Steady-States of Receptor–Ligand Dynamics: A Theoretical Framework

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Abstract

This paper studies aspects of the dynamics of a conventional mechanism of ligand-receptor interactions, with a focus on the stability and location of steady-states. A theoretical framework is developed, which is based upon the rich and deep formalism of irreducible biochemical networks. When represented in this manner, the mass action kinetics of biochemical processes can be clearly seen in terms of their component biochemical interactions, their kinetic rate constants, and the stoichiometry for the system. A minimal parametrization is provided for models for two- or multi-state receptor interaction with ligand, and an "affinity quotient" is introduced, which allows an elegant classification of ligands into agonists, neutral agonists, and inverse agonists.

Key words: multi-state receptor models, agonist classes, biochemical networks
PACS:

1 Introduction

Models of receptor–ligand interactions play an important role in understanding the biochemical mechanisms that initiate cellular signaling. They also serve the practical purpose of guiding the identification and optimization of new therapies that interact at receptors.

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[1] Supported in part by Fundação Calouste Gulbenkian and by a grant from Aventis.
[2] Supported in part by NIH Grants P20 GM64375 and R01 GM46383

The earliest models were based on the specific receptor–ligand interaction that results in Langmuir saturation [15]. Subsequently, it was realized that receptor–ligand interaction can have at least three outcomes [8,10]. First, a ligand can function as an *agonist*, resulting in a distinct biological consequence, such as contraction, secretion, or chemotaxis. Second, a ligand can bind to a receptor with no effect, i.e., as a *neutral agonist*, but this neutral activity can be used to *block* or *antagonize* an agonist. And third, if the receptor produces an intrinsic or constitutive amount of activity, a ligand can suppress this constitutive response by functioning as an *inverse agonist*. Down-stream biochemical feedback loops and other processes that modulate or limit the initial receptor–ligand interaction can further complicate the ligand–receptor interaction; these secondary events will not be discussed here.

Many models have been developed to explain ligand–receptor interactions (for reviews, see, inter alia, [16], in [9]). For these models, the biochemical reactions are delineated and their interactions diagrammed. A system of differential equations is then formulated to represent the time-dependent events that result from mass action kinetics. Experimental data for receptor–ligand interactions are obtained at relatively long times that are taken to be at steady-state, and for this reason, the representative differential equations are converted to algebraic equations for the steady-state condition. The final results are expressed in terms of equilibrium constants derived from kinetic constants. Even with a modest increase in the number of biochemical interactions, these models produce complex expressions, that can require the use of computer-based equation solvers [1]. The formulas obtained in this manner are complicated and virtually impossible to interpret in biological terms, which suggests the appeal of a more theoretical and conceptual approach. In this paper, we introduce such an approach.

Our approach is based upon the "complex balancing" ideas described by Horn and Jackson [7] and Feinberg [5,6]. It allows a systematic and concise description of the mass action kinetics of biochemical processes, expressed in terms of their component biochemical interactions, their kinetic rate constants, and the stoichiometry for the system, and it greatly simplifies the study of their dynamical behavior, steady-states, and stability properties. Among other benefits of this approach, we will be able to:

(1) guarantee existence and uniqueness (subject to stoichiometry constraints) of positive steady-states,
(2) guarantee global (subject to stoichiometry constraints) stability of these unique steady-states,
(3) provide an explicit and simple parametric analysis of the dependence of the steady-state values on the kinetic constants and initial concentrations, and
(4) introduce an *affinity quotient* which allows the classification of ligands into agonists, neutral agonists, and inverse agonists.

Using this mathematical formalism, the response curves of a receptor model that consists of two receptor conformations and corresponding receptor–ligand complexes will be studied in detail. For example, the two-state model has been used to describe the responses

of the chemotactic cAMP receptor of the slime mold amoeba *Dictyostelium* [4]. We show that this model can exhibit the dose-response curves corresponding to inverse agonists, as well as those of positive and neutral agonists, depending on the relative values of the kinetic constants. We will derive equations that characterize agonism classes in terms of the kinetic constants. These results will be extended to the multi-state receptor case, and will show that allowing more than two receptor conformations introduces *no qualitatively new behavior* into the system, in agreement with previous observations [10,16].

As already mentioned, our approach is based upon the rich and deep theory developed by Horn, Jackson, and Feinberg for irreducible biochemical networks, and more specifically, in the language of [6], for *zero-deficiency and weakly-reversible chemical networks* (we will call such networks *HJF networks*, so as to reflect the contributions of the above authors). For convenience, we employ the formalism and notations introduced in [14], and also appeal to theoretical results on global convergence shown in that reference and in [3].

2 Theoretical background

Our approach to mathematical models of receptor–ligand interactions begins by formulating the system graphically in terms of nodes consisting of elemental events or "complexes," and of edges comprised of reaction rates. In order to illustrate the formalism, let us consider first the two-state receptor model which is depicted in Figure 1. Here,

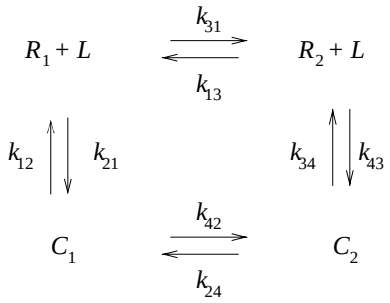

Fig. 1. A two-state receptor–ligand network.

$R_1 = [R_1]$ represents the concentration of free receptors in an inactive state, $R_2 = [R_2]$ represents the concentration of free receptors in an active state, $L = [L]$ represents the concentration of free ligand, and $C_1 = [R_1L]$, $C_2 = [R_2L]$ represent the two corresponding receptor–ligand complexes. From this diagram, and based on the principles of mass action kinetics, one derives in a routine fashion the following set of differential equations:

$$\frac{d\,R_1}{d\,t} = -(k_{21} + k_{31})R_1L + k_{12}C_1 + k_{13}R_2L$$
$$\frac{d\,R_2}{d\,t} = -(k_{13} + k_{43})R_2L + k_{31}R_1L + k_{34}C_2$$

$$\begin{aligned}
\frac{d\,L}{d\,t} &= -k_{21}R_1L - k_{43}R_2L + k_{12}C_1 + k_{34}C_2 \\
\frac{d\,C_1}{d\,t} &= -(k_{12}+k_{42})C_1 + k_{21}R_1L + k_{24}C_2 \\
\frac{d\,C_2}{d\,t} &= -(k_{34}+k_{24})C_2 + k_{42}C_1 + k_{43}R_2L
\end{aligned} \tag{1}$$

We now discuss the general formulation, for an arbitrary biochemical network which consists of reactions among n individual species $x_1, x_2, \ldots x_n$. In the example in Figure 1, there are five species: R_1, R_2, L, C_1, C_2. In such a general network, there will be a number m of nodes, representing each group of reactants, or group of products, in the network. In the example in Figure 1, there are four distinct nodes, corresponding to each of $R_1 + L, C_1, R_2 + L, C_2$. We will always assume that the number of nodes is no larger than the number of species: $m \leq n$. (This is a key condition needed for our theoretical results to be valid.)

We represent each node i, $i = 1, \ldots, n$ by a vector b_i. Each b_i contains the information on which individual species participate as reactants at that node. Thus each b_i is in fact a vector in $\mathbb{R}^n$, whose coordinates are $b_i = (b_{1i}, b_{2i}, \ldots, b_{ni})'$, with $b_{li} \neq 0$ if species x_l is part of the node b_i. The m vectors b_i form the column vectors of a matrix $B \in \mathbb{R}^{n\times m}$:

$$B := (b_1, b_2, \ldots, b_m)\,.$$

As an illustration, in the particular case of the network shown in Figure 1, the nodes are characterized as follows:

$$R_1 + L \rightsquigarrow b_1; \quad C_1 \rightsquigarrow b_2; \quad R_2 + L \rightsquigarrow b_3; \quad C_2 \rightsquigarrow b_4$$

where

$$b_1 = \begin{pmatrix} 1 \\ 0 \\ 1 \\ 0 \\ 0 \end{pmatrix}; \quad b_2 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 1 \\ 0 \end{pmatrix}; \quad b_3 = \begin{pmatrix} 0 \\ 1 \\ 1 \\ 0 \\ 0 \end{pmatrix}; \quad b_4 = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 1 \end{pmatrix}.$$

and

$$B = \begin{pmatrix} 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 1 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{pmatrix}.$$

For the next step in developing the model, links between nodes are represented by a matrix containing all of the kinetic constants. Specifically, if the reactants in node b_i are products resulting from the reactants in node b_j, then there is an arrow pointing from b_j to b_i, with a corresponding kinetic constant k_{ij}. A first matrix, representing reactions *ending at a node* is $K^{in} = (k_{ij}) \in \mathbb{R}^{m\times m}$, where $k_{ij} \neq 0$ if there is an arrow from b_j to b_i. A second matrix can be constructed, which contains in its i-th diagonal entry the information on all the reactions that *start from* the node b_i, that is, $K^{out} := \text{Diag}(\sum k_{j1}, \sum k_{j2}, \ldots, \sum k_{jm})$.

Thus, for the network in Figure 1, we write:

$$K^{in} := \begin{pmatrix} 0 & k_{12} & k_{13} & 0 \\ k_{21} & 0 & 0 & k_{24} \\ k_{31} & 0 & 0 & k_{34} \\ 0 & k_{42} & k_{43} & 0 \end{pmatrix}$$

and

$$K^{out} := \begin{pmatrix} k_{21}+k_{31} & 0 & 0 & 0 \\ 0 & k_{12}+k_{42} & 0 & 0 \\ 0 & 0 & k_{13}+k_{43} & 0 \\ 0 & 0 & 0 & k_{24}+k_{34} \end{pmatrix}.$$

The net contribution of both matrices is:

$$K := K^{in} - K^{out} := \begin{pmatrix} -(k_{21}+k_{31}) & k_{12} & k_{13} & 0 \\ k_{21} & -(k_{12}+k_{42}) & 0 & k_{24} \\ k_{31} & 0 & -(k_{13}+k_{43}) & k_{34} \\ 0 & k_{42} & k_{43} & -(k_{24}+k_{34}) \end{pmatrix}.$$

In the last step, a vector-valued function is constructed whose components consist of the mass action *elemental events* defined at each node as:

$$\theta_B(x) = \begin{pmatrix} x_1^{b_{11}} x_2^{b_{21}} \cdots x_n^{b_{n1}} \\ x_1^{b_{12}} x_2^{b_{22}} \cdots x_n^{b_{n2}} \\ \vdots \\ x_1^{b_{1m}} x_2^{b_{2m}} \cdots x_n^{b_{nm}} \end{pmatrix}.$$

For the model in Figure 1, with $x = (R_1, R_2, L, C_1, C_2)'$, this vector is:

$$\theta_B(x) := \begin{pmatrix} R_1 L \\ C_1 \\ R_2 L \\ C_2 \end{pmatrix}.$$

These elemental events, when multiplied by the suitable kinetic constants, provide the *reaction rates*: for instance, the reaction "$R_1 + L \to C_1$" has a reaction rate given by $k_{21} R_1 L$, as the mass action kinetics rate is usually expressed.

Finally, the time-dependent evolution of the concentration of the n species of this receptor-ligand model can then be written compactly as the product of B, K and θ_B:

$$\frac{d\,x}{d\,t} = BK\theta_B(x) \tag{2}$$

or equivalently, for each species $\ell = 1, \ldots, n$,

$$\frac{d\,x_\ell}{d\,t} \;=\; \sum_{i,j=1}^{m} k_{ij}\, x_1^{b_{1j}} x_2^{b_{2j}} \cdots x_n^{b_{nj}}\, (b_{\ell i} - b_{\ell j}). \tag{3}$$

Expression (2) is equivalent to (1), but has the advantage that the information on the system is "condensed" into three objects: (1) the matrix B, which defines the nodes involved in the reactions; (2) the matrix K, which specifies the kinetic constants; and (3) the vector $\theta_B(x)$, which specifies the elemental events.

Throughout this paper the following assumptions are required:

(A1) the matrix B has full column rank, i.e., the vectors b_1, ..., b_m are linearly independent, and none of its rows vanish ;
(A2) the matrix K^{in} is irreducible, i.e., $(K^{in} + I)^m$ has all entries positive, where I is the identity matrix.

When they are satisfied, we shall say that the network is an *HJF network*. The first condition translates into a "zero-deficiency" constraint, in the language of [6]. The second condition amounts to the requirement ("weakly-reversibility" in the language of [6]) that *there is a chemical pathway connecting each pair of nodes*. For instance, in the example in Figure 1, there exists a chemical pathway leading from the node "$R_1 + L$" to the node "C_2", by passing through "C_1". Similarly, it is possible to travel from "C_2" back to "$R_1 + L$" by another chemical pathway. (In the example, the pathways happen to be all reversible but, in general, complete reversibility is not needed.) We need these assumptions in order to conclude the existence and uniqueness of steady-states of (2) [6,14]. (Actually, a somewhat weaker condition, *block-irreducibility*, which asks that each connected component of the reaction graph should be weakly reversible, would be sufficient.)

2.1 Conservation laws and positive classes

The conservation laws for the networks described by (2) can be found by constructing a subspace from the differences of the column vectors of B. These differences, called *reaction vectors* [7], form the *stoichiometric space*, given by

$$\mathcal{D} := \text{ span } \{b_i - b_j : i, j = 1, \ldots, m\} \equiv \text{ span } \{b_1 - b_j : j = 2, \ldots, m\}.$$

The significance of $\mathcal{D}$ is that the concentrations of receptor, ligand, and receptor–ligand complexes are represented as trajectories constrained to evolve in a subspace which is a parallel translate of $\mathcal{D}$. That is, if we compute all the vectors which are perpendicular to that subspace $\mathcal{D}$:

$$\mathcal{D}^{\perp} := \{g \in \mathbb{R}^n : \ g \ \text{ is perpendicular to all } \ (b_1 - b_j)\},$$

it is not difficult to see (from equation (3)) that the inner product

$$g \ \cdot \ \frac{d\,x}{d\,t} \equiv 0.$$

Integrating, it follows that the linear combination "$g \cdot x$" is constant throughout time:

$$g \ \cdot \ x \ \equiv \ g \ \cdot \ x(0)$$

where $x(0)$ is the vector of initial concentrations. So, each vector g in $\mathcal{D}^{\perp}$ expresses a conservation law of the system. By assumption (A1), the b_i's are linearly independent, which implies that the space $\mathcal{D}$ has dimension $m - 1$. As a result, there are exactly $n - (m - 1) \geq 1$ other linearly independent vectors (g) perpendicular to $\mathcal{D}$, and hence, there are also $n - (m - 1)$ distinct conservation laws.

For the model in Figure 1, the space $\mathcal{D}$ can be computed to give

$$\mathcal{D} = \text{ span } \{b_1 - b_j : j = 2, 3, 4\} = \text{ span } \left\{ \begin{pmatrix} 1 \\ 0 \\ 1 \\ -1 \\ 0 \end{pmatrix}, \begin{pmatrix} 1 \\ -1 \\ 0 \\ 0 \\ 0 \end{pmatrix}, \begin{pmatrix} 1 \\ 0 \\ 1 \\ 0 \\ -1 \end{pmatrix} \right\}$$

and two ($= 5 - 3$) linear independent vectors perpendicular to $\mathcal{D}$ can be picked as:

$$\begin{pmatrix} 1 \\ 1 \\ 0 \\ 1 \\ 1 \end{pmatrix} \quad \text{and} \quad \begin{pmatrix} 0 \\ 0 \\ 1 \\ 1 \\ 1 \end{pmatrix},$$

corresponding to the following conservation equations

$$\begin{aligned} L(t) + C_1(t) + C_2(t) &= \alpha \\ R_1(t) + R_2(t) + C_1(t) + C_2(t) &= \beta, \end{aligned}$$

for some positive constants α and β. As expected, these equations reflect the conservation of the total amount of ligand and of the total amount of receptors. In other words, one can say that

$$\alpha = L_{\text{total}} = L(0) + C_1(0) + C_2(0) \tag{4}$$

$$\beta = R_{\text{total}} = R_1(0) + R_2(0) + C_1(0) + C_2(0). \tag{5}$$

Formally, for each pair of positive constants α, β, the pair of equations (4,5) defines a subspace of $\mathbb{R}^5$, where the trajectories of system (1) evolve whenever the initial conditions satisfy $L_{\text{total}} = \alpha$ and $R_{\text{total}} = \beta$. We call a *positive class* any set that is the intersection of one such subspace with the positive orthant:

$$\mathcal{S}_{x_0} := \{x \in \mathbb{R}^n_{\geq 0} : \quad g^{(i)} \cdot x = g^{(i)} \cdot x_0, \quad i = 1, \ldots, n-m+1 \},$$

where the vectors $\{g^{(1)}, g^{(2)}, \ldots, g^{(n-m+1)}\}$ form a basis of $\mathcal{D}^\perp$ and where $x_0 \in \mathbb{R}^n_{>0}$. Each positive class may also be represented as a parallel translate of the stoichiometric space $\mathcal{D}$, since

$$\mathcal{S}_{x_0} = (x_0 + \mathcal{D}) \cap \mathbb{R}^n_{\geq 0} = \{x \in \mathbb{R}^n_{\geq 0} : \ x = x_0 + d, \text{ for some } d \in \mathcal{D}\}.$$

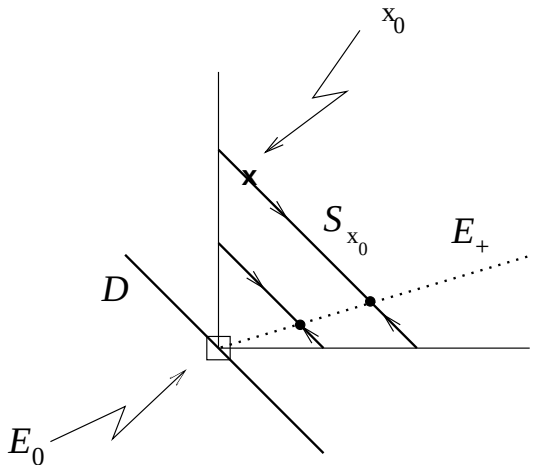

Fig. 2. A schematic representation of the stoichiometric space $\mathcal{D}$, the positive classes $\mathcal{S}_{x_0}$, and the positive steady-state set E_+.

2.2 Steady-states

The steady-states of system (2) (i.e., the steady-state concentrations of the component biochemical species) are the vectors $\bar{x} \in \mathbb{R}^n$ defined by

$$f(\bar{x}) = BK\theta_B(\bar{x}) = 0,$$

and can be divided into *positive and boundary steady-states*:

$$E_+ = \{\bar{x} : \ f(\bar{x}) = 0, \quad \text{and } \bar{x}_i > 0, \quad \text{for all coordinates } i\}$$

$$E_0 = \{\bar{z} : \ f(\bar{z}) = 0, \quad \text{and } \bar{z}_l = 0, \quad \text{for some coordinate } l\}.$$

A boundary steady-state corresponds to a situation when at least one of the species becomes completely depleted. The boundary steady-states for system (2), can be found by solving the equation $\theta_B(\bar{z}) = 0$. For model (1) the boundary steady-states are determined according to:

$$R_1 L = 0, \quad C_1 = 0, \quad R_2 L = 0, \quad C_2 = 0,$$

so that the set E_0 is given by

$$E_0 = \{(r_1, r_2, 0, 0, 0)', (0, 0, r_3, 0, 0)' : \ r_1, r_2, r_3 > 0\}.$$

For our results, in addition to assumptions (A1)-(A2), we also require:

(A3) There exist no boundary steady-states in each positive class, i.e.,

$$\mathcal{S} \ \cap \ E_0 \ = \ \emptyset.$$

Assumption (A3) is often satisfied for biochemical networks. This is indeed the case for this two-state model, and can be verified as follows. Upon substitution into equations (4) and (5), note that points of the type

$$(r_1, r_2, 0, 0, 0)' \quad \text{imply that} \quad R_{\text{total}} = r_1 + r_2, \quad \text{and} \quad L_{\text{total}} = 0,$$

so this would be an experiment involving no ligand, and thus no reactions would occur. Similarly, points of the type

$$(0, 0, r_3, 0, 0)' \quad \text{imply that} \quad R_{\text{total}} = 0, \quad \text{and} \quad L_{\text{total}} = r_3,$$

corresponding to an experiment where only molecules of ligand are present, and again no reactions would occur. In both cases, the pair $(\alpha, \beta) = (L_{\text{total}}, R_{\text{total}})$ *does not* define a positive class, because either $L_{\text{total}} = 0$, or $R_{\text{total}} = 0$.

On the other hand, it can be shown (see [6,7]) that each positive class contains exactly one positive steady-state, and that this positive steady-state is globally asymptotically stable (see [14]) with respect to the class. In other words, for HJF networks, i.e. under the assumptions (A1)-(A3) (as in the case of the two-state model, and later on for the

multi-state model), the trajectory of system (2) with a given initial condition $x(0) = x_0$, converges to the unique positive steady-state $\bar{x}$ in the same class of x_0.

The positive steady-states (E_+) can be further characterized in terms of the kinetic constants k_{ij}. In order to give this characterization, we need to introduce the set

$$\text{nullspace}(K) := \{v = (v_1, v_2, v_3, v_4)' : \ Kv = 0\}.$$

The steady-states satisfy

$$\bar{x} \in E_+ \Leftrightarrow BK\theta_B(\bar{x}) = 0 \Leftrightarrow K\theta_B(\bar{x}) = 0 \Leftrightarrow \theta_B(\bar{x}) \in \ \text{nullspace}(K),$$

where the second equivalence is justified because, by assumption (A1), the matrix B has full column rank, and the third equivalence is simply the definition of the nullspace of K.

Then the following statement ("complex balancing") is immediate from the assumptions; see e.g. [7] or Lemma V.1 in [14]:

Lemma 1 The point $\bar{x}$ is a positive steady-state if and only if the vector $\theta_B(\bar{x})$ belongs to the nullspace of K. □

Assumption (A2) states that the matrix K^{in} is irreducible (as was mentioned earlier, this assumption is essentially a mathematical way to describe the property of "weak reversibility" of the biochemical network). This irreducibility property allows a very useful characterization of the nullspace of K:

(1) the nullspace of K has dimension one,
(2) the nullspace of K is spanned by a positive vector.

This means that the nullspace of K can be characterized by a scaling factor σ and positive constants v_2, v_3 and v_4 as

$$\text{nullspace}(K) \ = \{\sigma(1, v_2, v_3, v_4)' : \ \sigma \in \mathbb{R}\}.$$

The positive constants v_2, v_3 and v_4 depend only on the kinetic constants k_{ij}. A computation of the nullspace of K for the model in Figure 1 is presented in Appendix A, where explicit expressions for the parameters v_2, v_3 and v_4 in terms of the k_{ij} are obtained. This characterization of the nullspace of K is obtained as a routine application of the Perron-Frobenius Theorem from linear algebra, see e.g. [2]; for ease of reference, a sketch of the proof is also presented in Appendix A. For each steady-state $\bar{x} \in E_+$, there is an appropriate, positive, value of σ so that

$$\theta_B(\bar{x}) = \sigma \begin{pmatrix} 1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}. \tag{6}$$

where the factor σ depends on the initial conditions $x(0)$.

To summarize, the steady-states for the receptor–ligand model of Figure 1 are completely characterized by (6), and (4), (5):

$$BK\theta_B(\bar{x}) = 0 \quad \Leftrightarrow \quad \begin{pmatrix} \bar{R}_1\bar{L} \\ \bar{C}_1 \\ \bar{R}_2\bar{L} \\ \bar{C}_2 \end{pmatrix} = \sigma \begin{pmatrix} 1 \\ v_2 \\ v_3 \\ v_4 \end{pmatrix}, \tag{7}$$

and

$$\bar{L} + \bar{C}_1 + \bar{C}_2 = L_{\text{total}} \tag{8}$$
$$\bar{R}_1 + \bar{R}_2 + \bar{C}_1 + \bar{C}_2 = R_{\text{total}}, \tag{9}$$

so there are 6 independent equations to determine 6 distinct quantities $\bar{L}, \bar{C}_1, \bar{C}_2, \bar{R}_1, \bar{R}_2$ and σ (which also depends on L_{total} and R_{total}).

The steady-states can be parametrized by the three numbers v_2, v_3, v_4 which summarize all the information needed about the kinetic constants, together with the two numbers L_{total} and R_{total} which summarize all the information needed about the initial states.

2.3 Remarks on the scaling factor σ and parameters v_2, v_3 and v_4

In essence, the factor σ has recast the receptor–ligand model in terms of the product of the steady-state amounts of the basic conformation R_1 and free ligand L. And, as we shall see, the three numbers v_2, v_3, v_4 lump the eight kinetic constants k_{ij} and, together with σ, they provide a complete description of the steady-state condition for the model *with a minimal number of parameters*. It had already been remarked in [16] that only 3 out of 8 constants that describe the network of reactions would be independent. The formalism described in this Section shows one possible way of extracting the independent constants, as well as providing them with a physical meaning. According to (7), the v_i's are *equilibrium constants* that give the fraction of steady-state values of the elemental events relative to one another: for instance, v_2 is the fraction of the steady-state concentration of the receptor–ligand complex $\bar{C}_1$ relative to the value $\bar{R}_1\bar{L}$. As will be seen in Section 3.3, in the case the reaction $R_1 + L \to C_1$ is much faster then its reverse, then v_2 is the inverse of the dissociation constant for that reaction.

3 Steady-state activity of the two-state receptor model

In this Section, the two–state model is examined in detail, using the formalism described earlier. Our steady-state analysis will show that this model provides a good description for receptor–ligand interactions not only for the case of agonists, but also for the case of neutral and inverse agonists, by varying the relative values of the kinetic constants. We will develop explicit expressions for several quantities of interest and provide a characterization of the different classes of ligand affinity in terms of the system's parameters. In Section 5, the same analysis will be extended to a multi-state receptor model with p receptor conformations and corresponding receptor–ligand complexes.

The steady-state response for different initial ligand concentrations is determined experimentally using ligand binding assays [16]. What is observed in these experiments is usually some combination of the concentration of the species in the model, or as introduced by Segel, Goldbeter *et. al.* in [12], one may consider the *final steady-state activity* as a linear combination

$$\mathcal{A} = a_1\bar{R}_1 + a_2\bar{C}_1 + a_3\bar{R}_2 + a_4\bar{C}_2.$$

Here the *activity coefficients* a_1, a_2, a_3 and a_4 are arbitrary nonnegative constants. For the general case of arbitrary (nonnegative) activity coefficients, we will provide a complete and exact analysis of the final steady-state activity, $\mathcal{A}$, as a function of the initial amount of ligand, L_{total}. This analysis will then lead to a characterization of affinity classes based on the values of the activity coefficients a_i (as well as the kinetic constants). We will assume, from now on, that the initial conditions are of the form

$$R_1(0) = R_{10}, \quad R_2(0) = R_0 - R_{10}, \quad L(0) = L_0, \quad C_1(0) = 0, \quad C_2(0) = 0,$$

that is, initially there are as yet no receptor–ligand complexes. In particular, note that

$$L_{\text{total}} = L_0 \quad \text{and} \quad R_{\text{total}} = R_0.$$

As an example, we remark that a typical "response" may be determined as the fraction of receptors in one of the two possible states [1,4], and plotted as a concentration-response curve, that is,

$$[\bar{R}_2 + \bar{C}_2]/R_{\text{total}}, \quad \text{vs.} \quad \log L_{\text{total}},$$

corresponding to the choice

$$a_1 = 0, \quad a_2 = 0, \quad a_3 = 1, \quad a_4 = 1,$$

in the final steady-state activity, $\mathcal{A}$.

Since the steady-state values $\bar{R}_1$, $\bar{R}_2$, $\bar{L}$, $\bar{C}_1$ and $\bar{C}_2$ are uniquely characterized by a set of algebraic equations (7)-(9), in principle, it is possible to obtain the exact values for these constants in terms of the kinetic constants (k_{ij}), and the initial conditions ($R_1(0)$, $R_2(0)$, $L(0)$, $C_1(0)$ and $C_2(0)$). However, the use of direct substitution to solve this set of algebraic equations can lead to very complex expressions (see [1]). Alternatively, one may solve the set of differential equations (1) numerically, since one knows that the solutions do converge to a (unique, positive) steady-state. However, focusing only on a numerical solution would not allow for general conclusions about the *actual functional dependence* of $\bar{R}_1$,...,$\bar{C}_2$, on the parameters k_{ij} and the initial conditions R_0, L_0. The knowledge of this functional dependence would enable one to show whether the model does indeed exhibit the experimental curves $\mathcal{A}$ versus $\log L_0$, characteristic of the three classes of ligand affinity. For this specific system, a closed explicit expression for $\bar{R}_1$, $\bar{R}_2$, $\bar{L}$, $\bar{C}_1$ and $\bar{C}_2$, can be given, using the techniques developed in [6], [14] and later in [3], and summarized in Section 2.

3.1 Steady-state response

We will now analyze the steady-state values and their dependence on the initial conditions and other parameters. From equation (7) it is immediate to see that:

$$\bar{C}_1 = v_2\sigma, \qquad \bar{C}_2 = v_4\sigma, \tag{10}$$

and then from the conservation equation (8) it follows that:

$$\bar{L} = L_0 - (v_2 + v_4)\sigma. \tag{11}$$

Substituting this expression for $\bar{L}$ back into (7) we have

$$\bar{R}_1 = \frac{\sigma}{L_0 - (v_2 + v_4)\sigma}, \qquad \bar{R}_2 = \frac{v_3\sigma}{L_0 - (v_2 + v_4)\sigma}\ . \tag{12}$$

As we noted above, the factor σ depends on the initial conditions, and to compute this dependence we will use the second conservation equation (9):

$$\frac{\sigma}{L_0 - (v_2 + v_4)\sigma} + \frac{\sigma v_3}{L_0 - (v_2 + v_4)\sigma} + v_2\sigma + v_4\sigma = R_0\ .$$

This leads to a quadratic polynomial on σ:

$$(v_2+v_4)^2\sigma^2-[(L_0+R_0)(v_2+v_4)+(1+v_3)]\sigma+R_0L_0=0,$$

together with the fact that $L_0-(v_2+v_4)\sigma>0$ (since $\bar{L}>0$). There are two possible solutions for this quadratic equation, but the correct one is found to be:

$$\sigma=\frac{1}{2(v_2+v_4)}\left[L_0+R_0+\frac{1+v_3}{v_2+v_4}-\sqrt{\left[L_0+R_0+\frac{1+v_3}{v_2+v_4}\right]^2-4R_0L_0}\,\right]. \tag{13}$$

We remark that the expression inside the square root can be simplified to

$$(L_0-R_0)^2+\left(\frac{1+v_3}{v_2+v_4}\right)^2+2(R_0+L_0)\frac{1+v_3}{v_2+v_4}$$

which is indeed a positive quantity, for all possible $L_0\geq 0$, $R_0\geq 0$. The other solution, $\sigma_+=\cdots+\sqrt{\cdots}$, would violate the conservation laws of the total amount of ligand and receptors. To see that this is so, we add up equations (8) and (9):

$$\bar{L}+\bar{R}_1+\bar{R}_2+2\bar{C}_1+2\bar{C}_2=L_0+R_0,$$

then use equations (10):

$$\bar{L}+\bar{R}_1+\bar{R}_2+2(v_2+v_4)\sigma=L_0+R_0$$

and finally substitute $\sigma=\sigma_+$ (note that the factors $2(v_2+v_4)$ cancel out), to obtain:

$$\bar{L}+\bar{R}_1+\bar{R}_2+L_0+R_0+\frac{1+v_3}{v_2+v_4}+\sqrt{\left[L_0+R_0+\frac{1+v_3}{v_2+v_4}\right]^2-4R_0L_0}=L_0+R_0.$$

This equation says that

$$L_0+R_0+\text{ positive quantity }=L_0+R_0,$$

which is obviously not true, and thus we conclude that σ_+ cannot be the correct solution to the quadratic equation.

In this fashion, we have now computed explicit expressions for the steady-state values, in terms of L_0, R_0 and the parameters k_{ij}. The dependence on the kinetic constants k_{ij} is condensed into the three positive constants v_2, v_3 and v_4 (see Appendix A).

We are now interested in analysing the behavior of the activity $\mathcal{A}$ as a function of L_0. In order to do this, fix R_0 and recall that v_2, v_3 and v_4 are constant factors, as well as a_1, a_2, a_3, and a_4. Define $\sigma=\sigma(L_0)$ to be a function of L_0 as given by (13), and define another function

$$\tau(L_0) := \frac{\sigma(L_0)}{L_0 - (v_2 + v_4)\,\sigma(L_0)} \tag{14}$$

and observe that

$$\mathcal{A}(L_0) = (a_1 + a_3 v_3)\,\tau(L_0) + (a_2 v_2 + a_4 v_4)\,\sigma(L_0)\ .$$

For very small or very large amounts of L_0, the following limits may be computed:

$$\lim_{L_0 \to 0} \sigma(L_0) = 0, \qquad \lim_{L_0 \to +\infty} \sigma(L_0) = R_0 \frac{1}{v_2 + v_4}.$$

The limit as $L_0 \to 0$ is immediate. To compute the limit as $L_0 \to +\infty$ write

$$Z = L_0 + R_0 + \frac{1 + v_3}{v_2 + v_4} \ \Rightarrow\ \sigma(L_0) = \frac{1}{2(v_2 + v_4)} \left[Z - \sqrt{Z^2 - 4R_0 L_0} \right]$$

and then multiply and divide σ by the quantity $Z + \sqrt{Z^2 - 4R_0 L_0}$, use the identity $(a-b)(a+b) = a^2 - b^2$ which is true for every pair of real numbers a, b, to obtain:

$$\begin{aligned}
\lim_{L_0 \to +\infty} \sigma(L_0) &= \lim_{L_0 \to +\infty} \frac{1}{2(v_2 + v_4)} \frac{Z^2 - (Z^2 - 4R_0 L_0)}{Z + \sqrt{Z^2 - 4R_0 L_0}} \\
&= \lim_{L_0 \to +\infty} \frac{1}{2(v_2 + v_4)} \frac{4R_0 L_0}{Z + \sqrt{Z^2 - 4R_0 L_0}} \\
&= \lim_{L_0 \to +\infty} \frac{1}{2(v_2 + v_4)} \frac{4R_0}{Z/L_0 + \sqrt{(Z/L_0)^2 - 4R_0/L_0}} \\
&= \frac{1}{2(v_2 + v_4)} \frac{4R_0}{2} = R_0 \frac{1}{v_2 + v_4}
\end{aligned}$$

where we used the fact that $\lim_{L_0 \to +\infty} Z/L_0 = 1$. Similarly, we have

$$\lim_{L_0 \to 0} \tau(L_0) = R_0 \frac{1}{1 + v_3}, \qquad \lim_{L_0 \to +\infty} \tau(L_0) = 0,$$

where the limit of $\tau(L_0)$ as $L_0 \to +\infty$ follows from the limit of $\sigma(L_0)$, and the limit as $L_0 \to 0$ may be computed using the same technique as above:

$$\begin{aligned}
\lim_{L_0 \to 0} \tau(L_0) &= \lim_{L_0 \to 0} \frac{1}{2(v_2 + v_4)} \frac{Z - \sqrt{Z^2 - 4R_0 L_0}}{L_0 - \frac{1}{2}Z + \frac{1}{2}\sqrt{Z^2 - 4R_0 L_0}} \\
&= \lim_{L_0 \to 0} \frac{1}{2(v_2 + v_4)} \frac{\left[Z - \sqrt{Z^2 - 4R_0 L_0} \right] \left[L_0 - \frac{1}{2}Z - \frac{1}{2}\sqrt{Z^2 - 4R_0 L_0} \right]}{(L_0 - \frac{1}{2}Z)^2 - \frac{1}{4}(Z^2 - 4R_0 L_0)}
\end{aligned}$$

$$= \lim_{L_0 \to 0} \frac{1}{2(v_2+v_4)} \frac{L_0\left[Z - \sqrt{Z^2 - 4R_0L_0}\right] - \frac{1}{2}\left(Z^2 - (Z^2 - 4R_0L_0)\right)}{L_0^2 - ZL_0 + R_0L_0}$$
$$= \lim_{L_0 \to 0} \frac{1}{2(v_2+v_4)} \frac{L_0\left[Z - \sqrt{Z^2 - 4R_0L_0} - 2R_0\right]}{L_0\left[L_0 - Z + R_0\right]}$$
$$= \frac{1}{2(v_2+v_4)} \frac{-2R_0}{-\frac{1+v_3}{v_2+v_4}} = R_0 \frac{1}{1+v_3}$$

Therefore,

$$\lim_{L_0 \to 0} \mathcal{A}(L_0) = R_0 \frac{a_1 + a_3 v_3}{1+v_3}, \qquad \lim_{L_0 \to +\infty} \mathcal{A}(L_0) = R_0 \frac{a_2 v_2 + a_4 v_4}{v_2+v_4}.$$

We can define the *affinity quotient* as

$$\mathtt{q} = \frac{\mathcal{A}(\infty)}{\mathcal{A}(0)} = \frac{a_2 v_2 + a_4 v_4}{a_1 + a_3 v_3} \frac{1+v_3}{v_2+v_4}. \tag{15}$$

The affinity quotient, $\mathtt{q}$, is well defined for each set of activity coefficients, as long as $a_1 \neq 0$ or $a_3 \neq 0$. The numerator of $\mathtt{q}$ will be strictly positive, since, typically, either $a_2 \neq 0$ or $a_4 \neq 0$. Then, we postulate that in the case $a_1 = a_3 = 0$ (a situation when no free receptor in any state contributes to the final steady-state activity), the affinity quotient takes the value $+\infty$. The main results are summarized next.

Theorem 1 Let R_0 be a fixed constant. Let a_1, a_2, a_3, a_4 be arbitrary nonnegative constants, with $a_2 + a_4 \neq 0$. The following statements hold:

(i) $\sigma(L_0)$ is a strictly increasing function of L_0;
(ii) $\tau(L_0)$ is a strictly decreasing function of L_0;
(iii) as a function of L_0, $\mathcal{A}(L_0)$ is
- strictly decreasing whenever $\mathtt{q} < 1$,
- strictly increasing whenever $\mathtt{q} > 1$,
- constant whenever $\mathtt{q} = 1$.

The proof of this Theorem follows essentially by computing the derivatives of the functions σ and τ, and analyzing their signs, as a function of L_0. The details can be found in Appendix B.

The affinity quotient can be interpreted in terms of the notion of *weighted average*. In general, the weighted average of a set of values $X_1,\ldots,X_p$, with respect to a set of *weight factors* $w_1,\ldots,w_p$ is defined by

$$\langle X \rangle_w := \frac{X_1 w_1 + X_2 w_2 + \ldots + X_p w_p}{w_1 + w_2 + \cdots + w_p},$$

and if the weights are all equal, then the weighted average coincides with the usual notion of the average value. Observe that $\mathtt{q}$ may be written as

$$\mathtt{q} = \frac{a_2 v_2 + a_4 v_4}{v_2 + v_4} \;/\; \frac{a_1 + a_3 v_3}{1 + v_3}$$

and then, multiplying and dividing be the quantity σ, and recalling from equation (7) that $\sigma = \bar{R}_1 \bar{L}$, $v_2\sigma = \bar{C}_1$,$v_3\sigma = \bar{R}_2\bar{L}$ and $v_4\sigma = \bar{C}_2$, we have:

$$\mathtt{q} = \frac{a_2 v_2 \sigma + a_4 v_4 \sigma}{v_2 \sigma + v_4 \sigma} \;/\; \frac{a_1 \sigma + a_3 v_3 \sigma}{1\sigma + v_3 \sigma} = \frac{a_2 \bar{C}_1 + a_4 \bar{C}_2}{\bar{C}_1 + \bar{C}_2} \;/\; \frac{a_1 \bar{R}_1 \bar{L} + a_3 \bar{R}_2 \bar{L}}{\bar{R}_1 \bar{L} + \bar{R}_2 \bar{L}}.$$

In the second factor, the quantity $\bar{L}$ cancels out, so we finally obtain:

$$\mathtt{q} = \frac{a_2 \bar{C}_1 + a_4 \bar{C}_2}{\bar{C}_1 + \bar{C}_2} \;/\; \frac{a_1 \bar{R}_1 + a_3 \bar{R}_2}{\bar{R}_1 + \bar{R}_2} = \frac{\langle \text{activity of bound receptors} \rangle_v}{\langle \text{activity of free receptors} \rangle_v},$$

so we may view the affinity quotient as the ratio between the *weighted average of the activity of bound receptors* and the *weighted average of the activity of free receptors*. The equilibrium constants v_i play the role of weight factors for the activity coefficients a_i, thus "choosing" the level of contribution from each species to the final activity. For example, if $a_1 = a_2 = 0$ and $a_3 = a_4 = 1$ then

$$\mathtt{q} \;=\; \frac{\bar{C}_2}{\bar{C}_1 + \bar{C}_2} \;/\; \frac{\bar{R}_2}{\bar{R}_1 + \bar{R}_2}\,. \tag{16}$$

The results of Theorem 1 hold for any two-state receptor model formulated according to the framework described in Section 2. Specifically, for a network consisting of the four elemental events $R_1 + L$, $R_2 + L$, C_1 and C_2, possible formulations of a two-state receptor model are:

(a) a cycle,

$$\begin{array}{ccc} R_1 + L & \rightarrow & R_2 + L \\ \uparrow & & \downarrow \\ C_1 & \leftarrow & C_2, \end{array}$$

(b) a (reversible) acyclic network

$$C_1 \leftrightharpoons R_1 + L \leftrightharpoons R_2 + L \leftrightharpoons C_2,$$

(c) any such representation that maintains the connectivity of the network.

Each of these models is characterized by a different matrix K, and hence the corresponding parameters v_i also have different values, but all the conclusions of Theorem 1 are unchanged.

3.2 Ligand affinity characterization

Part (iii) in Theorem 1 provides a complete characterization of the responses according to the values of the kinetic constants and activity coefficients. The different qualitative responses for the model can now be related to the ligand affinity classes mentioned earlier. For each set of kinetic constants k_{ij}, the affinity quotient $\mathtt{q}$ characterizes the affinity class in the following way:

(a) Agonists: $\mathtt{q} > 1$
(b) Neutral Agonists (or antagonists): $\mathtt{q} = 1$
(c) Inverse Agonists: $\mathtt{q} < 1$

Thus, different agonist behavior is obtained depending on the relative values of the scaling factors v_2, v_3 and v_4 (for the meaning of these parameters, see Section 3.3 below), and also on the activity coefficients a_1, a_2, a_3, and a_4. These classes, when represented graphically, have the features of typical receptor–ligand binding curves [1,9,13,16].

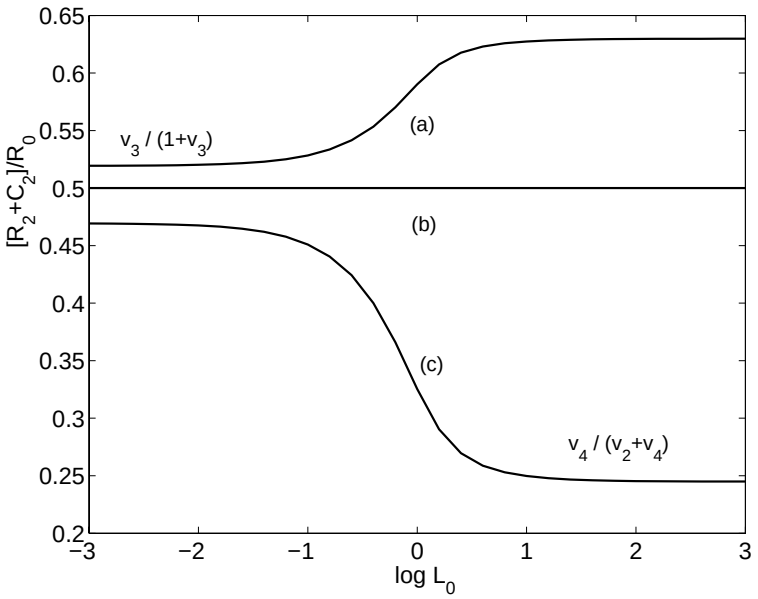

Fig. 3. Graphs of $\mathcal{A}(L_0)/R_0$ vs. $\log L_0$, when $a_1 = a_2 = 0$ and $a_3 = a_4 = 1$. Examples of: (a) an agonist ($\mathtt{q} = 1.21$), (b) a neutral agonist ($\mathtt{q} = 1.0$), and (c) an inverse agonist ($\mathtt{q} = 0.5217$).

As an example, we consider the case already mentioned above when $\mathcal{A} = \bar{R}_2 + \bar{C}_2$ (see [1,4]). In this case, the quotient takes the value (16), where the activity of free and bound receptors is measured, respectively, by a_3 (or $\bar{R}_2$) and a_4 (or $\bar{C}_2$).

In Figure 3 it is immediate to see that

(1) As $L_0 \to 0$: the concentration-response curve tends to a value which reflects the partition of receptors between the two possible states in the absence of ligand, $(a_1 + a_3 v_3)/(1 + v_3)$ (as the amount of ligand decreases to zero, the amount of receptor–ligand complexes also decreases to zero);
(2) As $L_0 \to +\infty$: the concentration-response curve reflects the capacity of the ligand to saturate the receptors, $(a_2 v_2 + a_4 v_4)/(v_2 + v_4)$ (for large amounts of ligand, all the receptors tend to be bound).

Furthermore, the affinity quotient $\mathtt{q}$ relates to the following ratio (see [8])

$$\frac{\text{fraction of } R_2 \ (L_0 \to +\infty)}{\text{fraction of } R_2 \ (L_0 \to 0)} = \frac{\eta(1+\kappa)}{1+\eta\kappa}. \tag{17}$$

According to [8], in the case when a receptor exists only in two conformations (say R_1 and R_2), the effect of a ligand on changing the ratio between the two conformations is given by (17), where η measures the affinity of ligand L for the conformation R_2, and κ is an allosteric constant

$$\eta = \frac{\text{affinity of } L \text{ for } R_2}{\text{affinity of } L \text{ for } R_1}, \qquad \kappa = \frac{\text{active receptors}}{\text{inactive receptors}}.$$

When the ratio (17) is > 1 the presence of ligand enriches the conformation R_2, and when it is < 1, the presence of ligand leads to depletion of the conformation R_2. In this sense, the ratio (17) is equivalent to our affinity quotient $\mathtt{q}$ and one can make the correspondence

$$\mathtt{q} = \frac{[v_4/v_2 v_3](1+v_3)}{1+v_4/v_2},$$

with

$$\kappa \rightsquigarrow v_3 = \frac{\bar{R}_2\bar{L}}{\sigma} = \frac{\bar{R}_2\bar{L}}{\bar{R}_1\bar{L}} = \frac{\bar{R}_2}{\bar{R}_1},$$

and

$$\eta \rightsquigarrow \frac{v_4}{v_2 v_3} = \frac{C_2/\sigma}{C_1/\sigma}\frac{1}{v_3} = \frac{\bar{C}_2}{\bar{C}_1}\frac{\bar{R}_1}{\bar{R}_2}.$$

3.3 Biochemical significance of the scalars v_2, v_3, v_4

The constants v_2, v_3, v_4 can be regarded as a concise parametrization of the biochemical networks being considered. Consider the case where the reactions

$$R_1 + L \rightleftharpoons C_1, \quad \text{with dissociation constant } \mathcal{K}_{D12}$$

and

$$R_2 + L \rightleftharpoons C_2, \quad \text{with dissociation constant } \mathcal{K}_{D34}$$

are uncoupled. Remembering that v_1 is set to unity, the constants v_2, v_3 and v_4 satisfy (from Appendix A)

$$\mathcal{K}_{D12} = \frac{v_1}{v_2} = \frac{1}{v_2}, \quad \text{and} \quad \mathcal{K}_{D34} = \frac{v_3}{v_4}.$$

Using the experimental evidence (see [9], Chapter 2) that the forward binding constants (such as k_{21} and k_{43} in Figure 1) are much larger (of order 10^6, 10^7) than comparable dissociation constants (of order 10^{-1}, 10^{-2}), we can obtain estimates for the v_i. The equation for $d\,R_1/d\,t$, at steady-state, is:

$$-(k_{21} + k_{31})\bar{R}_1\bar{L} + k_{12}\bar{C}_1 + k_{13}\bar{R}_2\bar{L} = 0, \tag{18}$$

and using the fact that $k_{21} \gg k_{31}$ we obtain:

$$-k_{21}\bar{R}_1\bar{L} + k_{12}\bar{C}_1 + k_{13}\bar{R}_2\bar{L} \approx 0.$$

Since the model is symmetric with respect to R_1, R_2, without loss of generality, we can assume that $\bar{R}_1 \geq \bar{R}_2$, and again using $k_{21} \gg k_{13}$:

$$k_{21}\bar{R}_1\bar{L} \gg k_{13}\bar{R}_2\bar{L}.$$

So, equation (18) is reduced to:

$$-k_{21}\bar{R}_1\bar{L} + k_{12}\bar{C}_1 \approx 0,$$

and yields

$$\frac{\bar{C}_1}{\bar{R}_1\bar{L}} \approx \frac{k_{21}}{k_{12}} = \frac{1}{\mathcal{K}_{D12}}.$$

We also have, from (7), that

$$v_2 = \frac{\bar{C}_1}{\bar{R}_1 \bar{L}} \approx \frac{k_{21}}{k_{12}}.$$

Next, using the equations that provide the nullspace of K (see Appendix A), we may obtain expressions for v_3 and v_4 from v_2:

$$\begin{aligned} &-(k_{21}+k_{31}) + k_{12}v_2 + k_{13}v_3 = 0 \\ &\quad -(k_{21}+k_{31}) + k_{21} + k_{13}v_3 = 0 \quad \Rightarrow \quad v_3 = \frac{k_{31}}{k_{13}}, \end{aligned}$$

and:

$$\begin{aligned} &k_{21} - (k_{12}+k_{42})v_2 + k_{24}v_4 = 0 \\ &k_{21} - k_{21} - k_{42}\frac{k_{21}}{k_{12}} + k_{24}v_4 = 0 \quad \Rightarrow \quad v_4 = \frac{k_{42}}{k_{24}}\frac{k_{21}}{k_{12}}. \end{aligned}$$

So the constants v_i may be estimated from *dissociation constants* as

$$\frac{1}{\mathcal{K}_{D12}} = \frac{k_{21}}{k_{12}} = v_2, \quad \frac{1}{\mathcal{K}_{D13}} = \frac{k_{31}}{k_{13}} = v_3 \quad \text{and} \quad \frac{1}{\mathcal{K}_{D24}\mathcal{K}_{D12}} = v_4,$$

which can be measured.

Under these circumstances (namely, (a) the order of magnitude of k_{21} and k_{43} is much larger then the order of magnitude of the other kinetic constants, and (b) $\bar{R}_1 \geq \bar{R}_2$, meaning that $\mathcal{K}_{D12}$ is the dissociation constant associated to the more abundant conformation of R_1), the affinity quotient $\mathtt{q}$, associated with the final activity $\mathcal{A} = \bar{R}_2 + \bar{C}_2$, becomes:

$$\mathtt{q} = \frac{v_4}{v_3}\frac{1+v_3}{v_2+v_4} \approx \frac{1/(\mathcal{K}_{D12}\mathcal{K}_{D24})}{1/\mathcal{K}_{D13}} \, \frac{1+1/\mathcal{K}_{D13}}{1/\mathcal{K}_{D12}+1/(\mathcal{K}_{D12}\mathcal{K}_{D24})} = \frac{\mathcal{K}_{D13}+1}{\mathcal{K}_{D24}+1}\,.$$

This expression indicates that the affinity class of the ligand is ultimately decided by the balance between the final distribution of free and bound receptors among the two-states, since

$$\mathcal{K}_{D13} \approx \frac{\bar{R}_1}{\bar{R}_2} \quad \text{and} \quad \mathcal{K}_{D24} \approx \frac{\bar{C}_1}{\bar{C}_2}.$$

An inverse agonist is characterized by $\mathcal{K}_{D24} > \mathcal{K}_{D13}$, or equivalently $\bar{C}_1/\bar{C}_2 > \bar{R}_1/\bar{R}_2$, while an agonist is characterized by $\bar{C}_1/\bar{C}_2 < \bar{R}_1/\bar{R}_2$. For instance, the inverse agonist in

Figure 3 was obtained with $k_{43} = k_{21} = 5$, $k_{24} = 4$, $k_{31} = 3$ and all other kinetic constants equal to 1, corresponding to $\mathcal{K}_{D12} = 0.2$, $\mathcal{K}_{D13} = 0.33$ and $\mathcal{K}_{D24} = 4$; while the agonist was obtained with $k_{43} = k_{21} = 5$, $k_{42} = 2$, $k_{13} = 1.99$ and all other kinetic constants equal to 1, corresponding to $\mathcal{K}_{D12} = 0.2$, $\mathcal{K}_{D13} = 1.99$ and $\mathcal{K}_{D24} = 0.5$.

Thus, the scalars v_i can be seen to generalize the concept of the equilibrium constants in the context of biochemical networks. They capture, in addition to direct reversibility between reactants and their products, all other network routes that achieve the same outcome and are present in the stoichiometry.

3.4 Comparison with experimental data

Devreotes and Sherring [4] identify two receptor conformations for the cAMP receptor of *Dictyostelium*. Assuming that the interactions between cAMP (ligand) and its receptors can be described by the model depicted in Figure 1, and that the concentration-response curve is determined as $[\bar{R}_2 + \bar{C}_2]$, as a function of of L_0, the authors measured the dissociation constants:

$$\mathcal{K}_{D12} = \frac{k_{12}}{k_{21}} = 15 \times 10^{-9} M, \quad \mathcal{K}_{D34} = \frac{k_{34}}{k_{43}} = 30 \times 10^{-9} M,$$
$$k_{31} = 0.012 \text{ min}^{-1}, \quad k_{13} = 0.104 \text{ min}^{-1}, \quad k_{42} = 0.222 \text{ min}^{-1}, \quad k_{24} = 0.055 \text{ min}^{-1}.$$

Also from the experimental concentration-response curve, the values:

$$\frac{[\bar{R}_2 + \bar{C}_2]}{R_0}(0) \approx 0.15, \qquad \frac{[\bar{R}_2 + \bar{C}_2]}{R_0}(\infty) \approx 0.804 \tag{19}$$

can be obtained. We may now compute the values of our constants v_2, v_3, v_4 (as estimated in Section 3.3) from the k_{ij} obtained in this experiment, and then compare the ratios $v_3/(1 + v_3)$ and $v_4/(v_2 + v_4)$ with the values (19). We have:

$$v_2 = 6.67 \times 10^7 M^{-1}, \quad v_3 = 0.115, \quad v_4 = 2.69 \times 10^8 M^{-1}$$

and

$$\frac{v_3}{1 + v_3} = 0.103, \quad \frac{v_4}{v_2 + v_4} = 0.806,$$

which are in agreement with the values (19).

4 Application of HJF networks to a classical model

As further illustration of the flexibility of the theory described in Section 2, we now apply the HJF networks formalism to analyze a classical model in the literature, a model that was studied in great detail by Segel, Goldbeter *et. al.* in [12], and is depicted in Figure 4.

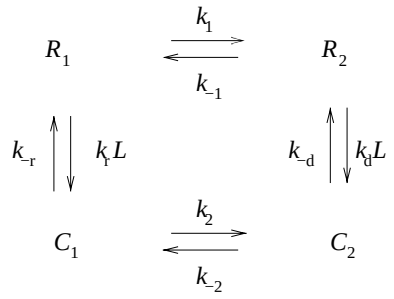

Fig. 4. The model studied in [12].

There are two essential differences between the models of Figures 1 and 4:

1. In the model of Figure 4, *the amount of ligand L is assumed to be constant*, i.e.,

   $$L \equiv L_0 \equiv \bar{L},$$

   and thus L is a parameter, but not a variable of the system, while in our two-state model (Figure 1) the amount of ligand is allowed to change, as it binds to the cell receptors, and therefore L is a variable of the system.
2. In the model of [12] (and also other references such as [9], Chapter 2), the exchange between receptor conformations occurs *independently* of the presence of ligand, whereas in our model (Figure 1), from the discussion of elemental events, the exchange between receptor conformations *may occur only in the presence of ligand*. This leads to the appearance of nonlinear terms ($R_1(t)L(t)$, $R_2(t)L(t)$) in the differential equations (1).

The HJF networks formalism also allows the rigorous analysis of the model in Figure 4. The equations that describe this model are (recall that L is assumed to be constant, and thus $dL/dt \equiv 0$, as opposed to our model (1))

$$\begin{aligned}
\frac{dR_1}{dt} &= -k_1\ R_1 + k_{-1}\ R_2 - k_r\ L\ R_1 + k_{-r}\ C_1 \\
\frac{dR_2}{dt} &= k_1\ R_1 - k_{-1}\ R_2 - k_d\ L\ R_2 + k_{-d}\ C_2 \\
\frac{dC_1}{dt} &= -k_2\ C_1 + k_{-2}\ C_2 + k_r\ L\ R_1 - k_{-r}\ C_1
\end{aligned}$$

$$\frac{dC_2}{dt} = k_2\, C_1 - k_{-2}\, C_2 + k_d\, L\, R_2 - k_{-d}\, C_2.$$

(These are equations (1a-d) in [12] and, in their notation, $R_1 \rightsquigarrow R$, $R_2 \rightsquigarrow D$, $C_1 \rightsquigarrow X$ and $C_2 \rightsquigarrow Y$.) There is only one conservation equation:

$$\bar{R}_1 + \bar{R}_2 + \bar{C}_1 + \bar{C}_2 = R_0.$$

Since the elemental events are simply R_1, R_2, C_1 and C_2, the positive steady-states of the system are given by:

$$\bar{R}_1 = \sigma, \quad \bar{C}_1 = \sigma\hat{v}_2, \quad \bar{R}_2 = \sigma\hat{v}_3, \quad \bar{C}_2 = \sigma\hat{v}_4.$$

One can solve for σ, using the conservation equation, to obtain

$$\sigma = R_0\, \frac{1}{1 + \hat{v}_2 + \hat{v}_3 + \hat{v}_4}\ .$$

Comparing Figures 1 and 4, there is the following correspondence between kinetic constants:

$$\begin{aligned} &k_{12} = k_{-r}, \quad k_{21} = k_r\, L, \quad k_{13} = k_{-1}, \quad k_{31} = k_1, \\ &k_{24} = k_{-2}, \quad k_{42} = k_2, \quad k_{34} = k_{-d}, \quad k_{43} = k_d\, L, \end{aligned} \tag{20}$$

so *in this case the scalars $\hat{v}_i$ depend on L.* Nevertheless, we may still define the steady-state activity and the affinity quotient as before, by carefully computating the limits $\mathcal{A}(L \to 0)$ and $\mathcal{A}(L \to +\infty)$. Following the expressions in Appendix A and the correspondence (20), the scalars $\hat{v}_i$ have the form

$$\hat{v}_3 = \frac{k_{-r}k_1(k_{-2} + k_{-d}) + k_{-d}k_2(k_r L + k_1)}{k_{-r}k_{-2}(k_{-1} + k_d L) + k_{-1}k_{-d}(k_{-r} + k_2)}$$

and

$$\hat{v}_2 = -\frac{k_{-1}}{k_{-r}}\hat{v}_3 + \frac{k_r L + k_1}{k_{-r}}, \qquad \hat{v}_4 = \frac{k_{-1} + k_d L}{k_{-d}}\hat{v}_3 - \frac{k_1}{k_{-d}}\ .$$

Then

$$\mathcal{A}(L) = R_0\, \frac{a_1 + a_2\hat{v}_2 + a_3\hat{v}_3 + a_4\hat{v}_4}{1 + \hat{v}_2 + \hat{v}_3 + \hat{v}_4}$$

$$= R_0 \, \frac{a_1 + a_2 \frac{k_r L + k_1}{k_{-r}} - a_4 \frac{k_1}{k_{-d}} + \hat{v}_3 \left(a_3 - a_2 \frac{k_{-1}}{k_{-r}} + a_4 \frac{k_{-1} + k_d L}{k_{-d}} \right)}{1 + \frac{k_r L + k_1}{k_{-r}} - \frac{k_1}{k_{-d}} + \hat{v}_3 \left(1 - \frac{k_{-1}}{k_{-r}} + \frac{k_{-1} + k_d L}{k_{-d}} \right)}.$$

Now, it is not difficult to see that

$$\lim_{L \to 0} \hat{v}_3 = \frac{k_1}{k_{-1}}, \qquad \lim_{L \to +\infty} \hat{v}_3 = \frac{k_r \; k_2 \; k_{-d}}{k_{-r} \; k_{-2} \; k_d},$$

and, upon substitution into $\mathcal{A}(L)$, standard limit computations yield:

$$\lim_{L \to 0} \mathcal{A}(L) = \frac{a_1 + a_3 \; k_1 / k_{-1}}{1 + k_1 / k_{-1}}, \quad \text{and} \quad \lim_{L \to +\infty} \mathcal{A}(L) = \frac{a_2 + a_4 \; k_2 / k_{-2}}{1 + k_2 / k_{-2}}.$$

Thus the affinity quotient for the model developed in [12] is

$$\mathtt{q} = \frac{a_2 \; K_2 + a_4}{K_2 + 1} \; / \; \frac{a_1 \; K_1 + a_3}{K_1 + 1}, \tag{21}$$

where $K_1 = k_{-1}/k_1$ and $K_2 = k_{-2}/k_2$. As a final remark, we point out that this affinity quotient in some sense expresses the concept of "sensory adaptation" introduced in [12]. This concept of adaptation involves choosing the activity coefficients a_i so that the final steady-state activity is always equal to the basal activity or, in other words, so that $\mathcal{A}(L_1) = \mathcal{A}(L_0)$, *for every pair of values* L_0, L_1. Equivalently, the choice of coefficients should satisfy

$$\mathcal{A}(0) = \mathcal{A}(\infty) \quad \Leftrightarrow \quad \mathtt{q} = 1,$$

and, indeed, setting $\mathtt{q} = 1$ in equation (21), yields precisely equation (26a) of [12], for exact adaptation.

5 Extension to multi-state receptor models

The versatility of the approach summarized in equation (2), where the receptor–ligand model is analyzed as an HJF network, can be seen in its extension to more complex systems. For example, consider the model in Figure 5, where a single ligand binds to multiple receptor states.

The results in Section 2 extend very naturally to the model of Figure 5. Now the vector of concentrations takes the form $x = (R_1, R_2, \ldots, R_p, L, C_1, C_2, \ldots, C_p)'$. The two conservation laws become

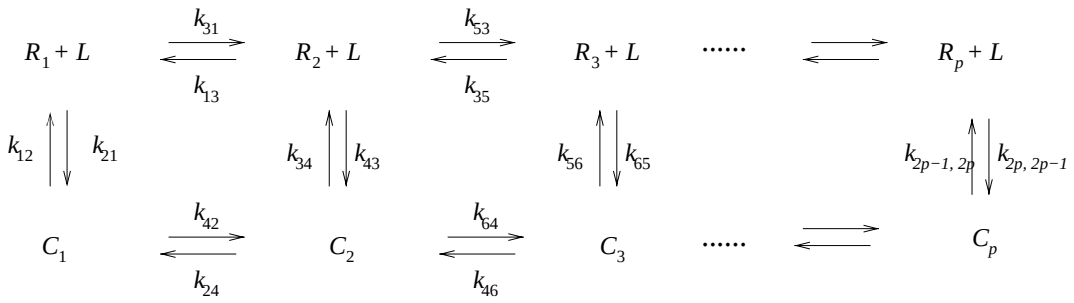

Fig. 5. A "ladder" receptor–ligand network, incorporating p receptor conformations.

$$\bar{L} + \bar{C}_1 + \bar{C}_2 + \cdots + \bar{C}_p = L_0$$
$$\bar{R}_1 + \bar{R}_2 + \cdots + \bar{R}_p + \bar{C}_1 + \bar{C}_2 + \cdots + \bar{C}_p = R_0,$$

while the matrix of reaction nodes B and the matrix of kinetic constants K extend in the obvious way, and the vector of elemental events becomes

$$\theta_B(x) = \begin{pmatrix} R_1L \\ C_1 \\ R_2L \\ C_2 \\ \vdots \\ R_pL \\ C_p \end{pmatrix} = \sigma \begin{pmatrix} 1 \\ v_2 \\ v_3 \\ v_4 \\ \vdots \\ v_{2p-1} \\ v_{2p} \end{pmatrix}.$$

The nullspace of K is now the set

$$\text{nullspace}(K) \ = \{\sigma(1, v_2, v_3, \ldots, v_{2p}) : \ \sigma \in \mathbb{R}\}$$

where $v_2, v_3, \ldots, v_{2p}$ are still positive scalars, given in terms of the k_{ij} only. Solving the new equations for the steady-state of the system, we find that, for each $\sigma > 0$,

$$\bar{C}_1 = v_2\sigma, \quad \bar{C}_2 = v_4\sigma, \ \ldots, \ \bar{C}_p = v_{2p}\sigma \ ,$$

$$\bar{L} = L_0 - (v_2 + v_4 + \cdots + v_{2p})\sigma \ ,$$

$$\bar{R}_1 = \frac{\sigma}{L_0 - (v_2 + v_4 + \cdots + v_{2p})\sigma}, \quad \bar{R}_2 = \frac{v_3\ \sigma}{L_0 - (v_2 + v_4 + \cdots + v_{2p})\sigma},$$
$$\bar{R}_3 = \frac{v_5\ \sigma}{L_0 - (v_2 + v_4 + \cdots + v_{2p})\sigma}, \quad \ldots, \quad \bar{R}_p = \frac{v_{2p-1}\ \sigma}{L_0 - (v_2 + v_4 + \cdots + v_{2p})\sigma} \ .$$

And finally, σ satisfies a quadratic polynomial very similar to the two state case (we only need to replace the sums of odd indexed v_i and even indexed v_i):

$$1 + v_3 \rightsquigarrow S_o = 1 + v_3 + v_5 + \cdots + v_{2p-1}$$
$$v_2 + v_4 \rightsquigarrow S_e = v_2 + v_4 + v_6 + \cdots + v_{2p}\ ,$$

so that

$$\sigma(L_0) = \frac{1}{2S_e}\left[L_0 + R_0 + \frac{S_o}{S_e} - \sqrt{\left[L_0 + R_0 + \frac{S_o}{S_e}\right]^2 - 4R_0L_0}\ \right]$$

and

$$\tau(L_0) = \frac{\sigma(L_0)}{L_0 - S_e\,\sigma(L_0)}$$

For this extended model, the final steady-state activity measurements would be given by:

$$\mathcal{A} = a_1\bar{R}_1 + a_2\bar{C}_1 + \ldots + a_{2p-1}\bar{R}_p + a_{2p}\bar{C}_p$$

as a function of L_0, and the affinity quotient is

$$\mathsf{q} = \frac{a_2v_2 + \ldots + a_{2p}v_{2p}}{a_1 + \ldots + a_{2p-1}v_{2p-1}}\ \frac{S_o}{S_e} \tag{22}$$

Under these conditions, the results in Theorem 1 are still valid, for any choice of constants $a_i \geq 0$, with $a_2 + a_4 + a_{2p} > 0$.

As before, the affinity classes are characterized by the affinity quotient, which can again be interpreted as

$$\begin{aligned}\mathsf{q} &= \frac{a_2\bar{C}_1 + \ldots + a_{2p}\bar{C}_p}{\bar{C}_1 + \bar{C}_2 + \bar{C}_3 + \cdots + \bar{C}_p}\ /\ \frac{a_1\bar{R}_1 + \ldots + a_{2p-1}\bar{R}_p}{\bar{R}_1 + \bar{R}_2 + \bar{R}_3 + \cdots + \bar{R}_p} \\ &= \langle\text{activity of bound receptors}\rangle_v\ /\ \langle\text{activity of free receptors}\rangle_v\end{aligned}$$

the ratio between the weighted averages of the activity of bound and free receptors, where the v_i's play the role of weight factors. Another interpretation for the affinity quotient is in terms of the distribution of the receptor conformation states (referred to as "allosteric constants" in [8]):

$$\kappa_i = \frac{\text{receptors in state } i}{\text{receptors in inactive state}} = \frac{\bar{L}\bar{R}_i}{\bar{L}\bar{R}_1} = \frac{\sigma v_{2i-1}}{\sigma v_1} = v_{2i-1},$$

(note that $\kappa_1 = 1$) and the relative affinity of ligand for each conformation:

$$\eta_i = \frac{v_{2i}}{v_2(a_1 + a_3 v_3 + \cdots + a_{2p-1} v_{2p-1})} = \frac{\bar{C}_i}{\bar{C}_1} \, \frac{\bar{R}_1}{a_1 \bar{R}_1 + a_3 \bar{R}_2 + \cdots + a_{2p-1} \bar{R}_p}.$$

Then

$$\mathsf{q} = \frac{(a_2\eta_1 + a_4\eta_2 + \cdots + a_{2p}\eta_p)(1 + \kappa_2 + \kappa_3 + \cdots + \kappa_p)}{1 + (\eta_1 + \eta_2 + \cdots + \eta_p)(a_1 + a_3\kappa_2 + \cdots + a_{2p-1}\kappa_p)}$$

or, in a more compact notation,

$$\mathsf{q} = \frac{(\sum a_{2i}\, \eta_i)\,(1 + \sum \kappa_i)}{1 + (\sum \eta_i)\,(\sum a_{2i-1}\, \kappa_i)}$$

generalizes expression (17)

$$\frac{\text{fraction of } R_2 \ (L_0 \to +\infty)}{\text{fraction of } R_2 \ (L_0 \to 0)} = \frac{\eta(1+\kappa)}{(1+\eta\kappa)},$$

which is indeed recovered for the particular case of the two-state model, with $\eta \equiv \eta_2$, $\kappa \equiv \kappa_2$ and $a_1 = a_2 = 0$, $a_3 = a_4 = 1$ (as we saw in Section 3.2).

As noted in Section 3, Theorem 1 implies that any number of reactions among the nodes may be added or removed (as long as the irreducibility property of the network is maintained), causing the values of the v_i's to change, but the general results and conclusions still hold. Consider, for instance a "star" network, as in Figure 6, in which only the "basic" receptor conformation (R_1) is allowed to change to other conformations (R_2, R_3, R_4). In this case, the nullspace of K is very simple to compute and the following values are obtained:

$$v_2 = \frac{k_{21}}{k_{12}}, \quad v_3 = \frac{k_{31}}{k_{13}}, \quad v_4 = \frac{k_{43}}{k_{34}}\,\frac{k_{31}}{k_{13}},$$
$$v_5 = \frac{k_{51}}{k_{15}}, \quad v_6 = \frac{k_{65}}{k_{56}}\,\frac{k_{51}}{k_{15}}, \quad v_7 = \frac{k_{71}}{k_{17}}, \quad v_8 = \frac{k_{87}}{k_{78}}\,\frac{k_{71}}{k_{17}},$$

so, in this "star" example the v_i are exactly given by dissociating constants, which is consistent with the notion that all receptors in the network are accessible via R_1.

6 Concluding remarks

Receptor–ligand interactions can be represented as HJF biochemical networks, in the form of equation (2), consisting of three essential objects (see also [7]): The vector $\theta_B(x)$ containing the elemental events; The matrix K of kinetic constants; And the matrix B

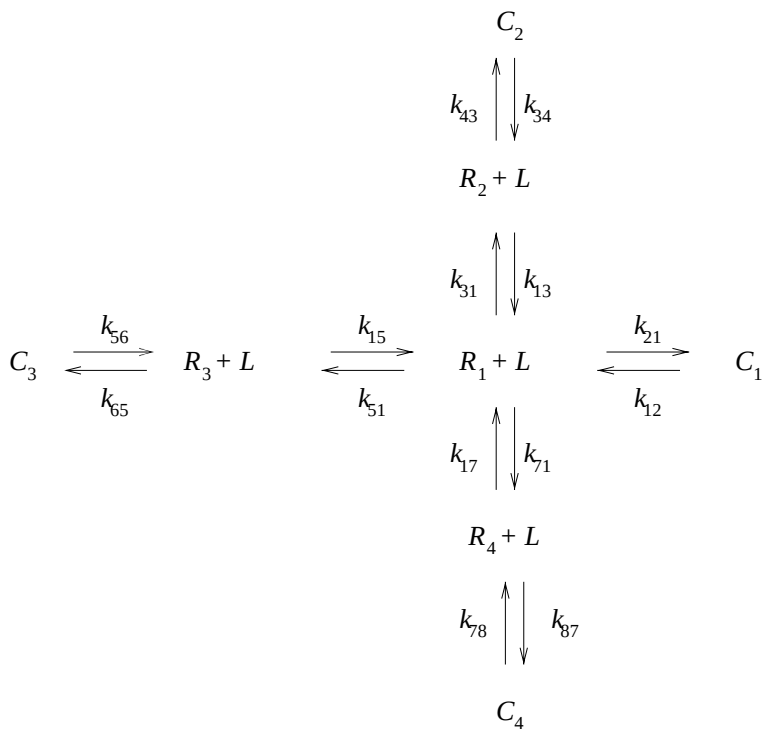

Fig. 6. A "star" receptor–ligand network.

that relates the nodes of the network to the rate of change of the individual species' concentrations. Formulated in this way, the conservation laws for this system are a consequence of the matrix B and establish a set of invariant subspaces for the system. The nullspace of the matrix K then identifies the set of steady-state points in these subspaces, using a minimal set of parameters. From our analysis, it becomes clear that this minimal set of parameters generalizes the role of the equilibrium constants in the context of biochemical networks, by incorporating the effect of the network as a whole (while it is often the case that the network is decoupled, for the purpose of computing the equilibrium constants of the "receptor+ligand $\leftrightarrow$ complex" reactions). With this minimal set of parameters, a detailed analysis of the steady-state activity of the two-state model is achieved, under general assumptions on the available biochemical pathways (which are identified by the nonzero entries of the matrix K^{in}).

Experimentally, steady-state measurements are a linear combination, $\mathcal{A}$, of contributing species, e.g., all sources of a receptor, both free and bound to ligand (see also [12]). This steady-state activity can also be expressed in terms of the minimal set of parameters, and depends on the activity coefficients and on the total amount of ligand present. The quotient concisely relating the final activity at the limiting conditions of zero and infinite amounts of ligand, summarizes the distribution of the species in the model. This affinity quotient can also be interpreted as the ratio of the weighted averages of, respectively, the activity of bound receptors and the activity of free receptors. The weight factors are, in fact, the set of minimal parameters, which are responsible for selecting the appropriate contribution from each species to the steady-state activity. The classification of the ligand

as agonist, neutral agonist or inverse agonist is then readily determined from the value of this affinity quotient. And finally, the flexibility of this formalism can be appreciated through its extension to multi-state receptor systems. All of the concepts can be directly generalized, and the characterizations of the steady-state activity and the corresponding affinity quotient are similarly preserved.

It is now well recognized that a simple mass action interaction between ligand and receptor is not the typical event initiating cell signaling. Rather, the response to ligand activation is a complex process that can eventuate in different receptor states and lead to a variety of functional consequences. The economic use of a single receptor type to initiate elaborate downstream signaling can be seen, for example, in the selective and sensitive response of cells to chemotactic factors and in the shifting responses to growth factors during different time points in development. Because of the potential for complex biological systems to be represented by equally complex sets of equations, significant progress in mathematical descriptions of these elaborate signaling processes will be best achieved with concise expressions that still capture the dynamics of the essential biochemical events taking place.

Despite their complexity, biochemical pathways still operate under the principles of mass action and stoichiometry. Additionally, metabolic networks and signaling pathways have been intuitively, but not formally, understood to be weakly reversible. Taken together, these basic concepts can lead to the formalism that has been presented here, in which brevity and flexibility are achieved through a minimal set of parameters that can ultimately be regarded as equilibrium constants for the signaling network. This generalization leads to the characterization of multiple receptor states in terms of weighted averages of its respective activities, with the generalized parameters as weighting factors. How these parameters can be further exploited to character drug receptor interactions and signaling in more complex biochemical networks is the subject of further investigations.

A The nullspace of K

A.1 Computing the scalars v_2, v_3, v_4

Consider the model in Figure 1 and the corresponding matrix K. The vectors in the nullspace of K satisfy $Kv = 0$. The vector $v = (1, v_2, v_3, v_4)'$ can be determined from the equations:

$$\begin{aligned} -(k_{21} + k_{31}) + k_{12}v_2 + k_{13}v_3 &= 0 \\ k_{21} - (k_{12} + k_{42})v_2 + k_{24}v_4 &= 0 \\ k_{31} - (k_{13} + k_{43})v_3 + k_{34}v_4 &= 0 \end{aligned}$$

which yield

$$v_3 = \frac{k_{31}k_{12}(k_{24} + k_{34}) + k_{34}k_{42}(k_{21} + k_{31})}{k_{12}k_{24}(k_{13} + k_{43}) + k_{13}k_{34}(k_{12} + k_{42})}$$

and from this expression both v_2 and v_4 can then be computed by

$$v_2 = -\frac{k_{13}}{k_{12}}v_3 + \frac{k_{21} + k_{31}}{k_{12}} \quad \text{and} \quad v_4 = \frac{k_{13} + k_{43}}{k_{34}}v_3 - \frac{k_{31}}{k_{34}} \ .$$

A.2 Characterization of the nullspace of K

We review here some standard facts about irreducible matrices. By construction, K is irreducible and it has negative entries only on its diagonal. So there is a constant $\gamma > 0$ such that $M = K + \gamma I$ has all entries nonnegative. Thus $M \geq 0$ and M is also irreducible. For such matrices, the Perron-Frobenius Theorem states that:

(1) the spectral radius of M, ρ, is an eigenvalue of M of multiplicity one;
(2) an eigenvector, v_ρ, corresponding to the eigenvalue ρ (so that $Mv_\rho = \rho v_\rho$) may be chosen with all entries positive.

Recall that the *spectral radius of M* is defined as the largest absolute value of all the eigenvalues of M ($\rho = \max\{|\lambda|,\ \lambda \text{ is an eigenvalue of } M\}$). In addition,

(3) any vector in the nullspace of K is an eigenvector of M, corresponding to the eigenvalue γ:

$$Mv = Kv + \gamma Iv = \gamma v;$$

(4) the columns of K add up to zero, a fact that can be written as $\bar{1}K = 0$ where $\bar{1} = (\,1 \quad 1 \quad \cdots \quad 1\,)$.

Then we have

$$\bar{1}Mv_\rho = \bar{1}(\rho v_\rho) = \rho(\bar{1}v_\rho), \tag{A.1}$$

where $\bar{1}v_\rho$ is a positive scalar, because all the entries of v_ρ are positive. On the other hand, because $\bar{1}K = 0$,

$$\bar{1}Mv_\rho = \bar{1}Kv_\rho + \gamma(\bar{1}v_\rho) = \gamma(\bar{1}v_\rho). \tag{A.2}$$

Comparing equations (A.1) and (A.2), it turns out that

$$\rho(\bar{1}v_\rho) = \gamma(\bar{1}v_\rho) \quad \Leftrightarrow \quad \rho = \gamma$$

Therefore:

$$\rho v_\rho = Mv_\rho = Kv_\rho + \gamma v_\rho = Kv_\rho + \rho v_\rho \quad \Leftrightarrow \quad Kv_\rho = 0,$$

meaning that v_ρ *is a vector in the nullspace of* K. Conversely, point (3) above shows that any element in the nullspace of K must be an eigenvector of M, corresponding to the eigenvalue $\gamma = \rho$. This is exactly what we wanted to conclude: the nullspace of K has dimension one and is spanned by a positive vector (v_ρ).

B Proof of Theorem 1

To show that σ is a strictly increasing function of L_0, we only need to compute its derivative and check that it is always positive. From expression (13) we see that

$$\frac{d\sigma}{dL_0} = \frac{1}{2(v_2+v_4)}\left[1 - \frac{2\left[L_0 + R_0 + \frac{1+v_3}{v_2+v_4}\right] - 4R_0}{2\sqrt{\left[L_0 + R_0 + \frac{1+v_3}{v_2+v_4}\right]^2 - 4R_0L_0}}\right]$$

$$= \frac{1}{2(v_2+v_4)}\left[1 - \frac{L_0 - R_0 + \frac{1+v_3}{v_2+v_4}}{\sqrt{(L_0-R_0)^2 + \left(\frac{1+v_3}{v_2+v_4}\right)^2 + 2(L_0+R_0)\frac{1+v_3}{v_2+v_4}}}\right].$$

If $(L_0 - R_0) + (1+v_3)/(v_2+v_4) \leq 0$, then $d\sigma/dL_0$ is clearly a positive quantity. Otherwise, if $(L_0 - R_0) + (1+v_3)/(v_2+v_4) > 0$, then notice that the negative term is of the form $(a+b)/\sqrt{a^2+b^2+c}$, with $c > 2ab$, and so:

$$\left(\frac{a+b}{\sqrt{a^2+b^2+c}}\right)^2 = \frac{a^2+b^2+2ab}{a^2+b^2+c} < 1.$$

implying that $d\sigma/dL_0$ is a positive quantity. Therefore, σ is an increasing function of L_0.

Next, recall the conservation equation (9), which may be written as:

$$(1+v_3)\ \tau(L_0) \quad + \quad (v_2+v_4)\ \sigma(L_0) \quad = \quad R_0$$

Note that v_2, v_3 and v_4 are constant factors, and that the left hand side of this equation is to remain constantly equal to R_0. Taking derivatives with respect to L_0 on both sides of this equation yields:

$$\frac{d\tau}{dL_0} = -\frac{v_2+v_4}{1+v_3}\ \frac{d\sigma}{dL_0}.$$

From (i) we know that $d\sigma/dL_0 > 0$ for all L_0, so it follows that $d\tau/dL_0 < 0$ for all L_0. This proves part (ii).

Finally, to prove part (iii), observe that

$$\begin{aligned}\frac{d\mathcal{A}}{dL_0} &= (a_1 + a_3 v_3)\ \frac{d\tau}{dL_0} + (a_2 v_2 + a_4 v_4)\ \frac{d\sigma}{dL_0} \\ &= \left(-(a_1 + a_3 v_3)\frac{v_2+v_4}{1+v_3} + (a_2 v_2 + a_4 v_4)\right)\frac{d\sigma}{dL_0}.\end{aligned}$$

Assume first that $\mathtt{q} < 1$. Then,

$$\frac{a_1 + a_3 v_3}{a_2 v_2 + a_4 v_4}\ \frac{v_2+v_4}{1+v_3} > 1 \ \Rightarrow\ (a_1 + a_3 v_3)\frac{v_2+v_4}{1+v_3} \ > \ (a_2 v_2 + a_4 v_4) \ \Rightarrow\ \frac{d\mathcal{A}}{dL_0} \ < \ 0$$

and therefore $\mathcal{A}$ is a strictly decreasing function of L_0. Assuming that $\mathtt{q} > 1$, we can conclude by a similar argument that $d\mathcal{A}/dL_0$ is positive and hence the function is strictly increasing. Finally, whenever $\mathtt{q} = 1$, it is clear that $d\mathcal{A}/dL_0 \equiv 0$, and so the function is constant.

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