]^2+\beta R_T[A]^2} \qquad \text{Eq. A32}$$

$$E = E_{MAX} \frac{\tau[A](\beta K_A+[A])}{K_A^2+2K_A[A]+K_A\tau[A]+[A]^2+\beta\tau[A]^2} \qquad \text{Eq. A33}$$

$$E = E_{MAX}\frac{[A]\tau(\beta[A]+K_A)}{K_A^2+2[A]K_A+[A]^2+\tau[A](\beta[A]+K_A)} \qquad \text{Eq. A34}$$

Apparent E'_{MAX} at $[A]>>K_A$ is then given by:

$$E'_{MAX} = E_{MAX}\frac{\beta\tau[A]^2}{2[A]K_A+[A]^2+\beta\tau[A]^2} \qquad \text{Eq. A35}$$

$$E'_{MAX} = E_{MAX}\frac{\beta\tau[A]^2}{[A](2K_A+[A]+\beta\tau[A])} \qquad \text{Eq. A36}$$

$$E'_{MAX} = E_{MAX}\frac{\beta\tau[A]}{[A]+\beta\tau[A]} \qquad \text{Eq. A37}$$

$$E'_{MAX} = E_{MAX}\frac{\beta\tau[A]}{[A](1+\beta\tau)} \qquad \text{Eq. A38}$$

$$E'_{MAX} = E_{MAX}\frac{\beta\tau}{1+\beta\tau} \qquad \text{Eq. A39}$$

To determine half-efficient concentration EC_{50}:

$$E_{MAX}\frac{EC_{50}\tau(\beta EC_{50}+K_A)}{K_A^2+2EC_{50}K_A+EC_{50}^2+\tau EC_{50}(\beta EC_{50}+K_A)} = \frac{E_{MAX}}{2}\frac{\beta\tau}{1+\beta\tau} \qquad \text{Eq. A40}$$

$$\frac{2EC_{50}(\beta EC_{50}+K_A)}{K_A^2+2EC_{50}K_A+EC_{50}^2+\tau EC_{50}(\beta EC_{50}+K_A)} = \frac{\beta}{1+\beta\tau} \qquad \text{Eq. A41}$$

$$EC_{50} = \pm\frac{\sqrt{K_A^2(4\beta^3\tau+\beta^2(\tau^2-4\tau+8)+4\beta(\tau-2)+4)}\pm K_A(\beta(\tau-2)+2)}{2\beta(\beta\tau+1)} \qquad \text{Eq. A42}$$

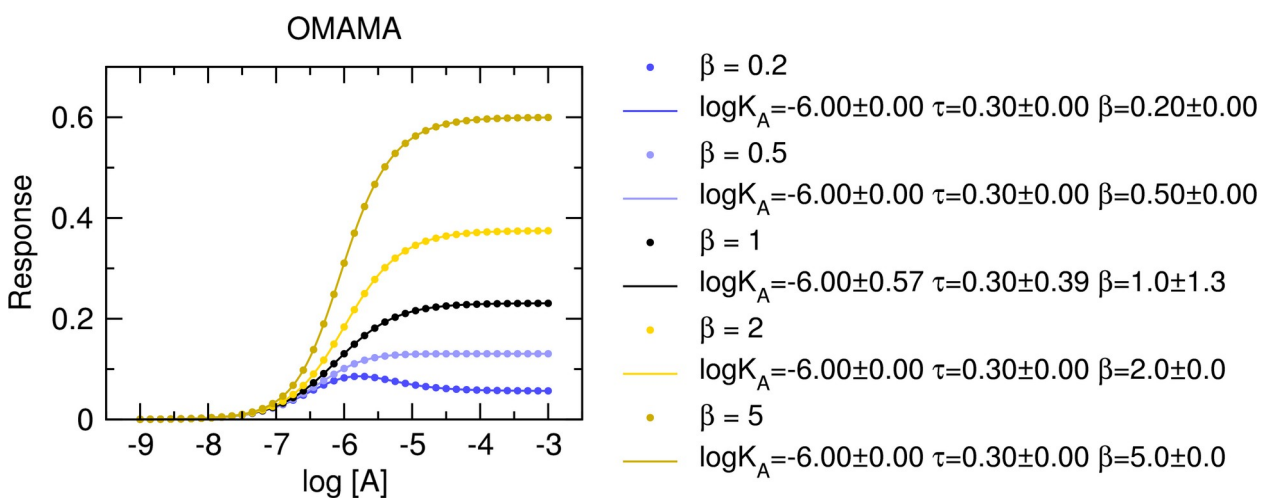

Figure S7 Fitting Eq. A34 to the model of homotropic allosteric modulation of efficacy
Dots, functional-response data modelled according to Eq. A31. E_{MAX} = 1, K_A = 10^{-6}M, τ = 0.3, R_T =1. Factors of cooperativity β are indicated in the legend. Lines, fits of Eq. A34 to the model data. E_{MAX} was fixed to 1. Parameter estimates of the fits are indicated in the legend.

Parameter estimates are correct, associated with low level of uncertainty, except for neutral cooperativity (β=1).

Substrate inhibition of functional response

In the case of substrate inhibition, functional response is given by Eq. A43.

$$E = E_{MAX}\frac{[AR]}{K_E+[AR](1+\frac{[AR]}{K_I})} \qquad \text{Eq. A43}$$

Substitution of Eq. A43 [AR] by binding function Eq. A5:

$$E = E_{MAX}\frac{\frac{R_T[A]}{[A]+K_A}}{K_E+\frac{R_T[A]}{[A]+K_A}(1+\frac{R_T[A]}{K_I([A]+K_A)})} \qquad \text{Eq. A44}$$

Apparent E'_{MAX} at [A]>>K_A is given by:

$$E'_{MAX} = \frac{R_T}{K_E+R_T(1+\frac{R_T}{K_I})} \qquad \text{Eq. A45}$$

After division of Eq. A45 by K_E and substitution $\tau=R_T/K_E$ and $\sigma=R_T/K_I$:

$$E'_{MAX} = E_{MAX}\frac{\tau}{1+\tau(1+\sigma)} \qquad \text{Eq. A46}$$

The apparent value of operational efficacy τ' is given by equation Eq. A47.

$$\tau'=\frac{\tau}{\sigma\tau+1} \qquad \text{Eq. A47}$$

Denominator of Eq. A44 can be rearranged to:

$$K_E+\frac{R_T[A]\frac{R_T[A]}{K_I([A]+K_A)}}{[A]+K_A}$$

The Eq. A44 can be rearranged to Eq. A48.

$$E = E_{MAX}\frac{R_T[A]}{([A]+K_A)\left(K_E+\frac{R_T[A]\left(\frac{R_T[A]}{K_I([A]+K_A)}+1\right)}{[A]+K_A}\right)} \qquad \text{Eq. A48}$$

Eq. A48 after bracket multiplication and rearrangement gives Eq. A49

$$E = E_{MAX}\frac{[A]K_I R_T([A]+K_A)}{[A]^2R_T^2+K_I K_E([A]+K_A)^2+[A]K_I R_T([A]+K_A)} \qquad \text{Eq. A49}$$

After division by K_E and K_I and substitution $\tau=R_T/K_E$ and $\sigma=R_T/K_I$:

$$E = E_{MAX}\frac{[A]\tau([A]+K_A)}{\sigma\tau[A]^2+([A]+K_A)^2+[A]\tau([A]+K_A)} \qquad \text{Eq. A50}$$

To determine half-efficient concentration EC_{50}:

$$E_{MAX}\frac{EC_{50}\tau(EC_{50}+K_A)}{EC_{50}^2\sigma\tau+(EC_{50}+K_A)^2+EC_{50}K_A\tau+K_A^2} = \frac{E_{MAX}}{2}\frac{\tau}{1+\tau(1+\sigma)} \qquad \text{Eq. A51}$$

$$E_{MAX}\frac{EC_{50}\tau(EC_{50}+K_A)}{EC_{50}^2\sigma\tau+(EC_{50}+K_A)^2+EC_{50}K_A\tau+K_A^2} = \frac{E_{MAX}}{2}\frac{\tau}{1+\tau(1+\sigma)} \qquad \text{Eq. A52}$$

$$EC_{50} = \pm\frac{K_A\sqrt{\sigma^2\tau^2+4\sigma\tau^2+8\sigma\tau+\tau^2+16\tau+8}-2K_A\sigma\tau-K_A\tau}{2(\sigma\tau+2\tau+1)} \qquad \text{Eq. A53}$$

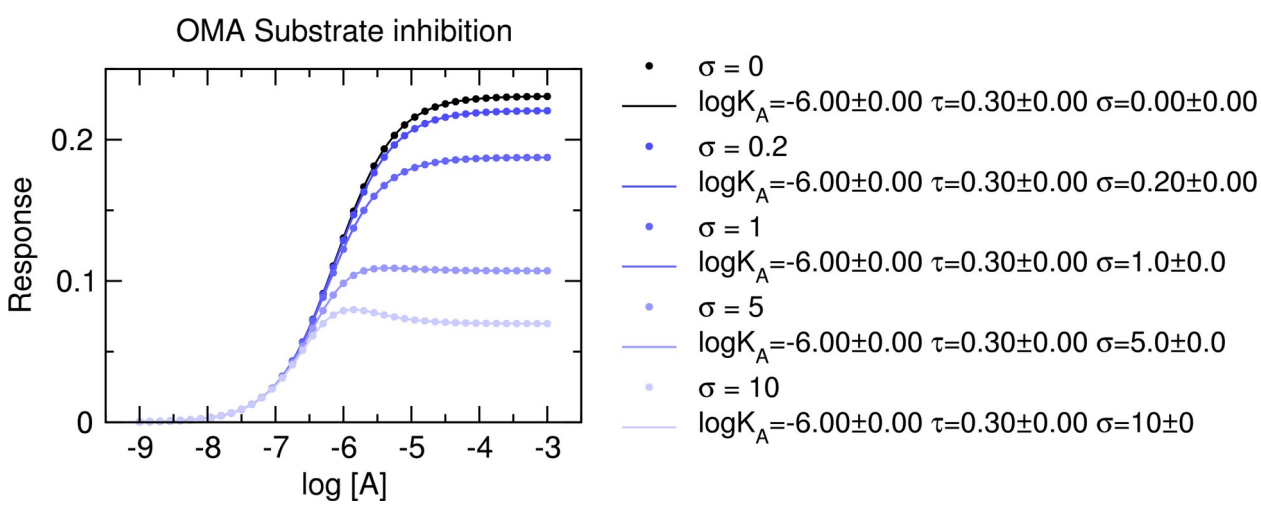

Figure S8 Fitting Eq. A44to the model of substrate inhibition
Dots, functional-response data modelled according to Eq. A44. E_{MAX} = 1, $K_A = 10^{-6}$M, τ = 0.3, R_T =1. Inhibition factors σ are indicated in the legend. Lines, fits of Eq. A500 to the model data. E_{MAX} was fixed to 1. Parameter estimates of the fits are indicated in the legend.

Parameter estimates are correct, associated with low level of uncertainty.

Non-competitive auto-inhibition

In the case of non-competitive auto-inhibition, functional response is given by Eq. A54.

$$E = E_{MAX} \frac{[AR]}{K_E + [AR]} \frac{[AR]}{K_I + [AR]} \qquad \text{Eq. A54}$$

Substitution of the [AR] complexes in Eq. A54 with binding function Eq. A5 yields Eq. A55.

$$E = E_{MAX} \frac{\frac{R_T[A]}{K_A + [A]}}{K_E + \frac{R_T[A]}{K_A + [A]}} \frac{\frac{R_T[A]}{K_A + [A]}}{K_I + \frac{R_T[A]}{K_A + [A]}} \qquad \text{Eq. A55}$$

That simplifies to Eq. A56

$$E = E_{MAX} \frac{R_T[A]}{K_A K_E + K_E[A] + R_T[A]} \frac{R_T[A]}{K_A K_I + K_I[A] + R_T[A]} \qquad \text{Eq. A56}$$

Division of Eq. A56 by K_E and K_I and substitution $\tau = R_T/K_E$ and $\sigma = R_T/K_I$ gives Eq. A57.

$$E = E_{MAX} \frac{\tau[A]}{K_A + [A](1+\tau)} \frac{\sigma[A]}{K_A + [A](1+\sigma)} \qquad \text{Eq. A57}$$

Apparent E'_{MAX} at $[A] >> K_A$ is given by Eq. A57.

$$E'_{MAX} = E_{MAX} \frac{\tau}{1+\tau} \frac{\sigma}{1+\sigma} \qquad \text{Eq. A57}$$

The apparent value of operational efficacy τ' is given by equation Eq. A58.

$$\tau' = \frac{\tau\sigma}{\tau + \sigma + 1} \qquad \text{Eq. A58}$$

For half-efficient concentration EC_{50}:

$$\frac{1}{2} E_{MAX} \frac{\tau}{1+\tau} \frac{\sigma}{1+\sigma} = E_{MAX} \frac{\tau EC_{50}}{K_A + EC_{50}(1+\tau)} \frac{\sigma EC_{50}}{K_A + EC_{50}(1+\sigma)} \qquad \text{Eq. A59}$$

$$\frac{1}{2} = \frac{EC_{50}(1+\tau)}{K_A + EC_{50}(1+\tau)} \frac{EC_{50}(1+\sigma)}{K_A + EC_{50}(1+\sigma)} \qquad \text{Eq. A60}$$

$$EC_{50} = K_A \frac{\sqrt{\sigma^2 + 6\sigma\tau + 8\sigma + \tau^2 + 8\tau + 8} + \sigma + \tau + 2}{2(1+\sigma)(1+\tau)} \qquad \text{Eq. A61}$$

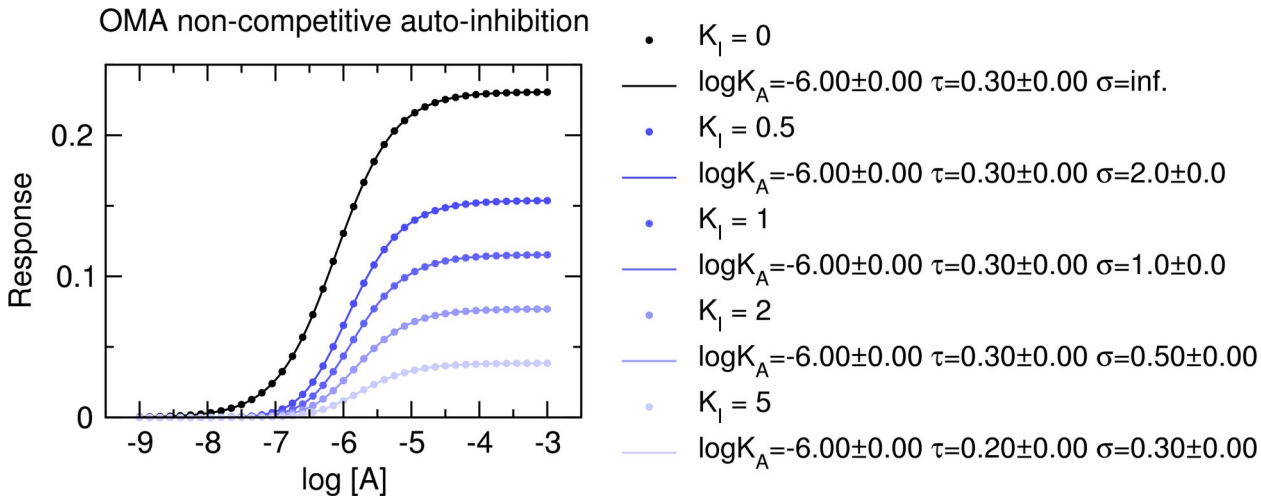

Figure S9 Fitting Eq. A55 to the model of non-competitive auto-inhibition
Dots, functional-response data modelled according to Eq. A55. E_{MAX} = 1, K_A = 10^{-6}M, τ = 0.3, R_T =1. Inhibition factors σ are indicated in the legend. Lines, fits of Eq. A57 to the model data. E_{MAX} was fixed to 1, initial estimate of τ was set to 0.3. Parameter estimates of the fits are indicated in the legend.

Parameter estimates are correct, associated with low level of uncertainty. However, it is result of initial estimate of τ. For K_I=5, estimates of operational efficacy τ and inhibition factor σ are swapped pointing to the symmetry of Eq. A57.

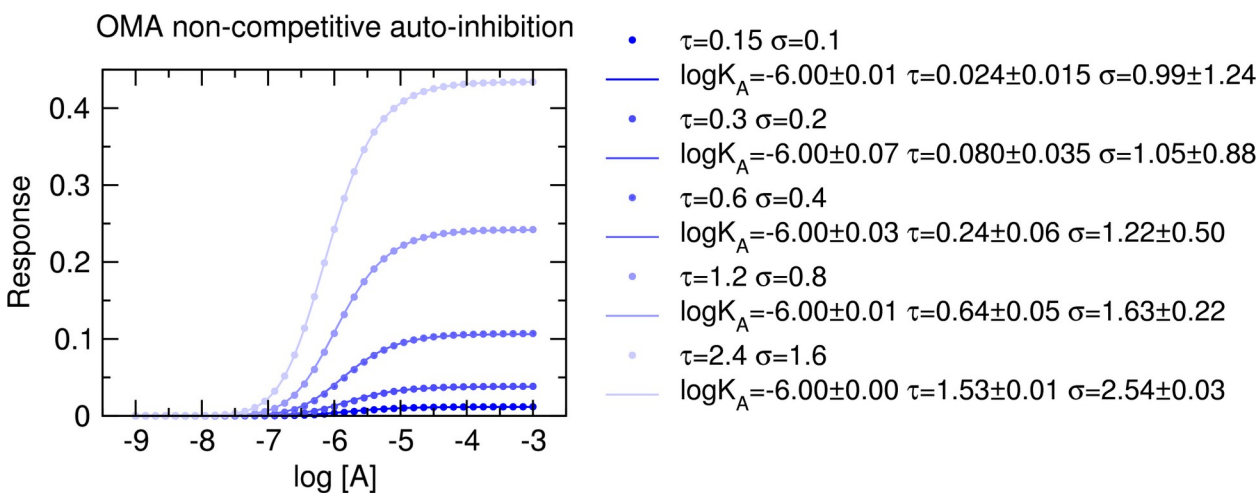

Figure S10 Fitting Eq. A55 to the model of non-competitive auto-inhibition with varying receptor number
Dots, functional-response data modelled according to Eq. A55. E_{MAX} = 1, K_A = 10^{-6}M, K_E = 3.333, K_I = 5. R_T varied from 0.5 to 8. Resulting operational efficacies τ and inhibition factors σ are indicated in the legend. Lines, fits of Eq. A57 to the model data. E_{MAX} was fixed to 1, initial estimate of τ was set to apparent operational efficacy τ' calculated as $E'_{MAX}/(E_{MAX}-E'_{MAX})$. Parameter estimates of the fits are indicated in the legend.

Parameter estimates are incorrect and for low values of τ and σ they are associated with high level uncertainty. However, calculated τ and σ values give correct apparent efficacy according to Eq. A58.

Python scripts

Scripts to model functional-response data and fit equations of Black & Leff, Hill and explicit models to them are provided in python.zip.

Requirements: python, numpy, matplotlib, scipy

Content of python.zip:

Folders:

- Black-Leff
- Hill
- OMAMA_alpha
- OMAMA_beta
- OMA_NCI
- OMA_Rtot
- OMA_SInh

Black-Leff

Black-Leff_generate_5_data_sets.py generates 5 datasets named Data_A.dat through Data_E.dat according to the Black & Leff operational model of agonism (OMA) with parameters in the header of the script. Parameters of the model are saved in Black-Leff.par.

Hill

Hill_generate_5_data_sets.py generates 5 datasets named Data_A.dat through Data_E.dat according to the OMA modified with the Hill coefficient with parameters in the header of the script. Parameters of the model are saved in Hill.par.

OMAMA_alpha

OMAMA_alpha_generate_5_data_sets.py generates 5 datasets named Data_A.dat through Data_E.dat according to the operational model of allosterically modulated agonism (OMAMA) of various factors of binding cooperativity alpha with parameters in the header of the script. Parameters of the model are saved in OMAMA_alpha.par.

Black-Leff_fit.py fits the Black & Leff OMA to the data. Fit results are saved in Black-Leff_fit.res Fit curves are saved as *_Black-Leff_fit.data.

Hill_fit.py fits the OMA modified with the Hill coefficient to the data. Fit results are saved in Hill_fit.res Fit curves are saved as *_Hill_fit.data.

OMAMA_alpha_fit.py fits the OMAMA of various alpha to the data. Fit results are saved in OMAMA_alpha_fit.res Fit curves are saved as *_OMAMA_beta_fit.data.

OMAMA_beta

OMAMA_beta_generate_5_data_sets.py generates 5 datasets named Data_A.dat through Data_E.dat according to the OMAMA of various cooperativity factors of efficacy beta with parameters in the header of the script. Parameters of the model are saved in OMAMA_beta.par.

Black-Leff_fit.py fits the Black & Leff OMA to the data. Fit results are saved in Black-Leff_fit.res Fit curves are saved as *_Black-Leff_fit.data.

Hill_fit.py fits the OMA modified with the Hill coefficient to the data. Fit results are saved in Hill_fit.res Fit curves are saved as *_Hill_fit.data.

OMAMA_beta_fit.py fits the OMAMA of various beta to the data. Fit results are saved in OMAMA_alpha_fit.res Fit curves are saved as *_OMAMA_beta_fit.data.

OMA_NCI

OMA_NCI_generate_5_data_sets.py generates 5 datasets named Data_A.dat through Data_E.dat according to the OMA of non-competitive auto-inhibition with parameters in the header of the script. Parameters of the model are saved in OMA_NCI.par.

Black-Leff_fit.py fits the Black & Leff OMA to the data. Fit results are saved in Black-Leff_fit.res Fit curves are saved as *_Black-Leff_fit.data.

Hill_fit.py fits the OMA modified with the Hill coefficient to the data. Fit results are saved in Hill_fit.res Fit curves are saved as *_Hill_fit.data.

OMA_NCI_fit.py fits the OMA of non-competitive auto-inhibition to the data. Fit results are saved in OMA_NCI_fit.res Fit curves are saved as *_OMA_NCI_fit.data.

OMA_NCI_Rtot

OMA_NCI_Rtot_generate_5_data_sets.py generates 5 datasets named Data_A.dat through Data_E.dat according to the OMA of non-competitive auto-inhibition with varying number of receptors Rtot with parameters in the header of the script. Parameters of the model are saved in OMA_NCI.par.

Black-Leff_fit.py fits the Black & Leff OMA to the data. Fit results are saved in Black-Leff_fit.res Fit curves are saved as *_Black-Leff_fit.data.

Hill_fit.py fits the OMA modified with the Hill coefficient to the data. Fit results are saved in Hill_fit.res Fit curves are saved as *_Hill_fit.data.

OMA_NCI_Rtot_fit.py fits the OMA of non-competitive auto-inhibition with varying Rtot to the data. Fit results are saved in OMA_NCI_Rtot_fit.res Fit curves are saved as *_OMA_NCI_Rtot_fit.data.

OMA_SInh

OMA_SInh_generate_5_data_sets.py generates 5 datasets named Data_A.dat through Data_E.dat according to the OMA of substrate inhibition with parameters in the header of the script. Parameters of the model are saved in OMA_SInh.par.

Black-Leff_fit.py fits the Black & Leff OMA to the data. Fit results are saved in Black-Leff_fit.res Fit curves are saved as *_Black-Leff_fit.data.

Hill_fit.py fits the OMA modified with the Hill coefficient to the data. Fit results are saved in Hill_fit.res Fit curves are saved as *_Hill_fit.data.

OMA_SInh_fit.py fits the OMA of substrate inhibition to the data. Fit results are saved in OMA_SInh_fit.res Fit curves are saved as *_OMA_SInh_fit.data.