Allosteric transition: a comparison of two models

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Abstract

Introduction: Two recent models are in use for analysis of allosteric drug action at receptor sites remote from orthosteric binding sites. One is an allosteric two-state mechanical model derived in 2000 by David Hall. The other is an extended operational model developed in 2007 by Arthur Christopoulos's group. The models are valid in pharmacology, enzymology, transportology as well as several other fields of biology involving allosteric concentration effects.

Results: I show here that Hall's model for interactions between an orthosteric, an allosteric, and a receptive unit is the best choice of model both for simulation and analysis of allosteric concentration-responses at equilibrium or steady-state.

Conclusions: As detailed knowledge of receptors systems becomes available, systems with several pathways and states and/or more than two binding sites should be analysed by extended forms of the Hall model rather than for instance a Hill type exponentiation of terms as introduced in non-mechanistic (operational) model approaches; yielding semi-quantitative estimates of actual system parameters based on Hill's unlikely simultaneity model for G protein-coupled receptors.

Background

A sizeable decline in development of classical agonists and antagonist for medication [1-3] has elicited a drug-hunt to construct and develop allosters in laboratories of academia [4-8] and industry (e.g., Novasite Pharmaceuticals Inc; Addex Pharmaceuticals), including positive and negative allosters as well as ortho-allosters for therapeutic purposes. In doing so, it has become important to simulate and analyse concentration-response data for allosters by models that are as close to the systems mechanistic function as possible.

Optimal allosteric models are in great demand, since mechanistic simulations may be combined with structural analysis of alloster binding, receptor multi-merization and association of molecules as G proteins, arrestins, and RAMPs into synthesis of QSARs for ligand binding and receptor activation [9-16].

Data from equilibrium concentration-response experiments involving allosteric modulators are presently interpreted by unlike choices of model. Therefore, with such schism in selection of model, especially true for data from cell-systems expressing subtype 7TMRs [17], it seems worth a discussion about which direction analysis of synagics data for allosters should take. For possible outcomes of including allosters consult Figure 1. For definitions of terms related to allostery see Table 1.

Two actual allosteric models - ATSM and EXOM. One model is the allosteric two-state model, ATSM, introduced by Hall in 2000, implemented and further discussed by others [5,17-25]. Another model we could call the “extended operational model”, EXOM for short [26], is based on combining the original operational model, BLM [27], with the ternary-complex model, TCM [28], as later further detailed [29-31]. EXOM is implemented and presently advocated by several lead-modellers [7,8,32-38]. There are other approaches taken to model the behaviour of allosters in the field of 7TMRs [20,33,39-42].

ATSM is a mechanistic model. ATSM-analysis with extracted numbers for model parameters supposes direct information about mechanical interactions between allosters, receptors and orthosters at a molecular scale. Thus, one might gain a quantitative and dynamic handle on molecular processes per se within receptors. The other model, EXOM, a non-mechanistic model, is a close relative of ATSM and has the same number of independent parameters to be determined. EXOM is used assuming that individual physical parameters of multistep processes as such cannot be extracted, as they are composite. EXOM may give quantified estimates on
elicited cooperative binding and efficacy for orthosters and allosters interacting at receptors [26,34]. By selecting similar assumptions for ATSM as for EXOM, ATSM may cover the EXOM-scenario and yield estimates of parameters for lumped multi-steps rather than single steps, and thus become a black-box model as the EXOM. In both ATSM and EXOM, allosters may behave as enhancers with ceiling and as competitive antagonists without ceiling. Furthermore, they are also efficient in simulating allo-agonism and allo-synergy both with ceiling effects; observed as lifts of concentration-response curves by allosters at low and high orthoster concentrations [17,26,37]. However, EXOM lacks ATSM’s advantage of being a mechanistic model and for describing spontaneous activity of receptive units. Additionally, from a theoretical point of view, a parameter in EXOM to describe cooperative activity is amputated, yielding illogic results. For this latter conclusion, see details in the next to last sections of Methods and Results and Discussion.

Here I focus on ATSM and EXOM and compare them for simulation and analysis of experimental data. It is demonstrated that there are no arguments as posited [8,17] for employing EXOM instead of ATSM, quite
the other way about. Therefore, my goal is to convince future modellers to use ATSM and possible extended forms for analysis and simulation of allosteric concentration-response relations rather than EXOM.

**Methods**

**One basic model - cTSM**

In simulation of synergics for orthosters and allosters, the basis of most models is often two simple reaction schemes; the cyclic-two-state model, cTSM, and the ternary-complex model, TCM. Since this paper is about modelling as opposed to general statements about ligand-receptor interactions it is paramount with precise definitions including aspects of cTSM and TCM. This has been discussed before [22] and may seem superfluous. However, in order to validate and compare newly derived ATSM and EXOM in a coherent fashion, concepts related to cTSM and TCM must be brought together and systematized. cTSM is dealt with first. The gist of the cTSM, Figure 2A, is its explicit description of a conformational switch between an inactive and active state of a non-bound receptor. It specifically includes spontaneous activity in form of non-liganded receptor R*. The behaviour of cTSM has been scrutinized [43,44]. cTSM has two interesting parameters. L describes the distribution between unliganded inactive and active receptor states, R ⇌ R*, such that

\[ L = \frac{R^*}{R} \]

Figure 2A. Deriving cTSM's distribution equation for activity, the free non-active receptor state R is equated with “1”. Thus, the unliganded, active receptor state R* is equal to L. The second parameter, a, is a concomitant constant for activation of receptor forms bound with ligand S, RS ⇌ R*S. This step has \( aL \) as its efficacy constant. By assuming multi-steps, \( aL \) is identical to Stephenson’s efficacy constant [45] and Black & Leff’s transducer ratio \( \tau \) [27]. \( A_1 \) is the equilibrium affinity constant for S binding to non-active forms of R, Figure 2A. Therefore, \( a \) is also a concomitant constant for binding of S to already activated receptors. The affinity constant for \( S+R^* \) ⇌ R*S is thus \( aA_2 \).

Arguments still appear on how to understand activation of protein molecules when ligands are applied - is it by *induction* after ligands bind or is it rather by ligand *selection* and stabilization of already activated molecules? Jacques Monod early on favoured a selection process [46] and this understanding crystallized in the famous MWC-model [47]. The MWC explicitly introduces an unliganded switch R ⇌ R* as the “allosteric transition” [48]. Contrary, Koshland argued for induction after binding [49]. “Selection” follows one leg of cTSM while “induction” follows another [50], Figure 2A. They are two views on a single process [18] chapter 5. Below, when either “induction” or “selection” is used on activation of receptive units as ligands bind, it covers both pathways in cTSM.

**Another basic model - TCM**

The TCM, Figure 2B, looks fairly simple, but possesses surprising allosteric regimes. Depending on which of the liganded complexes are included for activity, TCM can simulate enhancement with ceiling and competitive (“surmountable”) inhibition, besides allo-agonism without ceiling and “mixed competitive inhibition”. TCM
with tacit active conformations has no allo-synergy or spontaneous activity. Ten sub-models derived from TCM are characterized in Table 2. Three of these sub-models are further described in the Results section and some simulations by these three models are shown in a figure in the Results section.

Operational models
To understand the present use of “stimulus”, “efficacy” and “intrinsic efficacy” in operational models as EXOM, it is necessary to go back to their definitions [45,51,52]. Stephenson’s stimulus concept seems obsolete today by accepting two-step receptor schemes with straightforward derived distribution equations [18] chapter 2; [50] and when needed, apt assumptions of more than two steps. Two-step schemes yield equations identical to initially derived operational models based on the stimulus-response idea [27,51,53]. Concepts as “stimulus”, “transducer ratio” and “fitting parameter” are of course justified in selecting operational model approaches rather than mechanistic ones. Spontaneous activity often seen in studies with 7TMRs is not included in the realm of operational models, although recently serious attempts have appeared [54,55].

Meanwhile, users of operational models should recognize that their assumptions for derivation put a veil over underlying physical systems and that any involved “operational” assumption may just as well be applied to the ATSM. For instance, as mentioned, $a\rightarrow L$ can be conceived as equal to transducer ratio $r$.

Distribution equation for ATSM and EXOM
Reaction schemes of ATSM and EXOM are depicted in Figure 3A and 3B. The intention with EXOM was to derive a stimulus-equation for activating receptors, including alloster-activated units, while explicitly excluding non-ligated active conformations [26]. Thus, three bound species RS, MR, and MRS in EXOM can switch to active forms $R^*$, $M^*$, and $M^*$R. But, in order to exclude constitutive activity, non-ligated R is not allowed a switch to active $R^*$, Figure 3B. Thus, EXOM is a pure “induction” reaction scheme in Koshland-sense, as free forms of receptor $R$ must be bound before activation. The three bound and active forms of the receptor are equated as “stimulus” and transformed through a hyperbolic expression for activity, as for the BLM. The result is a distribution equation with three active conformations to a total of seven conformation, as even a possible inactive $R^*$-conformation is considered non-existent [26].

To simplify a comparison of EXOM with ATSM, distribution equations for both are expressed parallel to earlier expressions for ATSM [18] chapter 7.

Table 2 Phenotypic concentration-responses for allosters in 10 sub-models from TCM

| Type of TCM model | # | Enhancement $\leftarrow$ w/ ceiling $\rightarrow$ | Allo-agonism $\uparrow$ | w/ ceiling $\uparrow$ | Strict allo-synergy $\uparrow$ | Allo-modification w/ ceiling $\rightarrow$ | modifier - EC$_{50}$ ↓ |
|-------------------|---|----------------------------------|------------------|-----------------|--------------------------|--------------------------|-----------------|
| (S)/4             | 1 | no                               | na               | no              | na                       | no                       | na              |
| (S+MS)/4          | 2 | yes                              | yes              | no              | na                       | no                       | competitive     |
| (S+M+MS)/4        | 3 | yes                              | yes              | no              | yes                      | no                       | na              |
| (S)/4            | 4 | no                               | na               | inverse         | yes                      | no                       | yes             |
| (MS)/4            | 5 | yes                              | yes              | no              | (yes)                    | (yes)                    | na              |
| (S)/3            | 6 | no                               | na               | no              | na                       | no                       | modifier - EC$_{50}$ ↓ |
| (S+MS)/3          | 7 | yes                              | no               | na              | yes                      | no                       | no              |
| (S)/3*           | 8 | yes                              | no               | na              | (yes)                    | na                       | competitive**   |
| (S+M/3)          | 9 | no                               | yes              | no              | no                       | no                       | no              |
| (S+M)/3*         | 10 | no                              | na               | yes              | no                       | no                       | no              |

For model types in the left column, terms S, M, and MS in parenthesis indicate active forms of the liganded receptor as either $R^*$S, $M^*$R or $M^*$R, and with the total number of receptor conformations after the slash. In models 6–8, complex MR is not formed. Model 7 is the classical uncompetitive reaction scheme. * In models 9–10, complex MRS is not formed. ** Model 9 is classical type II reaction scheme for competitive inhibition with no ceiling, the same as assuming parameter $c = 0$. Arrows indicate direction of affinity change and direction of ceiling effects.

$na =$ not applicable, (yes) indicates that there is an effect in form of co-agonism, i.e., no response for ligand 5 alone.

Simulations of concentration-response relations for tabulated sub-models 1–4, in column 2, are shown in Figure 4 panels A–I. S stands for orthoster and M for alloster. Ceiling effects for enhancement (= parameter $c < 1$) in sub-model 2 starts at $A_{MS, M} > 1$, panel D in Figure 4. Allo-competitive antagonism (= parameter $c < 1$) in sub-model 2 requires $c_{A_{MS}, M} > 10$ for a ceiling effect to appear. Thus, sub-model 2 simulates genuine competitive antagonism as long as the product $c_{A_{MS}, M}$ is below 10, Figure 4 panel F. This dependence on product $A_{MS, M} > 1$ for ceiling effects of enhancement and on product $c_{A_{MS}, M} > 10$ for ceiling effects in allo-competitive-inhibition are also characteristics of both ATSM, Figure 5 panels A and C, and EXOM, Figure 5 panels D and E.

Tabulated ternary-complex sub-model 1 and 6 with parameter $c < 1$ are characterized as (mixed) modifier mechanisms in enzymology. Their mixed allo-modification includes a possible simulation of classical non-competitive antagonism with a fixed EC$_{50}$ when $c = 1$, Figure 4 panel B. Furthermore, both sub-models 1 and 6 have increasing affinity for increasing modifier concentration, indicated by EC$_{50}$ ↓ in column B. Sub-type model 4, excluding the ternary complex MRS as active, may show inverse agonism with decreasing ceiling values for the apparent affinity EC$_{50}$ when parameter $c > 1$ and increasing ceiling levels for EC$_{50}$ when parameter $c < 1$, Figure 4 panels J–L.

Sub-models 5 and 8 demonstrate co-agonism, which means that both ligand 5 and ligand M have to be present for an activity to show up, simulations not shown.

Sub-model 7 is identical to the classical un-competitive reaction scheme. Sub-models 9 and 10 are based on the classical type II competitive reaction scheme, excluding the double-ligated MRS conformation ([18] chapter 2), and therefore do not qualify as true TCMs.

Two characteristics for ATSM and EXOM are not covered by any of the listed TCM reaction schemes in Table 2, viz. a strict allo-synergy, Figure 5 panels M and N, and ceiling effects for allo-agonism, compare Figure 4 panels G–I with Figure 5 panels G–I, H–J, K–M, Q–R, and T–U.
Deviations between the two models are marked by bracketed and bolded symbols. Definitions of symbols listed below are followed by symbols in parenthesis from Leach [26] and Hall [22]: E = actual response; E\textsubscript{m} = maximal activity; S = orthoster (A; A); M = alloster (B; B); A\textsubscript{m} = equilibrium association constant for ligand S (1/K\textsubscript{Bm}, M); A\textsubscript{m} = equilibrium association constant for ligand M (1/K\textsubscript{Bm}, M); a = efficacy constant for S (τ\textsubscript{A}, a); b = efficacy constant for M (τ\textsubscript{B}, β); c = binding cooperativity constant (α; τ\textsubscript{A}); and d = activation cooperativity constant (β, δ). Parameter β for EXOM is only defined for cooperativity of an alloster on orthoster activation, but not reciprocally as in ATSM. Further, unlike ATSM, EXOM has a Hill type exponentiation parameter, n, for terms of summed activity and inactivity. The benefits of including such a Hill exponentiation may be questioned as discussed earlier [18] chapter 10. Indeed, Hill-type exponentiation may also be applied to ATSM. However, as ATSM is a mechanistic approach, it seems more logical to derive equations based on formulation for an extended ATSM with more than two binding sites [18,25].

In absence of an orthoster the initial efficacy, IntEff, for ATSM is given by: \[L/(L+1+ A\textsubscript{m}M)/((1+b⋅A\textsubscript{m}M)/1+(1+b⋅A\textsubscript{m}M))\], and for EXOM, assuming n = 1, by: \[1/(1+1+A\textsubscript{m}M)/((1+b⋅A\textsubscript{m}M)/1+(1+b⋅A\textsubscript{m}M))\].

For high values of the orthoster, S\rightarrow\infty, maximum activity, MaxEff, as a function of alloster concentration for ATSM is given by: \[L/[L+(1+c⋅A\textsubscript{m}M)/(a⋅(1+b⋅c⋅d⋅A\textsubscript{m}M))\], and for EXOM, assuming n = 1, by: \[1/[1+(1+c⋅A\textsubscript{m}M)/(a⋅(1+b⋅c⋅d⋅A\textsubscript{m}M))\]. Differences between ATSM and EXOM expressions are indicated with bolded types.

**Best-fit analyses to experimental data for ATSM and EXOM**

The analyses were performed in the following manner. Selected allosteric effects were obtained from data-figures in the literature, data-figure 1 ([38], Figure 2B), data-figure 2 ([37], Figure 2B), and data-figure 3 ([56], Figure 3). Model parameters a and A\textsubscript{s} were first evaluated by fitting the distribution equations for ATSM and EXOM to response data at zero alloster concentration. The obtained values for a and A\textsubscript{s} were then inserted into
the distribution functions for the two models and used for an ensuing fitting of the remaining parameters listed in the last Table, parameters b, c, d, and Am. By varying the initial values for each parameter in three steps, at least 12 fits were performed on each curve for every alloster concentration in all three data-figures. Only fitted parameter values with convergence to a tolerance of $10^{-10}$ in SigmaPlot software were accepted.

Thus, concentration-response curves at three different alloster concentrations yields three best-fit values for each of the four parameters. Obtained results for the single parameter in the last Table represent a ratio between the two best-fit values with the largest mutual difference of the three determinations for each parameter at different alloster concentrations. A global fit to data sets for all four parameters [57] was not possible.

A fourth data set, data-figure 4 ([36], Figure 1C), was also analysed but neither ATSM nor EXOM fitted well to these data with a 44% spontaneous activity and a 56% alloster/orthoster response. The failure of fitting was mostly due to a lack in obtaining a reasonable determination of maximal response for several of the concentration-response curves.

**Results and discussion**

**TCM - three and ten variants**

Three functional variants of TCM are briefly described below and examples of their simulations shown in Figure 4, while characteristics of ten different forms derived from TCM are listed in annotated Table 2.

In a first form, complex RS tacitly moves to R*S as the sole source of activity. Simulation of this allo-scheme can resemble classical non-competitive antagonism for orthosters in functional assays, where only the maximal effect attenuates as the concentration of an alloster increases while the dissociation constant for the agonist stays constant. This happens for activity when constant c is unity. An example is shown in Figure 4B. Note, that in TCM occupancy, alloster effects can never be non-competitive-like, i.e., with reduced activity and fixed EC$_{50}$.

In a second form, S-liganded conformations, RS and MRS, move tacitly to R*S and MR*S as source of activity. This reaction scheme gives us models of activity and occupancy that behave in an identical manner as their distribution equations are identical. This reaction scheme includes enhancement for constant $c > 1$ and with ceiling when $A_m \cdot M > 1$ and competitive inhibition when $c < 1$, but with a ceiling effects for both binding and activation by an alloster when $c \cdot A_m \cdot M > 10$, Figure 4D and 4F. This model is identical to the uncompetitive reaction scheme.

In a third form, all liganded conformations, i.e., RS, MR, and MRS, are sources of activity, Figure 2B. In EXOM, this is the basic TCM. TCM sub-type 3 may simulate allo-agonism for activity, but without ceiling effects as indicated by black circles for limiting EC$_{50}$ values as $M \to \infty$, Figure 4G-I.

Since the term “competitive inhibition”, according to an informative review [48], meant inhibition through an overlap or steric hindrance at binding sites [58], the term “allosteric inhibition” was used from the start of the 1960s merely to indicate negative feedback different from competitive inhibition. Nothing more. TCM with its two remote binding sites has no mutual exclusion by steric hindrance or by overlap. Meanwhile, TCM may still simulate “competitive inhibition”, either by its un-competitive form as shown in Figure 3E, or by mutual exclusion of triple complex MRS through remote or intermolecular conformational changes, not shown. Thus, TCM has allosteric inhibition in the MWC-sense. “Competitive inhibition” by mutual exclusion in TCM requires that the cooperative binding constant c goes to insignificantly small values, thus preventing detectable levels of MRS and of its tacitly active form, MR*S. Such allosteric mutual exclusion, as one type II competitive inhibition ([18], chapter 2) has been cartooned ([58], Figure III-1, panel 5). Thus, as “allosteric” solely refer to ligand binding at remote, non-overlapping binding sites and without steric hindrance, “allosteric” becomes a pleonasm in “allosteric ternary complex model”, ATCM, as TCM is defined by having two, non-overlapping binding sites without steric hindrance. As both acronyms cover the exact same model, it remains a matter of taste using either ATCM or TCM. Contrary, the signifier “allosteric” in “allosteric transition” [48] becomes indicative for two-state models as MWC and ATSM, involving cTSM.

**Comparison of simulations from ATSM and EXOM**

A comparison is made between ATSM and EXOM simulations of concentration-responses of activity with orthoster concentration as independent variable and with varying alloster concentration M. Thus, the following are principal statements about parameter influences on initial and maximal efficacies, on ceiling effects for enhancement, competitive and mixed inhibition, on allo-agonism and -synergy, as well as on apparent dissociation constant EC$_{50}$. To simplify the comparison, EXOM slope factor n is assumed unity. The results reveal a few crucial differences between the two models even based on homologous parameters as $A_v$, $A_m$, a, c, and d.

As indicated above, IntEff for EXOM is dependent on parameter b, while for ATSM it is dependent on both b and L. For ATSM, MaxEff is dependent on $L \cdot a$, whilst EXOM-MaxEff is only dependent on a. Thus, when comparing ATSM and EXOM, choice of values for a and b in EXOM should match with values for L-a and $L \cdot b$ in ATSM. Accordingly, in selection of parameter values for compared simulations with L for ATSM chosen as 0.01 in order to suppress spontaneous activity,
Figure 4 Reaction schemes of the allosteric two-state model, ATSM, and the extended operational model, EXOM. Panel A. The ATSM. Panel B. The EXOM. The models are presented with their basic simpler reactions schemes as the cTSM and TCM from Figure 2. The cubic ATSM has eight receptor conformations while the EXOM only has seven of those, as the spontaneous active represented by receptor conformation R* is excluded. The two models have the same total number of parameters, seven in all. Besides parameters defined in Figure 2, ATSM and EXOM have parameter $b$, an efficacy constant when the alloster M-bound receptor is activated, and parameter $d$ a cooperativity efficacy constant involving two ligands. The constants $L, A_c, A_{int}, a$ and $c$ are given as in Figure 2, and EXOM has a slope factor $n$, not shown.
values for $a$ and $b$ in EXOM are chosen 100 fold higher in ATSM, Figure 5.

IntEffs for both ATSM and EXOM are always completely independent of $A_s$, $a$, $c$, and $d$. ATSM-IntEff is dependent on $L$ and $b$-$A_m$-M. For more details see annotated Table 3. EXOM-IntEff only depends on $b$-$A_m$-M. Allo-agonism is a lift in the IntEff when supplying an alloster even before an orthoster is added. Various forms of allo-agonism are shown in Figure 5G-Z and with ceiling effects indicated by black circles for the limiting EC50 values as $M \to \infty$. Allo-agonism takes effect in both models when both $b$ and $b$-$A_m$-M are larger than unity. Furthermore, ATSM may simulate spontaneous activity before any ligand is added. Simulation of detectable spontaneous activity starts at values of $L$ above $10^{-2}$. This possibility is excluded from the EXOM theory.

MaxEff in ATSM is dependent on $L$-$a$ and $b$-$c$-$d$-$A_m$-M, Table 3, while MaxEff in EXOM is dependent on $a$ and $c$-$d$-$A_m$-M. In comparison, EXOM-MaxEff demonstrates complete independence of $b$, which is somewhat inconsistent. The independence is due to the definition of parameter $d$ ($\beta$) in EXOM, where an alloster only affects the efficacy of an orthoster with no reciprocity. Thus, synergy and mixed inhibition are different between ATSM and EXOM, since the MaxEff-ATSM has both parameter $b$ and $d$ involved while EXOM only depends on $d$.

As already indicated, more details on parameter influences on IntEff, enhancement, allo-agonism, allo-synergy, MaxEff, and mixed inhibition are given in comments to Table 3.

Ceiling effects of enhancement and allo-agonism by positive allosteric modulators (PAMs) are hallmarks and
Table 3 Conditions for alloster effects on initial efficacy and maximal efficacy in ATSM

| Assumptions for product parameters (M) | Reduced equation | Lower level product assumptions | Reduced equation at lower level product assumptions | IntEff / MaxEff their dependence on product of (M) |
|---------------------------------------|------------------|---------------------------------|-----------------------------------------------|----------------------------------|
| possible alla-agonism of IntEff for [orthoster] → 0: L/[L+(1+α M)/(1+b: A M)] | | | | |
| b: A M M > > 1 | L-b A M = X | A M M > > 1 | L-b /L+b | L-b vs 1 |
| | X/(X+1+ A M) M | | | |
| b: A M M = 1 | L-2(L-2+1+ A M) M | A M M >> 1 | L-2(L-2+ A M) M | L-2 vs A M M |
| | | A M M = 1 | L+L | L vs 1 |
| | | A M M < < 1 | L-2(L-2+1) M | L-2 vs 1 |
| b: A M M < < 1 | L/(L+1+ A M) M | A M M > > 1 | L/(L+1) M | L vs A M M |
| | | A M M = 1 | L/(L+2) M | L vs 2 |
| | | A M M < < 1 | L/(L+1) M | L vs 1 |
| possible alla-synergy of MaxEff for [orthoster] → 0: L:a/(L:a+(1+c A M)/(1+b c: A M))] | | | | |
| b: c d A M M > > 1 | with L-a b: c d A M = Y | c A M M > > 1 | L-a b: d/(L:a b: d+1) | L-a b: d vs 1 |
| | | c A M M = 1 | L-a b: d/(L:a b: d+2) | L-a b: d vs 2 |
| | | | | b d >> 1 |
| | | c A M M < < 1 | Y/(Y+1) | Y vs 1 |
| | | | | b d >> >> 1 |
| b: c d A M M = 1 | with L-a 2 = Z | c A M M > > 1 | Z/(Z+c A M) | Z vs c A M |
| | | c A M M = 1 | L/a(L-a+1) | L/a vs 1 |
| | | | | b d = 1 |
| | | c A M M < < 1 | L-a 2/(L-a+2) | L-a 2 vs 1 |
| b: c d A M M < < 1 | L-a/(L-a+1+c A M) M | c A M M > > 1 | L-a/(L-a+2) | L-a vs c A M |
| | | c A M M = 1 | L-a/(L-a+2) | L-a vs 2 |
| | | | | b d >> 1 |
| | | c A M M < < 1 | L-a/(L-a+1) | L-a vs 1 |

Initial and maximal response for ATSM with orthoster concentration as independent variable with an interfering alloster. M or [M] stands for alloster concentration. Conditions are listed with decreasing number of parameters from column 1 to 5 for products of M and parameters that affect the initial efficacy, IntEff, at very low concentrations of orthoster, S, and the final maximal efficacy, MaxEff, at very high concentrations of S. All conclusions for IntEff and MaxEff of ATSM are similar for the EXOM with the following exceptions: for EXOM 1) parameter L is replaced with 1 in all statements for ATSM and 2) parameter b disappears out of all MaxEff statements as listed for ATSM.

Below are further details about effects of parameters and alloster concentration on IntEff and MaxEff for ATSM and EXOM.

**Initial efficacy.** IntEff for ATSM or spontaneous activity:
For b = 1, IntEff = L/(L+1) and independent of the value of A M M. For b > 1, IntEff > L/(L+1) with increasing values of A M M above 1 the IntEff increases towards a ceiling value of L/(L+b-1), equal alla-agonism. For decreasing values of A M M below 1, the IntEff goes towards L/(L+1).
For b < 1, IntEff < L/(L+1) with increasing values of A M M above 1 the IntEff reduces towards a ceiling value of L/(L+b+1). For decreasing values of A M M below 1, the IntEff increases towards L/(L+1).

**Maximal efficacy. MaxEff for ATSM:**
When b: c d A M M > > 1 and as long as c A M M ≥ 1, ATSM-MaxEff is always dependent on the product b d and independent of the value of c A M M. For b d = 1, MaxEff = L/a(L+a+1), independent of c A M M. For b d > 1, MaxEff > L/a(L+a+1), = synergy. With increasing values of c A M M above 1, the MaxEff increases towards a ceiling value of 100%, i.e., above L/a(L+a+1) L-a(L+a+1) vs 1.

**MaxEff for EXOM:**
MaxEffs for EXOM are as well as for ATSM dependent on c d A M M. Further, for c A M M = 0 when b d > 1, EXOM-MaxEff goes to 100%, while for c A M M > > 1 but with c d A M M < < 1, it is determined by a/(a+c A M M). When c A M M ≤ 1 and c d A M M > > 1, EXOM-MaxEff goes to 1, while for d << 1, it goes to zero.
often detected in experiment [17,35-37]. These ceiling effects appear for $A_m \cdot M > 1$, panels A, D, G, J, M, Q, T, and X in Figure 5. Ceiling effects for competitive inhibition are determined by cooperative binding constant $c < 1$ and appears for $c \cdot A_m \cdot M > 10$, and best seen for $b \cdot d = 1$, panels C, F, I, L, P, S, V, and Z in Figure 5.

The ATSM was rejected as model for allo-competitive inhibition by gallamine at muscarinic subtype M2 receptors [20]. Meanwhile, both ATSM and EXOM can nicely simulate competitive inhibition with values of $c$ low enough to keep the parameter products $b \cdot c \cdot d \cdot A_m \cdot M$ for ATSM and $c \cdot d \cdot A_m \cdot M$ for EXOM less than 10, exemplified in Figure 5C and F.

Allo-synergy, seen in the presence of allosters as a lift in MaxEff above MaxEff for orthosters alone, is now commonly described for agonistic-PAMs as well [5,8,25,36]. In ATSM, these characteristics of PAMs with MaxEff above maximal response for endogenous ligands alone may be simulated with values of $b$ and $d$ when their product is $> 1$, Figure 5M-N, while EXOM can simulate allo-synergy for $d > 1$, not shown. Mixed inhibition, appearing as values of MaxEff lower than MaxEff with orthosters alone in the presence of NAMs, including pure non-competitive inhibition, may be simulated for $b \cdot d < 1$ in ATSM, Figure 5U, and for $d < 1$ in EXOM, Figure 5Y. Published examples of negative allosteric effects are now increasing as more interest is invested in development of NAMs [12,32,60].

In both allo-synergy and allo-inhibition, parameter $c$, as its value is lowered, will narrow the gap between MaxEff in the presence and absence of an alloster; compare panels M-P and panels T-Z in Figure 5.

The lack of effect of parameter $b$ on MaxEff in EXOM clearly weakens the theory, even though additional details have been presented on the behaviour of EXOM [34]. A variant of EXOM has been developed with lumped parameters thus avoiding the problem of a missing effect of parameter $b$ in MaxEff [24].

### Table 4 Parameter ratios from best-fits with ATSM and EXOM on three data sets

| Model for analysis | Data- figure # | Parameters ratios from best fits to concentration-response curves for orthosters at three different concentrations of allosters |
|--------------------|---------------|----------------------------------------------------------------------------------------------------------------|
|                    |              | $b$ | $c$ | $d$ | $A_m$ |
| ATSM               | 1            | 4.9 | 3.4 | 3.2 | 1.8 |
| EXOM               |              | 46  | 1.9 | 3.0 | 2.6 |
| ATSM               | 2            | 2.8 | 97  | 11  | 15  |
| EXOM               |              | 50  | 17  | 3.0 | 84  |
| ATSM               | 3            | 1.6 | 9.2 | 16  | 1.5 |
| EXOM               |              | 26  | 35  | 33  | 3.8 |

Each single parameter ratio from best fits with ATSM or EXOM is adapted from analysis of three sets of data in the literature, data-figures 1 to 3, see last section in Methods for references. Each data set consists of four concentration-response curves, where one curve is an orthoster concentration-response curve without an alloster present and the three other curves are orthoster concentration-responses experimentally obtained at three different alloster concentrations. Parameters $a$ and $A_s$ for both ATSM and EXOM were initially determined by model-fits to the basic orthoster concentration-response curves without an alloster present. Obtained values for $a$ and $A_s$ were inserted in the model equations, which were then used for fitting to experimental data of the parameters $b$, $c$, $d$, and $A_m$ in the theories. Each number in the table is a ratio between best-fit values with the largest deviation between two of three results from fits for the single parameter to three concentration-response curves at different alloster concentrations. * For responses indicating spontaneous activity as in data-figure 2, evaluation by EXOM theory was performed by assuming a level of 9% spontaneous activity, thus fitting the EXOM distribution equation to 91% activity for all results from fits for the single parameter to three concentration-response curves at different alloster concentrations.

Although exponentiation in form of a Hill coefficient may also be invoked for both models, such exponentiation was omitted in the present analysis. Also, an interpretation and detailed discussion of the actually obtained parameter values are beyond the scope of this paper.

Thus, based on the ratios in Table 4, it may be concluded that ATSM seems to be better than EXOM at evaluating possible parameter values with a requirement of consistency when determined at 3 different alloster concentrations, since in general most of the ratios are closer to unity when employing the ATSM.

### Conclusion

In a beautiful review, non-mechanistic EXOM against mechanistic ATSM is debated and further contrasted with an empirical general description of synagic behaviour of allosters in different experimental setups [17]. When system information is limited, analyses of allosteric behaviour by operational, empirical and mathematical approaches as Hill’s exponentiation are still valid. Meanwhile, analysing systems of allosteric synagics as discussed here, the best description of allosteric effects is by Hall’s millennium milestone mechanical model [22] due to shortcomings of EXOM. Limitations of mechanistic models as the ATSM are given with its
assumptions, which usually both exclude more than two binding sites and multi-steps or parallel pathways. The ATSM may still replace the EXOM as a phenomenological model by applying assumptions similar to those for EXOM. For the future, allosteric models should be developed based on ATSM and implicating multi-binding and diverse pathways of receptor activation when needed. Thus, instead of switching to non-mechanistic approaches as EXOM or reduce requirements for the basic TCM to analyse such systems [20,26], phenomenological or extended forms of the ATSM should be preferred (e.g., [25]).

**Abbreviations**

ATSM: Allosteric two-state model; EXOM: Extended operational model; cTSM: Cyclic two-state model; BLM: The Black & Leff operational model; ATCM: Allosteric ternary-complex model; EC50: Apparent dissociation constant at 50% activity; IffEff: Initial efficacy; MaxEff: Maximal efficacy; PAMs and NAMs: Positive and negative allosteric modulators; QSR: Quantitative structure-activity-relationship.

**Competing interest**

The author declares no conflicts of interest.

**Authors’ contribution**

NB developed and wrote the MS.

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