Response metrics of T-cell activation models

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Abstract

T-cell activation models describe the way in which antigens and T-cell receptors (TCRs) interact in order to trigger an immune response. A number of mathematical models of TCR-mediated T-cell activation have been proposed in the last decades. Often, the only measurable quantities available for the identification of these models are $E_{\max}$, the maximum achievable T cell activation level (over the background level), and EC_{50}, that is, the antigen concentration that results in 50% of $E_{\max}$. In order to analyse model properties such as identifiability, observability, and sensitivity, it is necessary to know how $E_{\max}$ and/or EC_{50} relate to the model parameters. However, their expressions are only available for a few of the published models of T-cell activation. Here we present a general method to derive the expressions of $E_{\max}$ and/or EC_{50}, and apply it to those models for which they are yet unknown. Our results expand the range of analyses that can be performed on T-cell activation models, contributing to enable their exploitation to understand and control the immune response.

1 Introduction

In vertebrates, the immune response consists of innate and adaptive strategies. The former is a first-line defense that reacts similarly to broad classes of pathogens, and may activate the latter one if a more targeted response is needed [1]. In contrast, the adaptive immune subsystem produces responses that are highly specific to each of the triggering antigens (Ag) [2]. Adaptive immune responses to protein Ags are based on interactions of T lymphocytes with Ag-presenting cells (APC) that lead to the activation of Ag-specific T cells. This lymphocyte activation is initiated by the binding of T-cell receptors (TCR) to their ligands, Ag-derived short peptides complexed with major histocompatibility complex proteins (pMHC) expressed on the membrane of APCs [2]. TCRs are membrane proteins composed of two chains, TCRα and TCRβ, which can indirectly transduce a signal intracellularly through their tight, non covalent association with a membrane protein complex composed of two heterodimers and one homodimer, collectively called CD3 [2].

The detailed mechanisms through which this T-cell signalling eventually leads to cell fate decisions remain only partially known. Such limited mechanistic knowledge could be exploited, however, to support the development of methods to control the immune response. To this end, many mathematical models have been proposed to describe these T-cell activation processes in a quantitative way [3, 4, 5]. These dynamic models consist of sets of ordinary differential equations (ODEs), whose integration over time yields time-courses of the system variables [6]. The models are nonlinear, and contain parameters that are not directly measurable; therefore, they must be calibrated by optimizing the fit of the model output to experimental data [7]. Calibration leads to correct estimates only if the model is identifiable, i.e., if it is possible to determine the values of its parameters uniquely [8]. A related property, observability, informs about the possibility of inferring the unmeasured state variables from the model output [9, 10].

These structural properties of dynamic models depend on the model equations and on the definition of the output functions, i.e., of the variables —or functions thereof— that can be measured.

Often, the experimentally measurable outputs consist of the maximum T-cell response level, *i.e.*, the peak responses that can be obtained ($E_{\max}$), and the Ag concentration that induces a response that is halfway between the baseline and the maximum (EC_{50}). Analysing these two outputs with respect to the aforementioned structural model properties requires mathematical expressions that relate these quantities to the model parameters and state variables. Furthermore, such expressions are also required for calculating the outputs' sensitivity to changes in parameter values [11, 12]. However, these formulas are only available for a few of the models published to date [3]. While they can be obtained relatively straightforwardly in some cases, their derivation is in general non-trivial.

In this paper we address this problem in the following way. We begin by presenting, in Section 2, a general methodology for deriving expressions of $E_{\max}$ and EC_{50} for all models in which the response R increases monotonically with the amount of ligand, until reaching a plateau. Then, in Section 3 we provide those expressions for a comprehensive set of phenotypic T-cell activation models (*i.e.*, models in which an output can be defined corresponding to the dynamics of a selected set of experimentally measurable quantities that determine a T-cell activation phenotype). This section also serves as a compendium of these models and their equations. For those models for which the expressions of $E_{\max}$ and EC_{50} had not been previously reported, their detailed derivations are given in the Appendix. Additionally, for one of the models —the serial triggering model described in subsection 3.13— we propose a modification of its equations, after discovering an important discrepancy between the conceptual description of the involved processes given in the original publication [13] and the equations presented therein. Lastly, we provide some concluding remarks in Section 4.

2 Methods

2.1 Phenotypic models of T-cell activation and parameters

The phenotypic models analysed in this work are summarized in Table 1, where references describing each model are included. Conceptual diagrams are shown in Fig. 1. All models have a minimum set of common parameters (most of which are kinetic rate constants). Both common and specific parameters are indicated in Fig. 1.

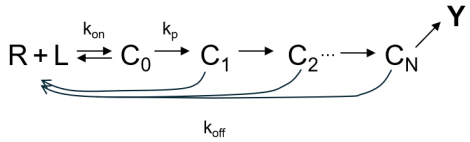
Table 1: Phenotypic models analysed in this work.

Model	Short name	Reference
Occupancy	**Occ**	[14]
Kinetic proofreading (McKeithan)	**KPR-1**	[15]
KPR with limited signalling	**KPR-LS**	[3]
KPR with sustained signalling	**KPR-SS**	[3, 16]
KPR with induced rebinding	**KPR-IR**	[17]
KPR with limited signalling and incoherent feedforward loop	**KPR-LS-IFF**	[4]
KPR with negative feedback I	**KPR-NF1**	[18]
KPR with negative feedback II	**KPR-NF2**	[19]
KPR with stabilizing activation chain	**KPR-SAC**	[5]
Zero-order ultrasensitivity	**ZU**	[20]
KPR with zero-order ultrasensitivity	**KPZU**	[20]
KPR with concentration compensation	**KPC**	[20]
Serial triggering	**ST**	[13]

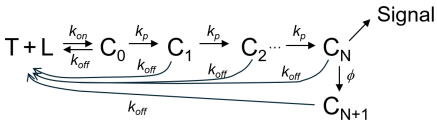
2.2 Modeling framework and notation

T-cell activation models are typically described by a system of ordinary differential equations (ODEs) that can be written in the following general form:

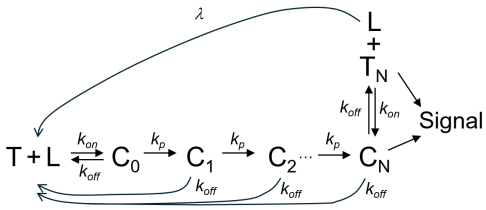
(1) Occ



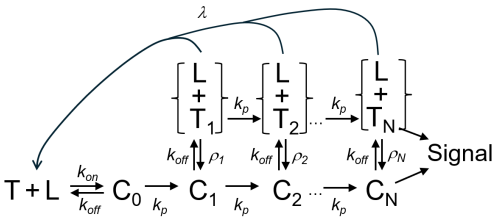
(2) KPR-1



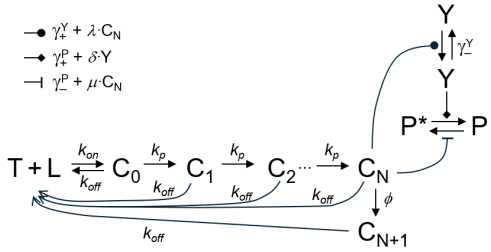
(3) KPR-LS



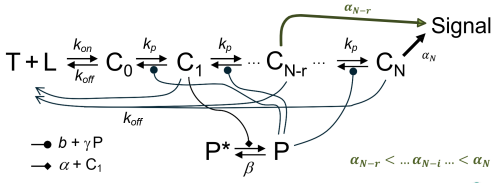
(4) KPR-SS



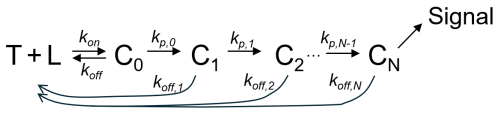
(5) KPR-IR



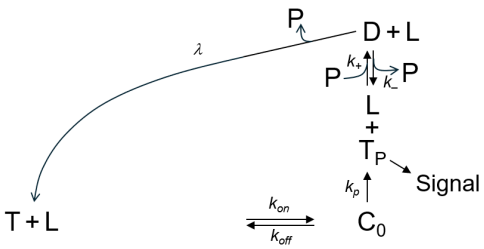
(6) KPR-LS-IFF



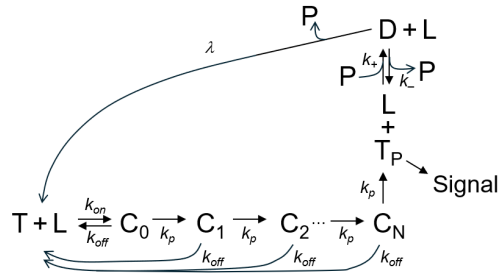
(7) KPR-NF1 and KPR-NF2 (green lines are exclusively for KPR-NF2).



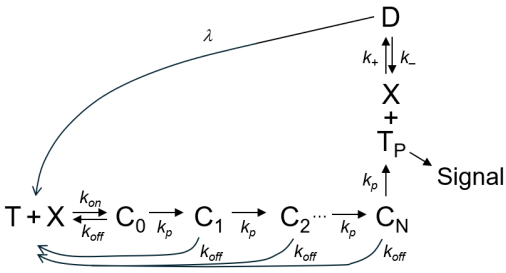
(8) KPR-SAC



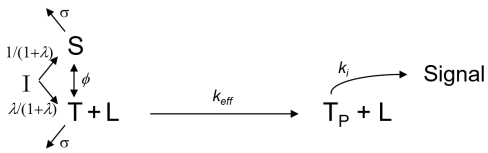
(9) ZU



(10) KPZU



(11) KPC



(12) ST

Figure 1: Diagrams describing the different models analysed in this paper.

$$\mathcal{M}: \begin{cases} \dot{x}(t) &= f(x(t), \theta), \\ y(t) &= h(x(t), \theta), \\ x(0) &= x^0 \end{cases} \tag{1}$$

where $\theta = (\theta_1, \ldots, \theta_p)$ is the parameter vector; $x(t) = (x_1(t), \ldots, x_n(t))$ is the state vector with initial conditions given by $x(0) = (x_1(0), \ldots, x_n(0))$; $y(t) = (y_1(t), \ldots, y_m(t))$ is the output vector; and $f(x(t), \theta)$ and $h(x(t), \theta)$ are non-linear functions. We will often simplify the notation by dropping the dependence on t. All vectors are real-valued, and typically have positive values.

The ODEs defining each phenotypic model are presented in Section 3 with a brief description of the corresponding model.

2.3 Calculation of $E_{\max}$ and EC_{50}

The phenotypic models considered in this work describe distinct processes, initiated by TCR-pMHC binding, resulting in the activation of TCRs (denoted also triggered TCRs) and the subsequent intracellular T cell signalling. In order to establish in each model a connection between total ligand per APC and T cell activation, we followed the approach in [3] and considered the outputs $E_{\max}$ and EC_{50} as two measures of T cell activation.

In this section we describe a procedure to derive, for all phenotypic models, the corresponding expressions for $E_{\max}$ and EC_{50} as functions of parameters and total ligand and TCR per cell. The main steps of this procedure are described in the following subsection.

2.3.1 Deriving the expressions of $E_{\max}$ and EC_{50} in T-cell activation ODE models

Step 1: *Steady-state.* First, assume that the system is in steady-state and, therefore, set to zero the differential equations given by $f(x(t), \theta)$. Also, consider the following conservation equations, respectively, for the ligands and receptors:

$$L_T = \widehat{L} + \widehat{C}_T$$

$$T_T = \widehat{T} + \widehat{C}_T$$

where L_T, T_T and $\widehat{C}_T$ are, respectively, the total cell density of pMHC, TCR and TCR-pMHC complexes, and $\widehat{L}$ and $\widehat{T}$ are, respectively, the cell density of unbound pMHC and TCR at steady-state.

Step 2: *Define $\widehat{R}$, the T-cell response level at steady-state, in terms of triggered TCRs, and obtain an expression for $\widehat{R}$ as a function of total ligand, L_T.* That expression can be obtained via two approaches:

- (2A): By incorporating the conservation equations within the equations that model the dynamics of free receptors and ligands, the cell density of free ligands and TCRs can be expressed in terms of $\widehat{C}_T$. Subsequently, using the remaining equations in the system at steady-state, derive an expression linking $\widehat{C}_T$ to R.
- (2B): If the equations that describe the evolution of free receptors and ligands contain some state variables in addition to $\widehat{C}_T$, it may be necessary to find a direct relationship between $\widehat{R}$ and L_T through each model equation.

Step 3: *Obtain an expression for E_{max}, the maximal response level.* For all models but four (KPR-LS-IFF, KPR-NF1, KPR-NF2, and KPC, considered below as special cases), the corresponding value of R grows monotonically with L_T, reaching a maximal level or plateau at ligand saturation. Following [3] we define $E_{\max}$ as the value of $\widehat{R}$ in the limit of excess ligand or ligand saturation, that is, in all these models $E_{\max} = \lim_{L_T \to \infty} \widehat{R}$.

To that end, in the first approach (2A), compute first the limit of $\widehat{C}_T$ for $L_T \to \infty$, and then substitute this result into the expression for R. In the second approach (2B), the limit $L_T \to \infty$ is directly computed after non-dimensionalizing the variables.

Step 4: *Obtain* EC_{50}. Impose the condition $\widehat{R} = E_{\max}/2$ and substitute the expressions obtained in steps 2 and 3 for $\widehat{R}$ and $E_{\max}$. This yields an expression in terms of L_T, T_T, and all or a subset of the system parameters. Then solve this expression for L_T in terms of T_T and parameters. Thus, $EC_{50} = L_T$ when $R = E_{\max}/2$.

2.3.2 Illustration of the methodology

Here we apply the above procedure to McKeithan's kinetic proofreading (KPR) model [15, 3] described below in subsection 3.2. Its corresponding ODE system is defined in that subsection by Eqns. (19). In this model the T-cell activation level is given by:

$$R(t) = C_N(t)$$

Step 1: As indicated above, we denote the system state variables at steady-state with a hat accent over the variable's name. Then the model equations at steady-state are:

$$\begin{aligned}
0 &= -k_{\text{on}}\widehat{L}\widehat{T} + k_{\text{off}}\widehat{C}_T && (2)\\
0 &= -k_{\text{on}}\widehat{L}\widehat{T} + k_{\text{off}}\widehat{C}_T && (3)\\
0 &= k_{\text{on}}\widehat{L}\widehat{T} - (k_{\text{off}} + k_p)\widehat{C}_0 && (4)\\
0 &= k_p\widehat{C}_{i-1} - (k_{\text{off}} + k_p)\widehat{C}_i; \quad 1 \le i \le N-1 && (5)\\
0 &= k_p\widehat{C}_{N-1} - k_{\text{off}}\widehat{C}_N && (6)\\
L_T &= \widehat{L} + \widehat{C}_T && (7)\\
T_T &= \widehat{T} + \widehat{C}_T && (8)
\end{aligned}$$

Step 2: From Eqn. (5) one has $\widehat{C}_i = \psi\,\widehat{C}_{i-1}$, with $\psi = \dfrac{k_p}{k_p + k_{\text{off}}}$, and applying this equation recursively, one obtains:

$$\widehat{C}_{N-1} = \psi^{N-1}\widehat{C}_0. \tag{9}$$

Taking into account that $k_{\text{off}}/k_p = (1-\psi)/\psi$, and inserting Eqn. (9) into Eqn. (6) yields $\widehat{C}_0 = \dfrac{1-\psi}{\psi^N}\widehat{C}_N$. Plugging now this equation into the equation that defines the total number of ligand-receptor complexes yields:

$$\widehat{C}_T = \sum_{i=0}^{N-1}\widehat{C}_i + \widehat{C}_N = \widehat{C}_0\sum_{i=0}^{N-1}\psi^i + \widehat{C}_N = \frac{1}{\psi^N}\widehat{C}_N. \tag{10}$$

Therefore:

$$\widehat{R} = \widehat{C}_N = \psi^N\widehat{C}_T. \tag{11}$$

Now, from Eqn. (2) one has:

$$\widehat{C}_T = \frac{k_{\text{on}}}{k_{\text{off}}}(L_T - \widehat{C}_T)(T_T - \widehat{C}_T). \tag{12}$$

Solving this equation for $\widehat{C}_T$ gives:

$$\widehat{C}_T = \frac{(L_T + T_T + K_D) - \sqrt{(L_T + T_T + K_D)^2 - 4L_T T_T}}{2}, \qquad \text{where } K_D = \frac{k_{\text{off}}}{k_{\text{on}}}, \tag{13}$$

and inserting this result into Eqn. (11) provides an expression for R in terms of L_T.

Step 3: Taking into account Eqn. (11), calculating $E_{\max} = \lim_{L_T \to \infty} R$ amounts to calculating $\widehat{C}_T$ for $L_T \to \infty$. In order to do that, it suffices to reorganise Eqn. (12) and take the limit $L_T \to \infty$:

$$\lim_{L_T \to \infty} \frac{k_{\text{on}}}{k_{\text{off}}}(T_T - C_T) = \lim_{L_T \to \infty} \frac{\widehat{C}_T}{L_T - \widehat{C}_T} = 0.$$

Therefore, $\widehat{C}_T = T_T$ in the limit $L_T \to \infty$. Hence:

$$E_{\max} = \lim_{L_T \to \infty} \widehat{R} = \psi^N \lim_{L_T \to \infty} \widehat{C}_T = \psi^N T_T. \tag{14}$$

Step 4: Let R_{50}, $\widehat{C}_{T_{50}}$, and $L_{T_{50}}$ denote, respectively, the half-maximal response, the total complexes at R_{50}, and the total ligand dose that induces the R_{50} response. Then, from Eqns. (11) and (14) one has:

$$R_{50} = \psi^N \widehat{C}_{T_{50}} = \frac{E_{\max}}{2} = \frac{\psi^N T_T}{2},$$

and, therefore,

$$\widehat{C}_{T_{50}} = \frac{T_T}{2} \tag{15}$$

Now, from Eqn. (13) and using Eqn. (15), one has at $\widehat{C}_T = \widehat{C}_{T_{50}}$:

$$\widehat{C}_{T_{50}} = \frac{(L_{T_{50}} + T_T + K_D) - \sqrt{(L_{T_{50}} + T_T + K_D)^2 - 4L_{T_{50}}T_T}}{2} = \frac{T_T}{2}. \tag{16}$$

Finally, solving Eqn. (16) for $L_{T_{50}}$ yields:

$$EC_{50} = L_{T_{50}} = \frac{T_T}{2} + \frac{k_{\text{off}}}{k_{\text{on}}}. \tag{17}$$

Figure 2 shows a graphical summary of these results for a particular set of parameter values.

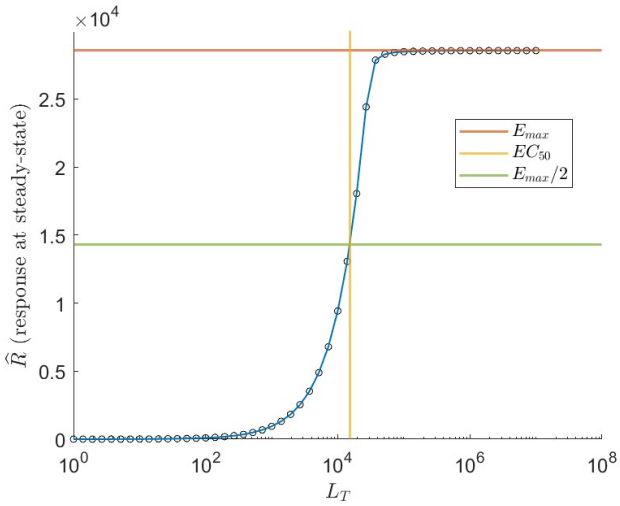


Figure 2: *KPR-1* model. The response at steady-state, $\widehat{R}$, as a function of total ligand. Black circles correspond to values of $R(t)$ obtained with the ODE system at $t = 300$ s. The horizontal red line shows the value of $E_{\max}$ computed with Eqn. (14), while the green line corresponds to $E_{\max}/2$. The vertical yellow line shows the value of EC_{50} obtained with Eqn. (17). Parameter values: $k_{on} = 10^{-5}$ $s^{-1}[]^{-1}$, $k_{\text{off}} = 0.01$ s^{-1}, $k_p = 1$ s^{-1}, and $T_T = 3 \times 10^4$ TCRs/cell. The symbol [] indicates a concentration unit (number of molecules per cell) for membrane and cytoplasmic molecules.

3 Results: calculation of $\widehat{R}$, $E_{\max}$ and EC_{50} for T-cell phenotypic models

In this section we briefly present the currently existing phenotypic models of T-cell activation (Table 1) with their defining equations, in approximate order of increasing complexity. For most of them we provide the corresponding expressions for $\widehat{R}$, $E_{\max}$ and EC_{50}. We start with those

few models for which these expressions were already available in the literature [3] (Sections 3.1 to 3.3). For them, we give the published results without further calculations. In section 3.4 we deal with the KPR with sustained signalling model. While this model was also analysed in reference [3], in that publication only an implicit-form expression was provided for $\widehat{R}$ and $E_{\max}$. Therefore, we reanalysed here this model, and in that section we provide the corresponding closed-form expressions for those two quantities in terms of L_T and T_T. For most of the remaining models (Sections 3.5 to 3.13) we give, for the first time, the corresponding expressions for R, $E_{\max}$ and EC_{50}. Since in general their derivation departs to varying degrees from the procedure outlined in subsection 2.3.2, we provide their detailed calculations in the Appendix.

To highlight the similarities and differences among the models, we show their conceptual schemes in Fig. 1 using a similar layout for all of them. The code and calculations used to produce the results can be accessed at the following repository: `https://github.com/Xabo-RB/Inmunology-analysis.git`.

3.1 Occupancy model (*Occ*)

The occupancy model presented in [14] assumes that T-cell activation is proportional to the number of TCRs occupied by pMHC complexes, without further delays. Its equations are given by:

$$\begin{cases} \dfrac{dL}{dt} = -k_{\text{on}} LT + k_{\text{off}} C_0 \\ \dfrac{dT}{dt} = -k_{\text{on}} LT + k_{\text{off}} C_0 \\ \dfrac{dC_0}{dt} = k_{\text{on}} LT - k_{\text{off}} C_0 \end{cases} \tag{18}$$

The response or signalling state in this model is defined as $R(t) = C_0(t)$. For this model, the expressions for $\widehat{R}$, $E_{\max}$ and EC_{50} can be found in the literature [3], and are as follows:

Occ: $\widehat{R}$, $E_{\max}$ and EC_{50}.

$$\widehat{R} = \frac{(L_T + T_T + \frac{k_{\text{off}}}{k_{\text{on}}}) - \sqrt{(L_T + T_T + \frac{k_{\text{off}}}{k_{\text{on}}})^2 - 4L_T T_T}}{2}$$

$$E_{\max} = T_T$$

$$EC_{50} = \frac{k_{\text{off}}}{k_{\text{on}}} + T_T/2$$

3.2 KPR McKeithan (*KPR-1*)

This is the first and simplest model of lymphocyte receptor activation through kinetic proofreading (KPR) proposed originally by McKeithan [15]. According to this mechanism, interaction between the TCR and the peptide-MHC complex does not immediately lead to signalling; instead, TCRs in complexes with pMHC ligands must undergo N sequential modifications (phosphorylation of the CD3 cytoplasmic tails) before triggering an effective immune response. In the KPR-1 model the response is defined as $R(t) = C_N(t)$. The ODE system defining this model is the following:

$$
\begin{cases}
\dfrac{dL}{dt} = -k_{\text{on}}LT + \displaystyle\sum_{i=0}^{N} k_{\text{off}}C_i \\
\dfrac{dT}{dt} = -k_{\text{on}}LT + \displaystyle\sum_{i=0}^{N} k_{\text{off}}C_i \\
\dfrac{dC_0}{dt} = k_{\text{on}}LT - (k_{\text{off}} + k_p)C_0 \\
\dfrac{dC_i}{dt} = k_pC_{i-1} - (k_{\text{off}} + k_p)C_i; \quad 1 \leq i \leq N-1 \\
\dfrac{dC_N}{dt} = k_pC_{N-1} - k_{\text{off}}C_N
\end{cases}
\tag{19}
$$

and the corresponding $\widehat{R}$, $E_{\max}$ and EC_{50} are:

KPR-1: $\widehat{R}$, $E_{\max}$ and EC_{50}.

$$
\widehat{R} = \left(\frac{k_p}{k_p + k_{\text{off}}}\right) \frac{\left(L_T + T_T + \frac{k_{\text{off}}}{k_{\text{on}}} - \sqrt{(L_T + T_T + \frac{k_{\text{off}}}{k_{\text{on}}})^2 - 4L_TT_T}\right)}{2}
$$

$$
E_{\max} = \left(\frac{k_p}{k_p + k_{\text{off}}}\right)^N T_T
$$

$$
EC_{50} = \frac{T_T}{2} + \frac{k_{\text{off}}}{k_{\text{on}}}
$$

3.3 KPR limited signalling (*KPR-LS*)

This extension of the KPR-1 model, proposed in [3], assumes that T-cell receptors signal for a limited time after reaching an activated state. For this model, $R(t) = C_N(t)$. This model is defined by the following ODE system:

$$
\begin{cases}
\dfrac{dL}{dt} = -k_{\text{on}}LT + \displaystyle\sum_{i=0}^{N+1} k_{\text{off}}C_i \\
\dfrac{dT}{dt} = -k_{\text{on}}LT + \displaystyle\sum_{i=0}^{N+1} k_{\text{off}}C_i \\
\dfrac{dC_0}{dt} = k_{\text{on}}LT - (k_{\text{off}} + k_p)C_0 \\
\dfrac{dC_i}{dt} = k_pC_{i-1} - (k_{\text{off}} + k_p)C_i \qquad \text{for } 1 \leq i \leq N-1 \\
\dfrac{dC_N}{dt} = k_pC_{N-1} - (k_{\text{off}} + \phi)C_N \\
\dfrac{dC_{N+1}}{dt} = \phi C_N - k_{\text{off}}C_{N+1}
\end{cases}
\tag{20}
$$

KPR-LS: $\widehat{R}$, $E_{\max}$ and EC_{50}.

$$\widehat{R} = \left(\frac{k_{\text{off}}}{k_{\text{off}} + \phi}\right)\left(\frac{k_p}{k_p + k_{\text{off}}}\right)^N \frac{\left(L_T + T_T + \frac{k_{\text{off}}}{k_{\text{on}}} - \sqrt{(L_T + T_T + \frac{k_{\text{off}}}{k_{\text{on}}})^2 - 4L_T T_T}\right)}{2}$$

$$E_{\max} = \left(\frac{k_{\text{off}}}{k_{\text{off}} + \phi}\right)\left(\frac{k_p}{k_p + k_{\text{off}}}\right)^N T_T$$

$$EC_{50} = \frac{T_T}{2} + \frac{k_{\text{off}}}{k_{\text{on}}}$$

3.4 KPR sustained signalling (*KPR-SS*)

This model [16] combines kinetic proofreading and serial engagement mechanisms, and defines the response as $R(t) = C_N(t) + T_N(t)$. Its ODE equations are:

$$\begin{cases} \dfrac{dL}{dt} = -k_{\text{on}}LT + \displaystyle\sum_{i=0}^{N} k_{\text{off}}C_i - k_{\text{on}}LT_N \\ \dfrac{dT}{dt} = -k_{\text{on}}LT + \displaystyle\sum_{i=0}^{N-1} k_{\text{off}}C_i + \lambda T_N \\ \dfrac{dC_0}{dt} = k_{\text{on}}LT - (k_{\text{off}} + k_p)C_0 \\ \dfrac{dC_i}{dt} = k_p C_{i-1} - (k_{\text{off}} + k_p)C_i \qquad \text{for } 1 \leq i \leq N-1 \\ \dfrac{dC_N}{dt} = k_p C_{N-1} - k_{\text{off}}C_N + k_{\text{on}}LT_N \\ \dfrac{dT_N}{dt} = k_{\text{off}}C_N - k_{\text{on}}LT_N - \lambda T_N \end{cases} \tag{21}$$

KPR-SS: $\widehat{R}$, $E_{\max}$ and EC_{50}.

$$\widehat{R} = \left(\frac{k_{\text{off}} + \lambda + k_{\text{on}}(L_T - C_T)}{\lambda + k_{\text{on}}\,\psi^N(L_T - C_T)}\right)\psi^N C_T,$$

with: $\psi = \dfrac{k_p}{k_{\text{off}} + k_p}$ and $\widehat{C}_T = \dfrac{(L_T + T_T + \frac{k_{\text{off}}}{k_{\text{on}}}) - \sqrt{(L_T + T_T + \frac{k_{\text{off}}}{k_{\text{on}}})^2 - 4L_T T_T}}{2}$

$$E_{\max} = T_T$$

$$EC_{50} = L_{T_{50}} = \frac{\psi^N\, C_T\,(k_{\text{off}} + \lambda - k_{\text{on}}C_T + k_{\text{on}}T_T/2) - T_T\lambda/2}{\psi^N\, k_{\text{on}}\,(T_T/2 - C_T)}$$

Note that the expression for EC_{50} or $L_{T_{50}}$ written above, which is taken from [3], depends implicitly on L_T through C_T. Therefore, given in this form, the equation for EC_{50} must be numerically solved for L_T. To solve this issue, we performed the corresponding calculations and derived the following closed-form expression for EC_{50} previously unavailable in the literature (see Appendix, subsection **KPR sustained signalling (KPR-SS)**):

KPR-SS: EC_{50}.

$$EC_{50} = \frac{\lambda\,(k_{\text{on}}\,T_T + 2\,\lambda) + (k_{\text{on}}\,T_T - 2\,\lambda)\big(a_1 + \psi^N(2\,\lambda + k_{\text{off}})\big)}{4\,k_{\text{on}}\,\lambda},$$

with,

$$a_1 = \sqrt{(1 - 2\,\psi^N)^2\,\lambda^2 + k_{\text{off}}\,\psi^N\big(k_{\text{off}}\,\psi^N + 2\,\lambda\,(1 + 2\,\psi^N)\big)}$$

3.5 KPR with induced rebinding (KPR-IR)

The model of *KPR with induced rebinding* assumes some changes in the receptor that could induce the rebinding to a pMHC [17]. Here we characterise this possible rebinding before fully dissociating by considering an intermediate ligand-receptor state denoted $\overline{C}_i(t)$ $(i = 1, ..., N)$. These are intermediate $[T_i\text{-}L]$ encounter complexes characterised by T_i and L being within the reaction distance but not bound [21]. The response is defined in this model as $R(t) = C_N(t) + \overline{C}_N(t)$, where $\overline{C}_N$ denotes the intermediate complex state $[T_N\text{-}L]$. The ODE system defining this model is:

$$\begin{cases} \dfrac{dL}{dt} = -k_{\text{on}}LT + k_{\text{off}}C_0 + \displaystyle\sum_{i=1}^{N} \lambda\overline{C}_i \\ \dfrac{dT}{dt} = -k_{\text{on}}LT + k_{\text{off}}C_0 + \displaystyle\sum_{i=1}^{N} \lambda\overline{C}_i \\ \dfrac{dC_0}{dt} = k_{\text{on}}LT - (k_{\text{off}} + k_p)C_0 \\ \dfrac{dC_i}{dt} = k_pC_{i-1} - (k_{\text{off}} + k_p)C_i + \rho_iC_i^*;\ 1 \leq i \leq N-1 \\ \dfrac{dC_N}{dt} = k_pC_{N-1} - k_{\text{off}}C_N + \rho_NC_N^* \\ \dfrac{d\overline{C}_1}{dt} = k_{\text{off}}C_1 - (\rho_1 + \lambda + k_p)\overline{C}_1 \\ \dfrac{d\overline{C}_i}{dt} = k_p\overline{C}_{i-1} + k_{\text{off}}C_i - (\rho_i + \lambda + k_p)\overline{C}_i;\ 2 \leq i \leq N-1 \\ \dfrac{d\overline{C}_N}{dt} = k_p\overline{C}_{N-1} + k_{\text{off}}C_N - (\rho_N + \lambda)\overline{C}_N \end{cases} \tag{22}$$

In order to derive an approximate version of this ODE system that could be solved analytically, we introduce the following hypothesis: due to experimental limitations, it is not possible to discriminate between the two molecular states C_i and $\overline{C}_i$. In particular, when re-binding after unbinding occurs fast enough, we cannot distinguish between unbound but encounter-oriented states and bound states [21]. In mathematical terms, this means that, in a coarse-grained observation timescale, we can safely assume that $k_{\text{off}}C_i = \rho_i\overline{C}_i$ (in statistical thermodynamics this hypothesis is often referred to as local steady state hypothesis). Then, defining $C_i^* = C_i + \overline{C}_i$, one has: $\overline{C}_i = \dfrac{k_{\text{off}}}{k_{\text{off}} + \rho_i}C_i^*$ and $C_i = \dfrac{\rho_i}{k_{\text{off}} + \rho_i}C_i^*$. Consequently, the above ODE system reduces to the following one:

$$
\begin{cases}
\dfrac{dL}{dt} = -k_{\text{on}} L\,T + k_{\text{off}} C_0 + \lambda \displaystyle\sum_{i=1}^{N} \alpha_i C_i^* \\
\dfrac{dT}{dt} = -k_{\text{on}} L\,T + k_{\text{off}} C_0 + \lambda \displaystyle\sum_{i=1}^{N} \alpha_i C_i^* \\
\dfrac{dC_0}{dt} = k_{\text{on}} L\,T - (k_{\text{off}} + k_p) C_0 \\
\dfrac{dC_i^*}{dt} = k_p (C_{i-1}^* - C_i^*) - \alpha_i C_i^*;\ 1 \le i \le N-1 \\
\dfrac{dC_N^*}{dt} = k_p C_{N-1}^* - \alpha_i C_N^*
\end{cases}
\tag{23}
$$

where $\alpha_i = \dfrac{k_{\text{off}}}{k_{\text{off}} + \rho_i}$. Note that in this approximate system $R(t) = C_N^*(t)$.

KPR-IR: $\widehat{R}$, $E_{\max}$ and EC_{50}.

$$
\widehat{R} = \frac{k_p}{\alpha_N} \left(\prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p} \right) \mu\, \widehat{C}_T.
$$

$$
E_{\max} = \frac{k_p}{\alpha_N} \left(\prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p} \right) \mu\, T_T
$$

$$
EC_{50} = \frac{k_{\text{off}} + k_p}{k_{\text{on}}}\, \mu + \frac{T_T}{2}
$$

Here the auxiliary parameter μ and state variable $\widehat{C}_T$ are as defined, respectively, in Eqns. (40) and (42) in the Appendix.

3.6 KPR limited signalling and incoherent feed-forward loop (*KPR-LS-IFF*)

An incoherent feed-forward loop is a signalling mechanism where an input activates the target signal through one route, and inhibits it through another one. In the KPR-LS-IFF, such a circuit is combined with the limited signalling mechanism described above [22, 4]. The response is the last state variable, $R(t) = P(t)$. This model is defined by the following ODE system:

$$
\begin{cases}
\dfrac{dL}{dt} = -k_{\text{on}} L T + \displaystyle\sum_{i=0}^{N} k_{\text{off}} C_i \\
\dfrac{dT}{dt} = -k_{\text{on}} L T + \displaystyle\sum_{i=0}^{N-1} k_{\text{off}} C_i \\
\dfrac{dC_0}{dt} = k_{\text{on}} L T - (k_{\text{off}} + k_p) C_0 \\
\dfrac{dC_i}{dt} = k_p C_{i-1} - (k_{\text{off}} + k_p) C_i & \text{for } 1 \le i \le N-1 \\
\dfrac{dC_N}{dt} = k_p C_{N-1} - (k_{\text{off}} + \phi) C_N \\
\dfrac{dC_{N+1}}{dt} = \phi C_N - k_{\text{off}} C_{N+1} \\
\dfrac{dY}{dt} = \gamma_+^y (Y_T - Y) - \gamma_-^y Y + \lambda C_N (Y_T - Y) \\
\dfrac{dP}{dt} = \gamma_+^p (P_T - P) - \gamma_-^p P + \delta Y (P_T - P) - \mu C_N P
\end{cases}
\tag{24}
$$

KPR-LS-IFF: $\widehat{R}$.

$$\widehat{R} = \frac{\left(\gamma_+^p\left(\gamma_-^y + \gamma_+^y + \lambda\,\alpha\,\widehat{C}_T\right) + \delta\left(\gamma_+^y + \lambda\,\alpha\,\widehat{C}_T\right)\cdot Y_T\right)\cdot P_T}{\left(\gamma_-^p + \gamma_+^p + \mu\,\alpha\,\widehat{C}_T\right)\left(\gamma_-^y + \gamma_+^y + \lambda\,\alpha\,\widehat{C}_T\right) + \delta\left(\gamma_+^y + \lambda\,\alpha\,\widehat{C}_T\right)\cdot Y_T},$$

with,

$$\alpha = \left(\frac{k_{\text{off}}}{k_{\text{off}} + \phi}\right)\psi^N,$$

and

$$\widehat{C}_T = \frac{(L_T + T_T + K_D) - \sqrt{(L_T + T_T + K_D)^2 - 4L_T T_T}}{2}.$$

In this model, the response $\widehat{R}$ as a function of L_T displays, for many reasonable parameter values, a bell-shaped behaviour (see Fig. 3). Therefore, to determine $E_{\max}$ it is necessary to compute first the derivative $d\widehat{R}/dL_T$, and subsequently solve the equation $d\widehat{R}/dL_T = 0$ to identify the corresponding value of L_T. Denoting this specific value as $L_{T_{\text{Emax}}}$, the expression for $E_{\max}$ is given by $E_{\max} = \widehat{R}(L_{T_{\text{Emax}}})$. Owing to their big size, the expressions for both, $E_{\max}$ and EC_{50} are only available in our online repository (`https://github.com/Xabo-RB/Inmunology-analysis.git`).

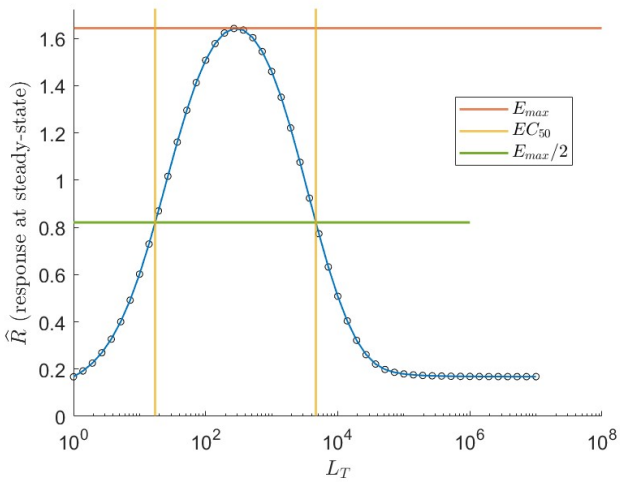


Figure 3: *KPR-LS-IFF* model. The response at steady-state, $\widehat{R}$, as a function of total ligand shows a bell-shape behaviour for many biologically reasonable parameter values. The blue line was obtained using the general equation for $\widehat{R}$. Black circles correspond to values of $R(t)$ obtained by solving the ODE system at $t = 300\ s$ for a large range of L_T values. The horizontal red and green lines show, respectively, the computed values of $E_{\max}$ and $E_{\max}/2$. The vertical yellow lines correspond to the two possible values of EC_{50}. Parameter values: $n = 5$ kinetic steps, $k_{\text{on}} = 10^{-5}\ s^{-1}[\,]^{-1}$, $k_{\text{off}} = 0.05\ s$, $k_p = 0.04\ s$, $\phi = 0.04\ s$, $\gamma_+ = 1\ s^{-1}$, $\gamma_- = 500\ s^{-1}$, $\lambda = 100\ s^{-1}[\,]^{-1}$, $\delta = 100\ s^{-1}[\,]^{-1}$, $\mu = 500\ s^{-1}[\,]^{-1}$, $Y_T = 100$ molec/cell, $P_T = 2.5$ molec/cell, and $T_T = 3 \times 10^4$ TCRs/cell. The symbol [] indicates a concentration unit (number of molecules per cell) for membrane and cytoplasmic molecules.

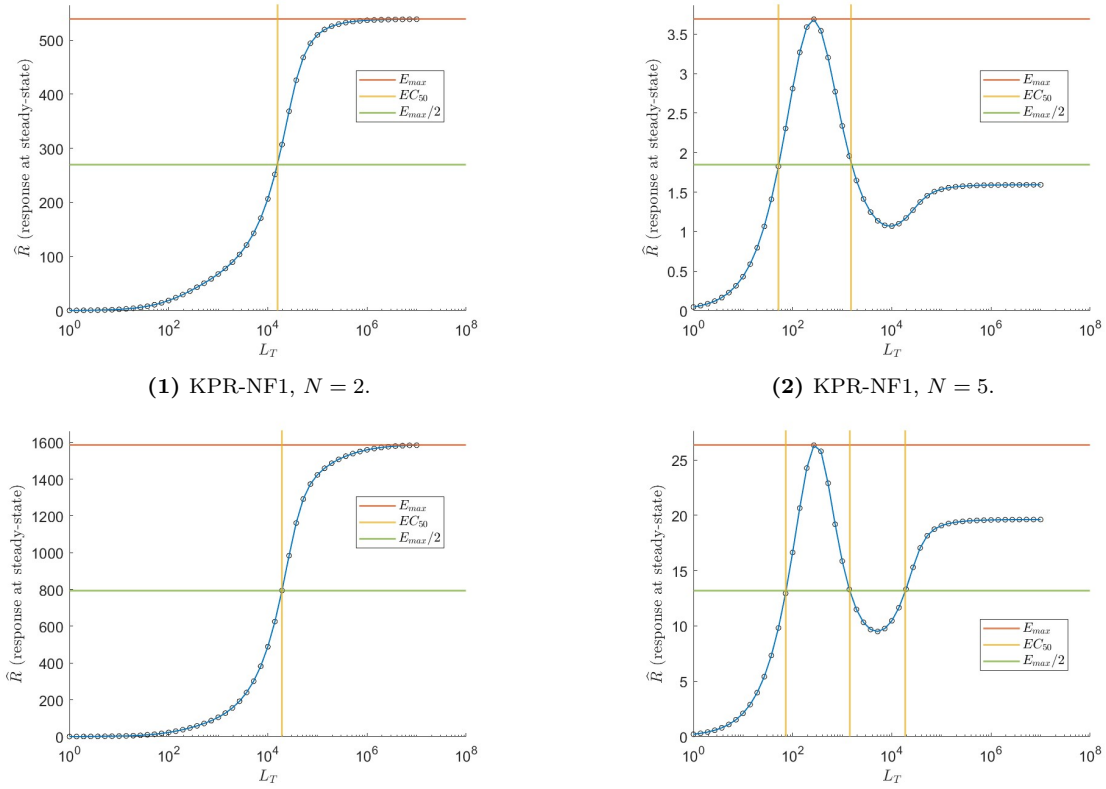
3.7 KPR with negative feedback 1 (*KPR-NF1*)

This model extends the basic KPR mechanism by adding a negative feedback loop mediated by Src homology 2 domain phosphatase-1 (SHP-1) [18, 23]. The response is defined here as $R(t) = C_N(t)$, and the corresponding ODE system is:

$$
\begin{cases}
\dfrac{dL}{dt} = -k_{\text{on}}LT + \displaystyle\sum_{i=0}^{N} k_{\text{off}}C_i \\
\dfrac{dT}{dt} = -k_{\text{on}}LT + \displaystyle\sum_{i=0}^{N} k_{\text{off}}C_i \\
\dfrac{dC_0}{dt} = k_{\text{on}}LT + (b+\gamma P)C_1 - (k_{\text{off}} + k_p)C_0 \\
\dfrac{dC_i}{dt} = k_pC_{i-1} - (k_{\text{off}} + k_p + b + \gamma P)C_i + (b+\gamma P)C_{i+1} \quad \text{for } 1 \leq i \leq N-1 \\
\dfrac{dC_N}{dt} = k_pC_{N-1} - (k_{\text{off}} + b + \gamma P)C_N \\
\dfrac{dP}{dt} = \alpha C_1(P_T - P) - \beta P
\end{cases}
\tag{25}
$$

As mentioned above and briefly discussed in [3, 4, 18], the *KPR-NF1* exhibits, for many parameter combinations and $N > 2$ kinetic steps, a bell-shaped behaviour in the relationship between the response $\widehat{R}$ and the total amount of ligands presented, L_T. This characteristic behaviour is shown, for $N = 5$, in panel (2) of Figure 4. For $N = 2$, the $E_{\max}$ value is attained when L_T approaches infinity, as in the other models. The expression for $\widehat{R}$ cannot be shown here due to its big size; we provide it at: `https://github.com/Xabo-RB/Inmunology-analysis.git`.

For $N > 2$, the $E_{\max}$ value must be obtained by analysing the derivative of an expression that describes, under steady-state conditions, the relationship between $\widehat{R}$ and L_T. For $N = 5$ and a standard set of parameter values (see Table 2 in the Appendix) we obtained the derivative numerically, using Mathematica [24]. In this case, the response R as function of L_T has five critical points, two of which are complex solutions and one is negative. Therefore, only two real positive possible solutions remain for the maximal response. Since $E_{\max}$ is, by definition, the solution with the largest numerical value, it corresponds to the local maximum of the curve shown in Fig. 4, panel (2). Thus, unlike most phenotypic models of TCR activation, in many reasonable parameter regimes the KPR-NF1 model does not reach $E_{\max}$ at ligand saturation but at relatively low ligand density in APCs. A limited analysis of the parameter impact on the behaviour of R *vs* L_T showed that the parameters that affect most this behaviour are k_{off}, k_p and the ratio α/β. Because the behaviour of the response R as a function of L_T has in many reasonable parameter regimes a bell shape with a rebound to a plateau, the ligand dose that produces a half-maximal T cell response, *i.e.* EC_{50}, may have up to three different positive solutions. In the present example (Fig. 4, panel (2)) there are two solutions: $EC_{50}^a = 52$ and $EC_{50}^b = 1551$ ligands per APC.



(1) KPR-NF1, $N = 2$.

(2) KPR-NF1, $N = 5$.

(3) KPR-NF2, $N = 2$, $n = 2$.

(4) KPR-NF2, $N = 5$, $n = 3$.

Figure 4: *KPR-NF1* and *KPR-NF2* models. Response level, R, as a function of L_T, for the KPR-NF1 model (first row) and the KPR-NF2 model (second row). Left panels, results for two kinetic proofreading steps ($N = 2$) for both models, and $n = 2$ for KPR-NF2. Right panels, results for $N = 5$ for both models, and $n = 3$ for KPR-NF2. Parameter values: $\alpha/\beta = 0.002$, $b = 0.04\ s^{-1}$, $\gamma = 1.0 \times 10^{-6}\ s^{-1}[\,]^{-1}$, $k_{\text{on}} = 1.0 \times 10^{-5}\ s^{-1}[\,]^{-1}$, $k_{\text{off}} = 0.05\ s^{-1}$, $k_p = 0.09\ s^{-1}$, $T_T = 3 \times 10^4$ TCRs/cell, and $S_T = 6 \times 10^5$ phosphatase molecules/cell. In panel (4) $k_p = 0.13$ and $k_{\text{off}} = 0.03$. The symbol [ ] indicates a concentration unit (number of molecules/cell) for membrane and cytoplasmic molecules.

3.8 KPR with negative feedback 2 (*KPR-NF2*)

The KPR-NF1 model was later relaxed [19] with respect to the definition of the response R. Nevertheless, the ODE system defining this model is still the same as that of the KPR-NF1 model and, therefore, all calculations but R remain in this model equal to the corresponding ones in the KPR-NF1 model. The KPR-NF2 model departs from the previous one by hypothesizing that not only TCRs in the C_N complexes contribute to T cell signalling, but also TCRs within the final n complexes of the proofreading chain (*i.e.*, from C_{N-n+1} to C_N), although with a contributing weight increasing from $N - n + 1$ to N. To account for this modification we define the following response, which assigns increasing weights α_i to the contributions of each C_i:

$$\widehat{R} = \sum_{i=N-n+1}^{N} \alpha_i \widehat{C}_i, \qquad \text{where } \alpha_i < \alpha_{i+1} \ \text{ and } \ N - n + 1 \leq i \leq N.$$

The second row of Figure 4 illustrates the same effect observed in the *KPR-NF1* model: for $N > 2$ and $n > 1$ the peak response does not occur at ligand saturation (*i.e.,* for $L_T \to \infty$), but at relatively low ligand levels. Similarly to the KPR-NF1 model, depending on parameter values, particularly those of k_{off}, k_p and the ratio α/β, there may be up to three different and biologically relevant solutions for EC_{50}. As in the previous model, the code for $\widehat{R}$ is available in our repository for $N = 2$ and $n = 2$ (`https://github.com/Xabo-RB/Inmunology-analysis.git`).

3.9 KPR with stabilizing activation chain (*KPR-SAC*)

The "Stabilizing Activation Chain" mechanism [5] relaxes the assumptions that all phosphorylation steps in the KPR mechanism (*i.e.*, $C_i \to C_{i+1}$) occur with the same rate constant k_p, and that all unbinding reactions (*i.e.*, $C_i \to L + T$) have the same rate constant k_{off}. This model was designed to lead to different responses for foreign and self-peptides. Thus, in this model it is assumed, on the one hand, that the phosphorylation steps $C_i \to C_{i+1}$ take place with rate constant k_{p_i}, with $k_{p_i} < k_{p_{i+1}}$, and, on the other hand, that the unbinding reactions $C_i \to L + T$ have rate constants $k_{\text{off}\,i}$, with $k_{\text{off}\,i} > k_{\text{off}\,i+1}$. In this model the response is defined as $R(t) = C_N(t)$, and the defining ODE system is:

$$\begin{cases} \dfrac{dL}{dt} = -k_{\text{on}} LT + \displaystyle\sum_{i=0}^{N} k_{\text{off}}(i) C_i \\ \dfrac{dT}{dt} = -k_{\text{on}} LT + \displaystyle\sum_{i=0}^{N} k_{\text{off}}(i) C_i \\ \dfrac{dC_0}{dt} = k_{\text{on}} LT - (k_{\text{off}}(0) + k_p(0)) C_0 \\ \dfrac{dC_i}{dt} = k_p(i-1) C_{i-1} - (k_{\text{off}}(i) + k_p(i)) C_i \quad \text{for } 1 \le i \le N-1 \\ \dfrac{dC_N}{dt} = k_p(N-1) C_{N-1} - k_{\text{off}}(N) C_N \\ k_{\text{off}}(i) = k_{\text{off}} \cdot \dfrac{1+i}{1+r \cdot i} \\ k_p(i) = k_p \cdot r_p^i \end{cases} \tag{26}$$

where r and r_p are constants.

KPR-SAC: $\widehat{R}$, $E_{\max}$ and EC_{50}.

$$\widehat{R} = \widehat{C}_T \left[1 + F_N \left(1 + \prod_{i=1}^{N-1} F_i + \sum_{i=1}^{N-2} \left(\prod_{j=1}^{i} F_{N-j} \right) \right) \right]^{-1}$$

$$E_{\max} = T_T \left[1 + F_N \left(1 + \prod_{i=1}^{N-1} F_i + \sum_{i=1}^{N-2} \left(\prod_{j=1}^{i} F_{N-j} \right) \right) \right]^{-1}$$

$$EC_{50} = \frac{T_T}{2} + \frac{k_{\text{off}} + k_p}{G\, k_{\text{on}}}.$$

Expressions for F_i, F_N, $\widehat{C}_T$, and G are given, respectively, in Eqns. (58), (59), (66) and (69) in the Appendix.

3.10 Zero-order ultrasensitivity model (*ZU*)

Zero-order ultrasensitivity [25] is a mechanism that enhances sensitivity to changes in ligand concentration and leads to fast responses, at the expense of decreasing specificity and the ability to discriminate foreign *vs* self-antigens [20]. In this model, $R(t) = T_p(t)$, and the ODE system defining this model is the following:

$$
\begin{cases}
\dfrac{dT}{dt} = -k_{\text{on}} T L + k_3 D + k_{\text{off}} C_0 \\
\dfrac{dL}{dt} = -k_{\text{on}} T L + (w + k_{\text{off}}) C_0 \\
\dfrac{dC_0}{dt} = k_{\text{on}} T L - (k_{\text{off}} + w) C_0 \\
\dfrac{dD}{dt} = k_+ T_p P - (k_- + k_3) D \\
\dfrac{dT_p}{dt} = -k_+ T_p P + k_- D + w C_0 \\
\dfrac{dP}{dt} = -k_+ T_p P + k_- D + k_3 D
\end{cases}
\tag{27}
$$

ZU: $\widehat{R}$ and $E_{\max}$.

$$
\widehat{R} = \frac{(k_- + \lambda)\, k_p\, (L_T - \widehat{L})}{k_+\, \lambda\, P_T - k_+\, k_p\, (L_T - \widehat{L})},
$$

$$
E_{\max} = \frac{\alpha + \sqrt{\alpha^2 + 4\, w^2\, k_3^2\, k_+\, (k_- + k_3)\, T_T}}{2\, w\, k_+\, k_3},
$$

where: $\alpha = \big(w\, k_+\, k_3\, T_T - k_+\, k_3\, (k_3 + w)\, P_T - w\, k_3\, (k_- + k_3)\big)$.

An expression for $\widehat{L}$ is given by a solution to Eqn. (77) in the Appendix. Note that for both $\widehat{R}$ and EC_{50} a general formula cannot be provided here because obtaining the corresponding results is computationally very expensive, involving quite large expressions. Therefore, in order to obtain values for these quantities one has to resort to numerical calculations using specific sets of parameter values.

3.11 KPR with zero-order ultraspecificity (*KPZU*)

The KPR with zero-order ultraspecificity model combines KPR and zero-order ultrasensitivity [20]. In this way it can balance specificity, sensitivity, and speed. The response is given by $R(t) = T_p(t)$, and the equations defining this model are the following:

$$
\begin{cases}
\dfrac{dT}{dt} = -k_{\text{on}} T L + k_3 D + k_{\text{off}} \displaystyle\sum_{i=0}^{n} C_i \\
\dfrac{dL}{dt} = -k_{\text{on}} T L + w C_N + k_{\text{off}} \displaystyle\sum_{i=0}^{n} C_i \\
\dfrac{dT_p}{dt} = w C_N - k_+ T_p P + k_- D \\
\dfrac{dC_0}{dt} = -k_{\text{off}} C_0 - w C_0 + k_{\text{on}} T L \\
\dfrac{dC_i}{dt} = -k_{\text{off}} C_i - w C_i + w C_{i-1} \quad \text{for } 1 \le i \le N \\
\dfrac{dP}{dt} = -k_+ T_p P + k_- D + k_3 D \\
\dfrac{dD}{dt} = k_+ T_p P - k_- D - k_3 D
\end{cases}
\tag{28}
$$

KPZU: $\widehat{R}$ and $E_{\max}$.

$$\widehat{R} = \frac{(k_- + \lambda)\Phi\,(L_T - \widehat{L})}{k_2\big(\lambda\,P_T - \Phi\,(L_T - \widehat{L})\big)}$$

$$E_{\max} = \frac{\alpha + \sqrt{\alpha^2 + 4\,\Phi^2\,\lambda^2\,k_+\,(k_- + \lambda)\,T_T}}{2\,\Phi\,k_+\,\lambda},$$

where:

$$\Phi = k_p\,\psi^N\left(\frac{1-\psi}{1-\psi^{N+1}}\right), \quad \psi = \frac{k_p}{k_{\text{off}} + k_p}, \quad \alpha = \big(\Phi\,k_+\,\lambda\,T_T - k_+\,\lambda\,(\lambda+\Phi)\,P_T - \Phi\,\lambda\,(k_- + \lambda)\big).$$

As was the case for the ZU model, an expression for $\widehat{L}$ is given by a solution to Eqn. (81) (see the Appendix), and a general formula for $\widehat{R}$ and EC_{50} cannot be provided here due to the large computational cost of their calculation.

3.12 KPR with concentration compensation (*KPC*)

The *KPC* model modifies the *KPZU* model by assuming a mechanism where the same molecule controls receptor phosphorylation and dephosphorylation [20]. Thus, the system can exhibit low sensitivity to high concentrations of non-target ligands and high sensitivity to low concentrations of target ligands. As in the previous model, $R(t) = T_p(t)$. The ODE system defining this model is:

$$\begin{cases} \dfrac{dT}{dt} = -k_{\text{on}}TX + k_3 D + k_{\text{off}}\displaystyle\sum_{i=0}^{N} C_i \\ \dfrac{dX}{dt} = -(k_{\text{on}}TX + k_+T_pX) + wC_N + k_{\text{off}}\displaystyle\sum_{i=0}^{N} C_i + (k_- + k_3)D \\ \dfrac{dC_0}{dt} = -(k_{\text{off}} + w)C_0 + k_{\text{on}}TX \\ \dfrac{dC_i}{dt} = -(k_{\text{off}} + w)C_i + wC_{i-1} \quad \text{for } 1 \le i \le N \\ \dfrac{dT_p}{dt} = wC_N - k_+T_pX + k_-D \\ \dfrac{dD}{dt} = k_+T_pX - (k_- + k_3)D \end{cases} \tag{29}$$

KPC: $\widehat{R}$, $E_{\max}$ and EC_{50}.

$$\widehat{R} = -\frac{(k_- + \lambda)\left(\beta_1 - \sqrt{\beta_2^2 - 4\,k_+^2\,k_{\text{on}}^2\,T_T\,X_T(1+\Phi)^2\psi^{2(N+1)}}\right)}{2\,k_+\,(1+\Phi)\Big(k_+\,\lambda + k_{\text{on}}\,(k_- + \lambda)\,\psi^{N+1}\Big)},$$

where:

$$\beta_1 = k_+\,\lambda\,\Phi + \Big((k_- + \lambda)\,\Phi + k_+\,(X_T - T_T)(1+\Phi)\Big)\,k_{\text{on}}\,\psi^{N+1},$$

$$\beta_2 = k_+\,\lambda\,\Phi + \Big((k_- + \lambda)\,\Phi + k_+\,(X_T + T_T)(1+\Phi)\Big)\,k_{\text{on}}\,\psi^{N+1},$$

$$\psi = \frac{k_p}{k_{\text{off}} + k_p}, \quad \text{and} \quad \Phi = \frac{k_p}{\lambda}\left(\frac{\psi^N - \psi^{N+1}}{1 - \psi^{N+1}}\right).$$

$$E_{\max} = \frac{k_{\text{on}}\,(k_- + \lambda)\,k_p^{N+1}\,T_T}{k_+\,\lambda\,(k_{\text{off}} + k_p)^{N+1} + k_{\text{on}}\,(k_- + \lambda)\,k_p^{N+1}},$$

$$EC_{50} = \frac{T_T}{2} + \frac{k_{\text{off}}\Big(k_-\,k_{\text{on}}\,k_p^{N+1} + \big(k_{\text{on}}\,k_p^{N+1} + k_+\,\lambda\,(k_{\text{off}} + k_p)^{N+1}\big)\Big)}{k_+\,k_{\text{on}}\Big(k_{\text{off}}\big(k_p^{N+1} + \lambda\,(k_{\text{off}} + k_p)^N\big) - \lambda\,k_p\,\big(k_p^N - (k_{\text{off}} + k_p)^N\big)\Big)}.$$

This is an atypical model in that the response at steady-state, $\widehat{R}$, as a function of total ligand shows an inverted sigmoidal behaviour (Fig. 5), with a maximum at $L_T \to 0$ and decreasing to 0 for $L_T \to \infty$.

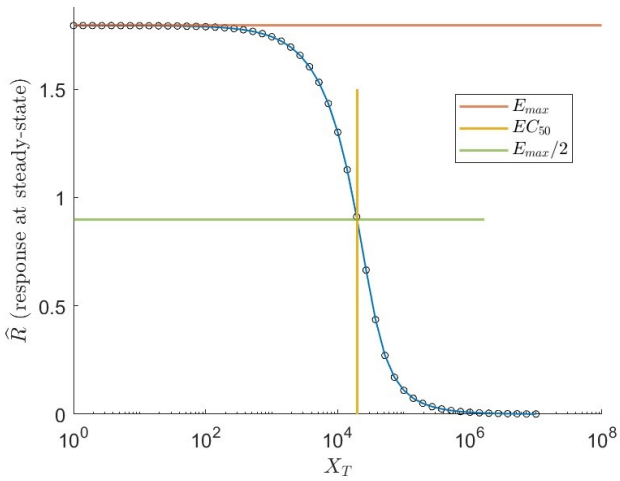


Figure 5: *KPC* model. The response at steady-state, $\widehat{R}$, as a function of total ligand (solid blue line) was obtained using the general equation for $\widehat{R}$ (Eqn. (86) in the Appendix). Black circles correspond to values of $R(t)$ obtained with the ODE system at $t = 2000\ s$ and a wide range of values of L_T. The horizontal red line shows the value of $E_{\max}$ computed with Eqn. (89) while the green line corresponds to $E_{\max}/2$. The vertical yellow line corresponds to EC_{50} obtained with equation Eqn. (90). Parameter values: $k_{on} = 10^{-5}\ s^{-1}[\,]^{-1}$, $\lambda = 0.04\ s^{-1}$, $k_{\text{off}} = 0.05\ s^{-1}$, $k_p = 0.09\ s^{-1}$, $k_+ = 0.1\ s^{-1}$, $k_- = 0.05\ s^{-1}$, $T_T = 3 \times 10^4$ TCRs/cell. The symbol [] indicates a concentration unit (number of molecules per cell) for membrane and cytoplasmic molecules.

3.13 Modified ST model (*ST-mod*)

The serial triggering model *(ST)* was presented in [26, 13] and it was defined there by the following ODE system:

$$
\begin{cases}
\dfrac{dS}{dt} = -\lambda\phi(S-T) + s(1-S) \\
\dfrac{dT}{dt} = \phi(S-T) + s(1-T) - k_{\text{eff}}T^h L^h \\
\dfrac{dA}{dt} = k_{\text{eff}}T^h L^h - k_i A
\end{cases}
\tag{30}
$$

According to the conceptual description of this model [13], two subsets of TCRs are distinguished: one within the interface pool and the other within the spare pool. TCRs within the interface pool (denoted T) are situated in an area, determined by the membrane interface between a T cell and an APC, where they can interact with pMHC molecules. These interactions, characterised by an effective kinetic rate k_{eff} and a kinetic order $h > 1$, result in the formation of triggered (phosphorylated) TCRs (denoted A), which are subsequently internalised with constant rate k_i [13]. Conversely, the spare pool contains TCRs located outside the interface area (denoted S), with the ratio of interface area to spare area characterized by the parameter λ. Free TCRs diffuse between the two pools with constant rate ϕ per unit membrane area, and undergo turnover between the membrane and the cytoplasm with constant rate s per unit membrane area. At close inspection, however, it can be seen that Eqns. (30) do not correctly translate into mathematical expressions the conceptual description of the model. For instance, in the absence of ligand, each pool ends up accumulating half of the total TCRs, independently of the ratio λ and of the initial values of S and T.

Here we propose a modification of the above equations, in an attempt to make them correspond accurately to the mechanism conceptually described in [13]. In this model variant, the rate of TCRs turnover between the membrane and the cytoplasm is denoted σ, and all other parameters are denoted as in the original model. This reformulation introduces a new definition for the flow between areas, represented by the first term in the equations for dS/dt and dT/dt. Additionally, it redefines the input rate of receptors, which is now proportional to the respective areas of the two pools. The new equations, with the state-variables adimensionalyzed with respect to the total amount of TCRs in the absence of ligand, that is, with respect to $S_0 + T_0$, are the following:

$$
\begin{cases}
\dfrac{dS}{dt} = -\phi\left(S - \dfrac{1}{\lambda}T\right) + \sigma\left(\dfrac{1}{1+\lambda} - S\right) \\
\dfrac{dT}{dt} = \phi\left(S - \dfrac{1}{\lambda}T\right) + \sigma\left(\dfrac{\lambda}{1+\lambda} - T\right) - k_{\text{eff}}T^h L^h \\
\dfrac{dA}{dt} = k_{\text{eff}}T^h L^h - k_i A
\end{cases}
\tag{31}
$$

In this model the response is defined by $R(t) = A(t)$. Notice that, according to the definition of λ, it verifies $\lambda = T_0/S_0$. We refer to this variant model as modified ST or *ST-mod*. Note, however, that both models, *ST* and *ST-mod*, share the same graphical scheme (see Fig. 1, panel 12).

ST-mod: $\widehat{R}$, $E_{\max}$ and EC_{50}.

For $h = 1$:

$$\widehat{R} = \frac{\lambda\,\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big)\,k_{\text{eff}}\,L}{k_i\,(1+\lambda)\,\Big(\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big) + \lambda\,(\sigma+\phi)\,k_{\text{eff}}L\Big)},$$

$$EC_{50} = \frac{\sigma\,\big(\sigma\,\lambda + \phi\,(1+\lambda)\big)}{\lambda\,k_{\text{eff}}\,(\sigma+\phi)}.$$

For $h = 2$:

$$\widehat{R} = \frac{\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big)\,\Big(\sigma\,\Omega - \sqrt{\sigma\,\Omega\,\big(4\,k_{\text{eff}}\,L^2\lambda^2(\sigma+\phi) + \sigma\,\Omega\big)}\Big)}{k_i\,(1+\lambda)(\sigma+\phi)\,\Big(\sigma\,\Omega + \sqrt{\sigma\,\Omega\,\big(4\,k_{\text{eff}}\,L^2\lambda^2(\sigma+\phi) + \sigma\,\Omega\big)}\Big)},$$

with: $\Omega = (1+\lambda)\big(\phi + \lambda\,(\sigma+\phi)\big)$.

$$EC_{50} = \frac{\sqrt{2\,\sigma\,(1+\lambda)\,\big(\phi + \lambda\,(\sigma+\phi)\big)}}{\lambda\,\sqrt{k_{\text{eff}}\,(\sigma+\phi)}}.$$

For $h \geq 1$ ($h \in \mathbb{N}$):

$$E_{\max} = \frac{\sigma\,\big(\sigma\,\lambda + \phi\,(1+\lambda)\big)}{k_i\,(1+\lambda)\,(\sigma+\phi)}.$$

Due to the complexity of the calculations we were unable to obtain a general expression of EC_{50} for $h = 3,\ 4$.

4 Conclusions

In this work we have contributed to filling a gap in the literature, by deriving the expressions of maximum drug effect ($E_{\max}$) and half-maximal effective concentration (EC_{50}) for the main T-cell activation models for which those expressions were previously unknown. Knowledge of these expressions is necessary to perform analyses of key model properties, including identifiability, observability, and sensitivity. Thus, the results presented in this paper enable a deeper analysis of the existing models of T-cell activation, and facilitate obtaining biological insights about the involved mechanisms.

Our results have revealed a clear trend. While the previously known expressions of $E_{\max}$ and EC_{50} were relatively simple, the formulas of these quantities become more involved as the complexity of the models increases. As a result, in some models the impact of specific parameters on the maximum drug effect and half-maximal effective concentration is less direct, and possibly easier to be compensated by changes in other parameters. This, in turn, implies that inferring the values of such parameters from measurements of $E_{\max}$ and EC_{50} is likely to be quite difficult, if not impossible. Since those are often the only measurable quantities, this fact hints at the possible existence of identifiability issues in the corresponding models. We are currently investigating this aspect, and the results will be presented elsewhere.

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Appendix: derivations of $\widehat{R}$, $E_{\mathbf{max}}$ and EC_{50} formulas

KPR sustained signalling (KPR-SS)

Proof. **Step 1:** The ODE system at steady-state:

$$\frac{dL}{dt} = 0 = -k_{\text{on}}LT + \sum_{i=0}^{N} k_{\text{off}}C_i - k_{\text{on}}LT_N$$

$$\frac{dT}{dt} = 0 = -k_{\text{on}}LT + \sum_{i=0}^{N-1} k_{\text{off}}C_i + \lambda T_N$$

$$\frac{dC_0}{dt} = 0 = k_{\text{on}}LT - (k_{\text{off}} + k_p)C_0$$

$$\frac{dC_i}{dt} = 0 = k_pC_{i-1} - (k_{\text{off}} + k_p)C_i, \qquad \text{for } 1 \leq i \leq N-1$$

$$\frac{dC_N}{dt} = 0 = k_pC_{N-1} - k_{\text{off}}C_N + k_{\text{on}}LT_N$$

$$\frac{dT_N}{dt} = 0 = k_{\text{off}}C_N - k_{\text{on}}LT_N - \lambda T_N$$

Conservation equations, with $C_T = \sum_{i=0}^{N} C_i$:

$$L_T = L + C_T$$

$$T_T = T + T_N + C_T$$

Step 2: In this model the response is defined as $\widehat{R} = \widehat{C}_N + \widehat{T}_N$. Firstly, we should express $\widehat{R}$ in terms of only one variable, $\widehat{C}_N$ or $\widehat{T}_N$. Thus, from the equation for dT_N/dt at steady-state and using the conservation equation for $\widehat{L}$,

$$\widehat{T}_N = \frac{k_{\text{off}}}{k_{\text{on}}(L_T - \widehat{C}_T) + \lambda} \cdot \widehat{C}_N \tag{32}$$

In order to find an expression for $\widehat{C}_N$ in terms of $\widehat{C}_T$ we proceed as follows. First, from Eqn. dC_i/dt one has,

$$\widehat{C}_i(k_p + k_{\text{off}}) = k_p\widehat{C}_{i-1};\ \widehat{C}_i = \psi\widehat{C}_{i-1}, \text{ with } \psi = \frac{k_p}{k_p + k_{\text{off}}},$$

and applying it recursively it yields $\widehat{C}_i = \psi^i\widehat{C}_0$, for $i = 0, \ldots, N-1$. Therefore:

$$\sum_{i=0}^{N-1} \widehat{C}_i = \frac{1-\psi^N}{1-\psi}\widehat{C}_0.$$

On the other hand,

$$\sum_{i=0}^{N-1} \widehat{C}_i = \widehat{C}_T - \widehat{C}_N., \tag{33}$$

hence:

$$\widehat{C}_0 = \frac{1-\psi}{1-\psi^N}(\widehat{C}_T - \widehat{C}_N). \tag{34}$$

Summing the steady-state equations for dT/dt and dC_0/dt, and using Eqns. (32) and (34) yields,

$$-\frac{(1-\psi)}{1-\psi^N}(\widehat{C}_T - \widehat{C}_N)(k_{\text{off}} + k_p) + k_{\text{off}}(\widehat{C}_T - \widehat{C}_N) + \frac{\lambda k_{\text{off}}}{k_{\text{on}}\widehat{L} + \lambda}\widehat{C}_N = 0,$$

and taking into account that $k_{\text{off}} + k_p = \dfrac{k_{\text{off}}}{1-\psi}$ one has,

$$-\frac{1}{1-\psi^N}(\widehat{C}_T - \widehat{C}_N) + (\widehat{C}_T - \widehat{C}_N) + \frac{\lambda}{k_{\text{on}}\widehat{L} + \lambda}\widehat{C}_N = 0,$$

which, after some algebraic rearrangements, yields:

$$\widehat{C}_N \quad = \quad \frac{(k_{\text{on}}\widehat{L} + \lambda)\psi^N}{(k_{\text{on}}\widehat{L}\psi^N + \lambda)}\, C_T.$$

And inserting there the conservation equation for $\widehat{L}$, yields:

$$\widehat{C}_N = \Big(\frac{k_{\text{on}}(L_T - \widehat{C}_T) + \lambda}{k_{\text{on}}(L_T - \widehat{C}_T)\psi^N + \lambda}\Big)\psi^N \widehat{C}_T. \tag{35}$$

Now, inserting Eqns. (32) and (35) in the definition of R one has,

$$\widehat{R} = \Big(\frac{k_{\text{off}} + k_{\text{on}}(L_T - \widehat{C}_T) + \lambda}{k_{\text{on}}(L_T - \widehat{C}_T)\psi^N + \lambda}\Big)\psi^N \widehat{C}_T. \tag{36}$$

In order to obtain an expression for $\widehat{C}_T$ in terms of L_T and T_T, it suffices to follow the procedure outlined in subsection 2.3.2 and substitute the conservation equations into the steady-state equation for dL/dt:

$$\begin{aligned} 0 &= -k_{\text{on}}(L_T - \widehat{C}_T)(T_T - \widehat{C}_T - \widehat{T}_N) + k_{\text{off}}\widehat{C}_T - k_{\text{on}}(L_T - \widehat{C}_T)\widehat{T}_N \\ &= -k_{\text{on}}(L_T - \widehat{C}_T)(T_T - \widehat{C}_T) + k_{\text{off}}\widehat{C}_T, \end{aligned} \tag{37}$$

hence,

$$\widehat{C}_T = \frac{(L_T + T_T + K_D) - \sqrt{(L_T + T_T + K_D)^2 - 4L_T T_T}}{2}, \qquad \text{where } K_D = \frac{k_{\text{off}}}{k_{\text{on}}}. \tag{38}$$

Finally, substituting Eqn. (38) into Eqn. (36) gives a closed-form expression for R in terms of L_T and T_T.

Step 3: To calculate $E_{\max} = \lim\limits_{L_T\to\infty} \widehat{R}$ we make use of Eqn. (37) and proceed as in subsection 2.3.2:

$$\lim_{L_T\to\infty} \frac{k_{\text{on}}}{k_{\text{off}}}(T_T - \widehat{C}_T) = \lim_{L_T\to\infty} \frac{\widehat{C}_T}{L_T - \widehat{C}_T} = 0.$$

Consequently, $\widehat{C}_T = T_T$ in that limit; and applying this limit also to Eqn. (36) one has:

$$\lim_{L_T\to\infty} \frac{R}{C_T} = \frac{\lim_{L_T\to\infty} R}{T_T} = \lim_{L_T\to\infty} \left(\frac{k_{\text{off}} + k_{\text{on}}(L_T - \widehat{C}_T) + \lambda}{k_{\text{on}}(L_T - \widehat{C}_T)\psi^N + \lambda}\right)\psi^N = 1,$$

therefore:

$$E_{\max} = \lim_{L_T\to\infty} \widehat{R} = T_T.$$

Step 4: In this model, EC_{50} is the value of L_T for which it verifies $\widehat{R} = E_{\max}/2 = T_T/2$, that is, it verifies:

$$\left(\frac{k_{\text{off}} + k_{\text{on}}(L_T - \widehat{C}_T) + \lambda}{k_{\text{on}}(L_T - \widehat{C}_T)\psi^N + \lambda}\right)\psi^N C_T = \frac{T_T}{2},$$

with C_T given by Eqn. (38).

Solving this equation for L_T gives the following closed-form expression for EC_{50} that was previously unavailable in the literature:

$$EC_{50} = \frac{\lambda\,(k_{\text{on}}\,T_T + 2\,\lambda) + (k_{\text{on}}\,T_T - 2\,\lambda)\big(a_1 + \psi^N(2\,\lambda + k_{\text{off}})\big)}{4\,k_{\text{on}}\,\lambda},$$

with,

$$a_1 = \sqrt{(1 - 2\,\psi^N)^2\,\lambda^2 + k_{\text{off}}\,\psi^N\big(k_{\text{off}}\,\psi^N + 2\,\lambda\,(1 + 2\,\psi^N)\big)}$$

□

KPR with induced rebinding (KPR-IR)

Proof. **Step 1:** The ODE system at steady-state:

$$\frac{dL}{dt} = 0 = -k_{\text{on}} L\,T + k_{\text{off}} C_0 + \lambda \sum_{i=1}^{N} \alpha_i C_i^*$$

$$\frac{dT}{dt} = 0 = -k_{\text{on}} L\,T + k_{\text{off}} C_0 + \lambda \sum_{i=1}^{N} \alpha_i C_i^*$$

$$\frac{dC_0}{dt} = 0 = k_{\text{on}} L\,T - (k_{\text{off}} + k_p) C_0$$

$$\frac{dC_i^*}{dt} = 0 = k_p (C_{i-1}^* - C_i^*) - \alpha_i C_i^*;\ 1 \le i \le N-1$$

$$\frac{dC_N^*}{dt} = 0 = k_p C_{N-1}^* - \alpha_N C_N^*$$

Conservation equations, with $C_T = \sum_{i=0}^{N} C_i$:

$$L_T = L + C_T$$

$$T_T = T + C_T$$

Step 2: In this model the response is given by $\widehat{R} = \widehat{C}_N^*$. From the steady-state equations for dC_i^*/dt and $dC_N^* dt$ we get,

$$\widehat{C}_i^* = \frac{k_p}{\alpha_i + k_p} \widehat{C}_{i-1}^*, \text{ with } 1 \le i \le N-1, \qquad \text{and} \qquad \widehat{C}_{N-1}^* = \alpha_i \widehat{C}_N^* / k_p.$$

This leads to,

$$\widehat{C}_{N-1}^* = \widehat{C}_0^* \prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p},$$

and

$$\widehat{C}_N^* = C_0^* \frac{k_p}{\alpha_N} \prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p} \tag{39}$$

This allows to obtain the following expression for $\widehat{C}_T$:

$$\widehat{C}_T = \widehat{C}_0 + \sum_{i=1}^{N-1} \widehat{C}_i^* + \widehat{C}_N^* = \widehat{C}_0 + \widehat{C}_0 \prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p} + \widehat{C}_0 \frac{k_p}{\alpha_N} \prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p},$$

and, therefore,

$$\widehat{C}_0 = \left(1 + \prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p} + \frac{k_p}{\alpha_N} \prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p} \right)^{-1} \widehat{C}_T = \mu\, \widehat{C}_T. \tag{40}$$

with $\mu = \left(1 + \prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p} + \frac{k_p}{\alpha_N} \prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p} \right)^{-1}$.

Introducing this last result into Eqn. (39) yields,

$$\widehat{R} = \widehat{C}_N^* = \frac{k_p}{\alpha_N} \left(\prod_{i=1}^{N-1} \frac{k_p}{\alpha_i + k_p} \right) \mu\, \widehat{C}_T. \tag{41}$$

To express $\widehat{R}$ in terms of only parameters and constants we need to express $\widehat{C}_T$ in terms of L_T and T_T. For that, it suffices to introduce Eqn. (40) into the steady-state equation for dC_0/dt giving,

$$k_{\text{on}}(L_T - \widehat{C}_T)(T_T - \widehat{C}_T) = (k_{\text{off}} + k_p)\widehat{C}_0 = (k_{\text{off}} + k_p)\,\mu\,\widehat{C}_T,$$

and solving that equation for $\widehat{C}_T$ yields,

$$\widehat{C}_T = \frac{\mu\,(k_{\text{off}} + k_p) + k_{\text{on}}(L_T + T_T) - \sqrt{\Big(\mu\,(k_{\text{off}} + k_p) + k_{\text{on}}(L_T + T_T)\Big)^2 - 4k_{\text{on}}^2 L_T T_T}}{2k_{\text{on}}}. \tag{42}$$

Step 3: From first principles we have $\widehat{C}_0 \leq \widehat{C}_T$. Now, from the equation for dC_0/dt at steady-state one has:

$$\frac{\widehat{C}_0}{L_T - \widehat{C}_T} = \frac{k_{\text{on}}}{k_{\text{off}} + k_p}(T_T - \widehat{C}_T),$$

and in the limit $L_T \to \infty$ it verifies:

$$\lim_{L_T\to\infty} \frac{k_{\text{on}}}{k_{\text{off}} + k_p}(T_T - \widehat{C}_T) = \lim_{L_T\to\infty} \frac{\widehat{C}_0}{L_T - \widehat{C}_T} \leq \lim_{L_T\to\infty} \frac{\widehat{C}_T}{L_T - \widehat{C}_T} = 0.$$

Consequently, in that limit $\widehat{C}_T = T_T$. Inserting this result into Eqn. (41), we find that:

$$E_{\max} = \lim_{L_T\to\infty} \widehat{R} = \lim_{L_T\to\infty} \widehat{C}_N^* = \frac{\frac{k_p}{\alpha_N}\prod_{i=1}^{N-1}\dfrac{k_p}{\alpha_i + k_p}}{1 + \prod_{i=1}^{N-1}\dfrac{k_p}{\alpha_i + k_p} + \frac{k_p}{\alpha_N}\prod_{i=1}^{N-1}\dfrac{k_p}{\alpha_i + k_p}} T_T \tag{43}$$

Step 4: EC_{50} can be determined by finding the concentration of L_T that satisfies the expression $\widehat{R} = E_{\max}/2$. Taking into account Eqns. (41) and (43), this implies finding the concentration of L_T that satisfies:

$$\widehat{C}_T = T_T/2.$$

Substituting there C_T by its value defined in Eqn. (42), and after some algebraic rearrangements, it yields:

$$EC_{50} = L_{T_{50}} = \frac{k_{\text{off}} + k_p}{k_{\text{on}}}\,\mu + \frac{T_T}{2} = \frac{k_{\text{off}} + k_p}{k_{\text{on}}\left(1 + \prod_{i=1}^{N-1}\dfrac{k_p}{\alpha_i + k_p} + \frac{k_p}{\alpha_N}\prod_{i=1}^{N-1}\dfrac{k_p}{\alpha_i + k_p}\right)} + \frac{T_T}{2}.$$

□

KPR with limited signalling and incoherent feed-forward loop (*KPR-LS-IFF*)

Proof. **Step 1:** The ODE system at steady-state, with $C_T = \sum_{i=0}^{N} C_i$, is:

$$\frac{dL}{dt} = 0 = -k_{\text{on}} LT + k_{\text{off}} C_T$$

$$\frac{dT}{dt} = 0 = -k_{\text{on}} LT + k_{\text{off}} C_T$$

$$\frac{dC_0}{dt} = 0 = k_{\text{on}} LT - (k_{\text{off}} + k_p) C_0$$

$$\frac{dC_i}{dt} = 0 = k_p C_{i-1} - (k_{\text{off}} + k_p) C_i$$

$$\frac{dC_N}{dt} = 0 = k_p C_{N-1} - (k_{\text{off}} + \phi) C_N$$

$$\frac{dC_{N+1}}{dt} = 0 = \phi C_N - k_{\text{off}} C_{N+1}$$

$$\frac{dY}{dt} = 0 = \gamma_+^y (Y_T - Y) - \gamma_-^y Y + \lambda C_N (Y_T - Y)$$

$$\frac{dP}{dt} = 0 = \gamma_+^p (P_T - P) - \gamma_-^p P + \delta Y (P_T - P) - \mu C_N P$$

Conservation equations:

$$L_T = L + C_T$$

$$T_T = T + C_T$$

Step 2: The response in this model is defined by $R(t) = P(t)$. To establish a relationship between the total number of complexes and the variable $P(t)$, we first obtain an expression for the total number of complexes at steady-state. For that we proceed as in subsection 2.3.2, and using the steady-state equation for dL/dt and the conservation equations, we get:

$$\widehat{C}_T = \frac{(L_T + T_T + K_D) - \sqrt{(L_T + T_T + K_D)^2 - 4 L_T T_T}}{2}, \qquad \text{with } K_D = \frac{k_{\text{off}}}{k_{\text{on}}}. \tag{44}$$

Next, we seek to express $\widehat{C}_N$ in terms of $\widehat{C}_T$. For that we first notice that by definition:

$$\widehat{C}_T = \widehat{C}_0 + \sum_{i=1}^{N-1} \widehat{C}_i + \widehat{C}_N + \widehat{C}_{N+1}. \tag{45}$$

Following again the procedure in subsection 2.3.2 and using the steady-state equations for dC_i/dt we have $\widehat{C}_i = \psi^i \, \widehat{C}_0$, where $\psi = \dfrac{k_p}{k_{\text{off}} + k_p}$. Therefore,

$$\sum_{i=0}^{N-1} \widehat{C}_i = \sum_{i=0}^{N-1} (\psi^i \, \widehat{C}_0) = \frac{1 - \psi^N}{1 - \psi} \, \widehat{C}_0,$$

Also, from the equation for dC_{N+1}/dt we have:

$$\widehat{C}_{N+1} = \frac{\phi}{k_{\text{off}}} \widehat{C}_N$$

Last, adding the steady-state equations for dC_0/dt and dL/dt, leads to:

$$\widehat{C}_0 = \frac{k_{\text{off}}}{k_{\text{off}} + k_p}\widehat{C}_T = (1-\psi)\widehat{C}_T.$$

Replacing the above three results in Eqn. (45) yields:

$$\widehat{C}_T = \left(\frac{1-\psi^N}{1-\psi}\right)(1-\psi)\widehat{C}_T + \widehat{C}_N + \frac{\phi}{k_{\text{off}}}\widehat{C}_N,$$

and solving for $\widehat{C}_N$ gives:

$$\widehat{C}_N = \alpha\,\widehat{C}_T, \qquad \text{where} \quad \alpha = \left(\frac{k_{\text{off}}}{k_{\text{off}} + \phi}\right)\psi^N. \tag{46}$$

Now, from the equations for dY/dt and dP/dt at steady-state we have:

$$(\gamma_-^y + \gamma_+^y + \lambda\widehat{C}_N)\cdot\widehat{Y} = (\gamma_+^y + \lambda\widehat{C}_N)\cdot Y_T$$

and

$$(\gamma_-^p + \mu\widehat{C}_N + \gamma_+^p + \delta\widehat{Y})\cdot\widehat{P} = (\gamma_+^p + \delta Y)\cdot P_T$$

From the first of these two equations we have:

$$\widehat{Y} = \frac{(\gamma_+^y + \lambda\widehat{C}_N)\cdot Y_T}{(\gamma_-^y + \gamma_+^y + \lambda\widehat{C}_N)},$$

and inserting this result into the second equation we get:

$$\widehat{P} = \frac{\left(\gamma_+^p + \delta\cdot\frac{(\gamma_+^y+\lambda\widehat{C}_N)\cdot Y_T}{(\gamma_-^y+\gamma_+^y+\lambda\widehat{C}_N)}\right)\cdot P_T}{\left(\gamma_-^p + \mu\widehat{C}_N + \gamma_+^p + \delta\cdot\frac{(\gamma_+^y+\lambda\widehat{C}_N)\cdot Y_T}{(\gamma_-^y+\gamma_+^y+\lambda\widehat{C}_N)}\right)}.$$

Finally, using Eqn. (46), we have:

$$\begin{aligned}\widehat{R} = \widehat{P} &= \frac{\left(\gamma_+^p + \delta\cdot\frac{(\gamma_+^y+\lambda\alpha\,\widehat{C}_T)\cdot Y_T}{\gamma_-^y+\gamma_+^y+\lambda\alpha\,\widehat{C}_T}\right)\cdot P_T}{\left(\gamma_-^p + \mu\,\alpha\,\widehat{C}_T + \gamma_+^p + \delta\cdot\frac{(\gamma_+^y+\lambda\,\alpha\,\widehat{C}_T)\cdot Y_T}{\gamma_-^y+\gamma_+^y+\lambda\,\alpha\,\widehat{C}_T}\right)} \\ &= \frac{\left(\gamma_+^p(\gamma_-^y + \gamma_+^y + \lambda\,\alpha\,\widehat{C}_T) + \delta(\gamma_+^y + \lambda\,\alpha\,\widehat{C}_T)\cdot Y_T\right)\cdot P_T}{\left(\gamma_-^p + \gamma_+^p + \mu\,\alpha\,\widehat{C}_T\right)\left(\gamma_-^y + \gamma_+^y + \lambda\,\alpha\,\widehat{C}_T\right) + \delta\left(\gamma_+^y + \lambda\,\alpha\,\widehat{C}_T\right)\cdot Y_T},\end{aligned} \tag{47}$$

with:

$$\alpha\,\widehat{C}_T = \left(\frac{k_{\text{off}}}{k_{\text{off}} + \phi}\right)\psi^N\left(\frac{(L_T + T_T + K_D) - \sqrt{(L_T + T_T + K_D)^2 - 4L_T T_T}}{2}\right).$$

Step 3: In this model, the response $\widehat{R}$ as a function of L_T displays, for many reasonable parameter values, a bell-shaped behaviour (see Fig. 3 in the main text), therefore, in general $E_{\max}$ does not correspond to $\lim_{L_T\to\infty}\widehat{R}$.

To obtain $E_{\max}$ one has to proceed in two steps, first calculate $d\widehat{R}/dL_T$ using Eqn. (47), and then calculate L_T from the equation $d\widehat{R}/dL_T = 0$. Denoting $L_{T_{\text{Emax}}}$ this particular value of L_T, one has:

$$E_{\max} = \widehat{R}(L_{T_{\text{Emax}}}).$$

According to that procedure, we obtained the following equation for $L_{T_{\text{Emax}}}$:

$$L_{T_{\text{Emax}}} = \frac{\sqrt{-a^2\delta\gamma_-^y\lambda^2\mu Y_T b_4\left(\delta\gamma_-^p\gamma_-^y\lambda Y_T - b_1\right)^2} - a\lambda\mu(\mu(\gamma_+^p(\gamma_-^y + \gamma_+^y) + \delta\gamma_+^y Y_T)(b_3 + b_5) - b_2)}{a^2\lambda^2\mu(\gamma_+^p + \delta Y_T)\left(\gamma_+^p\mu(a\lambda T_T + \gamma_-^y + \gamma_+^y)^2 + \delta\mu Y_T\left((a\lambda T_T + \gamma_+^y)^2 + \gamma_-^y\gamma_+^y\right) - \delta\gamma_-^p\gamma_-^y\lambda Y_T\right)}$$

where:

$$b_1 = \mu\left(a^2\lambda^2 T_T(K_D+T_T)(\gamma_+^p+\delta Y_T) + 2a\lambda T_T(\gamma_+^p(\gamma_-^y+\gamma_+^y)+\delta\gamma_+^y Y_T) + (\gamma_-^y+\gamma_+^y)(\gamma_+^p(\gamma_-^y+\gamma_+^y)+\delta\gamma_+^y Y_T)\right),$$

$$b_2 = \delta\gamma_-^p\gamma_-^y\lambda Y_T(\gamma_+^p(a\lambda K_D+\gamma_-^y+\gamma_+^y)+\delta Y_T(a\lambda K_D+\gamma_+^y)),$$

$$b_3 = \gamma_+^p(a\lambda T_T+\gamma_-^y+\gamma_+^y)(a\lambda(K_D+T_T)+\gamma_-^y+\gamma_+^y),$$

$$b_4 = (-\gamma_-^p\lambda(\gamma_+^p+\delta Y_T)+\gamma_+^p\mu(\gamma_-^y+\gamma_+^y)+\delta\gamma_+^y\mu Y_T),$$

$$b_5 = \delta Y_T(\gamma_-^y(a\lambda K_D+\gamma_+^y)+(a\lambda T_T+\gamma_+^y)(a\lambda(K_D+T_T)+\gamma_+^y)),$$

and

$$a = \left(\frac{k_p}{k_{\text{off}}+k_p}\right)^N\left(\frac{k_{\text{off}}}{k_{\text{off}}+\phi}\right).$$

Substituting this result into Equation (47) yields an expression for $E_{\max}$. Owing to its big size, however, this expression is only available in our online repository (`https://github.com/Xabo-RB/Inmunology-analysis.git`).

Step 4: EC_{50} is the ligand concentration at which $\widehat{R} = E_{\max}/2$, that is, it is the value of L_T that verifies:

$$\frac{\left(\gamma_+^p\left(\gamma_-^y+\gamma_+^y+\lambda\,\alpha\,\widehat{C}_T\right)+\delta\left(\gamma_+^y+\lambda\,\alpha\,\widehat{C}_T\right)\cdot Y_T\right)\cdot P_T}{\left(\gamma_-^p+\gamma_+^p+\mu\,\alpha\,\widehat{C}_T\right)\left(\gamma_-^y+\gamma_+^y+\lambda\,\alpha\,\widehat{C}_T\right)+\delta\left(\gamma_+^y+\lambda\,\alpha\,\widehat{C}_T\right)\cdot Y_T} = E_{\max}/2,$$

with $\widehat{C}_T$ given by Eqn. (44). As for the case of $E_{\max}$, due to its big size, the complete expression for EC_{50} is available only in our online repository.

□

KPR with negative feedback (*KPR-NF1*)

Proof. **Step 1:** The ODE system at steady-state, with $C_T = \sum_{i=0}^{N} C_i$, is:

$$\frac{dL}{dt} = 0 = -k_{\text{on}}LT + k_{\text{off}}C_T$$

$$\frac{dT}{dt} = 0 = -k_{\text{on}}LT + k_{\text{off}}C_T$$

$$\frac{dC_0}{dt} = 0 = k_{\text{on}}LT + (b+\gamma P)\,C_1 - (k_{\text{off}}+k_p)\,C_0$$

$$\frac{dC_i}{dt} = 0 = k_pC_{i-1} - (k_{\text{off}}+k_p+b+\gamma P)\,C_i + (b+\gamma P)C_{i+1}$$

$$\frac{dC_N}{dt} = 0 = k_pC_{N-1} - (k_{\text{off}}+b+\gamma P)\,C_N$$

$$\frac{dP}{dt} = 0 = \alpha\,C_1(P_T-P) - \beta\,P$$

The conservation equations are:

$$\begin{aligned} L_T &= L + C_T \\ T_T &= T + C_T \end{aligned} \tag{48}$$

Step 2: We have to relate the response of the system, defined in this model as $R(t) = C_N(t)$, to the total number of complexes, C_T. For that we need to proceed in a way different from the previous models. First, from the steady-state equations for dC_i/dt, we have:

$$\widehat{C}_i = a\,\widehat{C}_{i-1} - d\,\widehat{C}_{i-2}$$

and

$$\widehat{C}_{i+1} = a\,\widehat{C}_i - d\,\widehat{C}_{i-1},$$

with $a = \dfrac{k_{\text{off}} + k_p + b + \gamma\,\widehat{P}}{b + \gamma\,\widehat{P}}$ and $d = \dfrac{k_p}{b + \gamma\,P}$.

Writing those equations in vector notation for $i = N-1$:

$$\begin{pmatrix} \widehat{C}_{N-1} \\ \widehat{C}_N \end{pmatrix} = \begin{pmatrix} 0 & 1 \\ -d & a \end{pmatrix} \begin{pmatrix} \widehat{C}_{N-2} \\ \widehat{C}_{N-1} \end{pmatrix} = A \begin{pmatrix} \widehat{C}_{N-2} \\ \widehat{C}_{N-1} \end{pmatrix}$$

Proceeding recursively, it yields:

$$\begin{pmatrix} \widehat{C}_{N-1} \\ \widehat{C}_N \end{pmatrix} = A^{N-1} \begin{pmatrix} \widehat{C}_0 \\ \widehat{C}_1 \end{pmatrix} \tag{49}$$

On the other hand, matrix A can be diagonalised to give:

$$A = \begin{pmatrix} 0 & 1 \\ -d & a \end{pmatrix} = \begin{pmatrix} \dfrac{a+r}{2\,d} & \dfrac{a-r}{2\,d} \\ 1 & 1 \end{pmatrix} \begin{pmatrix} \dfrac{a-r}{2} & 0 \\ 0 & \dfrac{a+r}{2} \end{pmatrix} \begin{pmatrix} \dfrac{d}{r} & \dfrac{-a+r}{2\,r} \\ -\dfrac{d}{r} & \dfrac{a+r}{2\,r} \end{pmatrix} = B \cdot D \cdot B^{-1}$$

where $r = \sqrt{a^2 - 4\,d}$. Therefore:

$$A^i = B \cdot \begin{pmatrix} \left(\dfrac{a-r}{2}\right)^i & 0 \\ 0 & \left(\dfrac{a+r}{2}\right)^i \end{pmatrix} \cdot B^{-1}.$$

From Eqn. 49, we get:

$$\sum_{i=0}^{N-1} \begin{pmatrix} \widehat{C}_i \\ \widehat{C}_{i+1} \end{pmatrix} = \begin{pmatrix} \displaystyle\sum_{i=0}^{N-1} \widehat{C}_i \\ \displaystyle\sum_{i=1}^{N} \widehat{C}_i \end{pmatrix} = \begin{pmatrix} \widehat{C}_T - \widehat{C}_N \\ \widehat{C}_T - \widehat{C}_0 \end{pmatrix} = \sum_{i=0}^{N-1} A^i \begin{pmatrix} \widehat{C}_0 \\ \widehat{C}_1 \end{pmatrix},$$

and using the following known expression for the geometric sum of a diagonalizable matrix:

$$\sum_{i=0}^{N-1} A^i = (I - A)^{-1}(I - A^N) = B \cdot (I - D)^{-1}(I - D^N) \cdot B^{-1}.$$

Then, after some straightforward matrix operations, we have:

$$\begin{pmatrix} C_T - \widehat{C}_N \\ C_T - \widehat{C}_0 \end{pmatrix} = B \cdot \begin{pmatrix} \dfrac{2^N - (a-r)^N}{(2-a+r)\,2^{N-1}} & 0 \\ 0 & \dfrac{2^N - (a+r)^N}{(2-a-r)\,2^{N-1}} \end{pmatrix} \cdot B^{-1} \cdot \begin{pmatrix} \widehat{C}_0 \\ \widehat{C}_1 \end{pmatrix} = \begin{pmatrix} f_1(\widehat{C}_0, \widehat{C}_1, \widehat{P}) \\ f_2(\widehat{C}_0, \widehat{C}_1, \widehat{P}) \end{pmatrix}. \tag{50}$$

Therefore, from the above equation we have:

$$\widehat{C}_T - \widehat{C}_N = f_1(\widehat{C}_0, \widehat{C}_1, \widehat{P}), \tag{51}$$

$$\widehat{C}_T - \widehat{C}_0 = f_2(\widehat{C}_0, \widehat{C}_1, \widehat{P}). \tag{52}$$

On the other hand, from the steady-state equations for dL/dt and dT/dt the following expressions for $\widehat{L}$ and $\widehat{T}$ in terms of L_T and T_T can be obtained:

$$\widehat{L} = \frac{L_T - T_T - K_D + \sqrt{(L_T - T_T - K_D)^2 + 4\,L_T\,T_T}}{2},$$

$$\widehat{T} = \frac{T_T - L_T - K_D + \sqrt{(L_T - T_T - K_D)^2 + 4\,L_T\,T_T}}{2},$$

with $K_D = k_{\text{on}}/k_{\text{off}}$; and proceeding as in subsection 2.3.2 from the steady-state equation for dL/dt one has:

$$K_D\,\widehat{C}_T - (L_T - \widehat{C}_T)(T_T - \widehat{C}_T) = 0,$$

and therefore:

$$\widehat{C}_T = \frac{L_T + T_T + K_D - \sqrt{(L_T + T_T + K_D)^2 - 4\,L_T\,T_T}}{2}.$$

We need now to express $\widehat{C}_0$, $\widehat{C}_1$ and $\widehat{P}$ in terms of $\widehat{C}_T$, which will link them directly through the above equation to L_T and T_T. For that we first use the steady-state equation for dP/dt to get:

$$\widehat{C}_1 = \frac{m\,\widehat{P}}{P_T - \widehat{P}}, \qquad \text{where} \quad \mathrm{m} = \beta/\alpha.$$

Next, we turn to the steady-state equation for dC_0/dt:

$$k_{\text{on}}\widehat{L}\widehat{T} = (k_{\text{off}} + k_p)\widehat{C}_0 - (b + \gamma\,\widehat{P})\frac{m\,\widehat{P}}{P_T - \widehat{P}}.$$

Solving this equation for $\widehat{P}$ yields:

$$\widehat{P} = \frac{(k_{\text{on}}\,\widehat{L}\,\widehat{T} - \widehat{C}_0(k_{\text{off}} + k_p) - b\cdot m) + \sqrt{\left(\widehat{C}_0(k_{\text{off}} + k_p) + b\cdot m - k_{\text{on}}\,\widehat{L}\,\widehat{T}\right)^2 + 4\,P_T\,m\,\left(\widehat{C}_0(k_{\text{off}} + k_p) - k_{\text{on}}\,\widehat{L}\,\widehat{T}\right)}}{2\,m\,\gamma}$$

After having obtained an expression for $\widehat{C}_1$ and $\widehat{P}$ in terms of $\widehat{C}_0$, we go on to obtain an expression for $\widehat{C}_0$ in terms of $\widehat{C}_T$. For that we notice that Eqn. (52) can be written as:

$$\widehat{C}_T - \widehat{C}_0 = f(\widehat{C}_0), \tag{53}$$

where $f(\widehat{C}_0) = f_2(\widehat{C}_0, \widehat{C}_1, \widehat{P})$ with $\widehat{C}_1$ and $\widehat{P}$ given by the above equations. Solving Eqn. (53) for $\widehat{C}_0$ and inserting the obtained expressions for $\widehat{C}_0, \widehat{C}_1$ and $\widehat{P}$ into $f_1(\widehat{C}_0, \widehat{C}_1, \widehat{P})$ in Eqn. (51) leads to:

$$\widehat{C}_N = f_1(\widehat{C}_0, \widehat{C}_1, \widehat{P}) - \widehat{C}_T. \tag{54}$$

Hence, since in this model $\widehat{R} = \widehat{C}_N$, we obtained the expression for $\widehat{R}$ we were looking for.

A drawback of this result is that as N increases the formulas become exceedingly large, so that even for $N = 3$ one has to resort to numerical calculations.

Step 3: In references [3, 4, 18]it was mentioned that in this model the response $\widehat{R}$ as a function of L_T exhibits a bell-shaped behaviour. We have confirmed by numerical calculations that this is the case for many parameter combinations and more than two kinetic steps ($N > 2$). Therefore, like with the KPR-LS-IFF loop model, in this model $E_{\max}$ does not correspond in general to $\lim_{L_T\to\infty} \widehat{R}$. Instead, we have to specify a particular set of parameter values, leaving L_T as a free parameter, and define numerically $\widehat{R}(L_T)$. This allows to obtain the peak of this function for each particular set of parameters.

Step 4: EC_{50} can be determined by finding the concentration of L_T that satisfies the expression: $\widehat{R} = E_{\max}/2$. Because in this model a general expression for $E_{\max}$ cannot be obtained, the value of EC_{50} has to be calculated numerically, which requires defining a particular set of parameter values.

□

KPR with negative feedback II (*KPR-NF2*)

Proof. Given that the structure of this model is identical to that of the *KPR-NF1* model, all results from Eqn. (48) to (54) hold also in this model. However, in this model the response is defined as:

$$R(t) = \sum_{i=N-r}^{N} \alpha_i C_i(t),$$

with $\alpha_i < \alpha_{i+1}$, for $i = N - n$ to $N - 1$, and $\alpha_N = 1$.

The procedure developed for the KPR-NF1 model allows to calculate $\widehat{C}_i$ from $i = 1$ to N and, therefore, an expression for $\widehat{R}$ can be obtained also in this model (albeit a quite complex one) once n and α_i are specified.

□

KPR stabilizing activation chain (*KPR-SAC*)

Proof. **Step 1:** The ODE system at steady-state is:

$$\frac{dL}{dt} = 0 = -k_{\text{on}}LT + \sum_{i=0}^{N} k_{\text{off}} \cdot \frac{1+i}{1+r\cdot i} C_i$$

$$\frac{dT}{dt} = 0 = -k_{\text{on}}LT + \sum_{i=0}^{N} k_{\text{off}} \cdot \frac{1+i}{1+r\cdot i} C_i$$

$$\frac{dC_0}{dt} = 0 = k_{\text{on}}LT - (k_{\text{off}}(0) + k_p(0))C_0$$

$$\frac{dC_i}{dt} = 0 = k_p(i-1)C_{i-1} - (k_{\text{off}}(i) + k_p(i))C_i, \quad \text{for } 1 \le i \le N-1$$

$$\frac{dC_N}{dt} = 0 = k_p(N-1)C_{N-1} - k_{\text{off}}(N)C_N$$

$$k_{\text{off}}(i) = k_{\text{off}} \cdot \frac{1+i}{1+r\cdot i} \qquad \text{with r} \equiv \text{constant}$$

$$k_p(i) = k_p \cdot r_p^i \qquad \text{with r}_\text{p} \equiv \text{constant}$$

Conservation equations, with $C_T = \sum_{i=0}^{N} C_i$:

$$\begin{aligned} L_T &= L + C_T \\ T_T &= T + C_T \end{aligned} \tag{55}$$

Step 2: In this model the response is defined as $R(t) = C_N(t)$. In order to calculate C_N at steady-state, that is, $\widehat{C}_N$, we proceed as follows. We first substitute $k_p(i)$ and $k_{\text{off}}(i)$ with their corresponding expressions in the equations for dC_i/dt and dC_N/dt at steady-state:

$$\widehat{C}_i = \frac{k_p \cdot r_p^{i-1}}{k_{\text{off}} \cdot \dfrac{1+i}{i\cdot r+1} + k_p \cdot r_p^i} \cdot \widehat{C}_{i-1}, \tag{56}$$

$$\widehat{C}_N = \frac{k_p \cdot r_p^{N-1}}{k_{\text{off}} \cdot \dfrac{1+N}{N\cdot r+1}} \cdot \widehat{C}_{N-1}. \tag{57}$$

To simplify the notation we define:

$$F_i = \left[\frac{k_{\text{off}} \dfrac{1+i}{i\cdot r+1} + k_p \cdot r_p^i}{k_p \cdot r_p^{i-1}} \right] \qquad \text{for} \quad i = 1 \text{ to } N-1, \tag{58}$$

and

$$F_N = \left[\frac{k_{\text{off}} \cdot \dfrac{1+N}{N\cdot r+1}}{k_p \cdot r_p^{N-1}} \right] \tag{59}$$

Applying induction to Eqn. (56) one gets:

$$\widehat{C}_i = \widehat{C}_0 \prod_{j=1}^{i} \frac{1}{F_j} \qquad \text{for } 1 \le i \le N-1, \tag{60}$$

and, in particular:

$$\widehat{C}_{N-1} = \widehat{C}_0 \prod_{i=1}^{N-1} \frac{1}{F_i}.$$

Inserting the above equation into Eqn. (57) yields:

$$\widehat{C}_N = \widehat{C}_0 \cdot \frac{1}{F_N} \cdot \prod_{i=1}^{N-1} \frac{1}{F_i}, \tag{61}$$

and isolating variable $\widehat{C}_0$ we get,

$$\widehat{C}_0 = \widehat{C}_N \cdot F_N \cdot \prod_{i=1}^{N-1} F_i. \tag{62}$$

The total number of complexes is given by: $\widehat{C}_T = \sum_{i=0}^{N-1} \widehat{C}_i + \widehat{C}_N$. Therefore, inserting here Eqns. (60) and (61), and after some algebraic rearrangement, we find:

$$\widehat{C}_T = \widehat{C}_N \left[1 + F_N \cdot \prod_{i=1}^{N-1} F_j \cdot \left(1 + \sum_{i=1}^{N-1} \left(\prod_{j=1}^{i} \frac{1}{F_i} \right) \right) \right]. \tag{63}$$

Using now the straightforward result:

$$\prod_{i=1}^{N-1} F_j \cdot \sum_{i=1}^{N-1} \left(\prod_{j=1}^{i} \frac{1}{F_i} \right) = 1 + \sum_{i=1}^{N-2} \left(\prod_{j=1}^{i} F_{N-j} \right),$$

we can rewrite Eqn. (63) in this way:

$$\widehat{C}_T = \widehat{C}_N \left[1 + F_N \left(1 + \prod_{i=1}^{N-1} F_i + \sum_{i=1}^{N-2} \left(\prod_{j=1}^{i} F_{N-j} \right) \right) \right]. \tag{64}$$

Finally, since in this model $R(t) = C_N(t)$, we have at steady-state:

$$\widehat{R} = \widehat{C}_N = \widehat{C}_T \left[1 + F_N \left(1 + \prod_{i=1}^{N-1} F_i + \sum_{i=1}^{N-2} \left(\prod_{j=1}^{i} F_{N-j} \right) \right) \right]^{-1} \tag{65}$$

An expression for C_T can be derived using the steady-state equation for dC_0/dt and inserting there Eqns. (62) and (65). In this way it is obtained:

$$(L_T - C_T)(T_T - C_T) = \alpha\, \widehat{C}_T$$

Therefore,

$$\begin{aligned} \widehat{C}_T &= \frac{(L_T + T_T + \alpha) - \sqrt{(L_T + T_T + \alpha)^2 - 4 L_T T_T}}{2} \\ \text{with:} \quad \alpha &= \left(\frac{k_{\text{off}} + k_p}{k_{\text{on}}} \right) F_N \left(\prod_{i=1}^{N-1} F_i \right) \left[1 + F_N \left(1 + \prod_{i=1}^{N-1} F_i + \sum_{i=1}^{N-2} \left(\prod_{j=1}^{i} F_{N-j} \right) \right) \right]^{-1} \end{aligned} \tag{66}$$

Step 3: Similarly to previous models, from the equation for dC_0/dt at steady-state and the conservation equations, and considering that $\widehat{C}_0 \leq \widehat{C}_T$, one has:

$$\frac{\widehat{C}_0}{L_T - \widehat{C}_T} = \frac{k_{\text{on}}}{k_{\text{off}} + k_p} (T_T - \widehat{C}_T) \leq \frac{\widehat{C}_T}{L_T - \widehat{C}_T},$$

thus:

$$\lim_{L_T \to \infty} \frac{k_{\text{on}}}{k_{\text{off}} + k_p} (T_T - \widehat{C}_T) \leq \lim_{L_T \to \infty} \frac{\widehat{C}_T}{L_T - \widehat{C}_T} = 0.$$

Therefore, in that limit it verifies $\widehat{C}_T = T_T$. Hence:

$$E_{\max} = T_T \left[1 + F_N \left(1 + \prod_{i=1}^{N-1} F_i + \sum_{i=1}^{N-2} \left(\prod_{j=1}^{i} F_{N-j} \right) \right) \right]^{-1}. \tag{67}$$

Step 4: EC_{50} can be determined by finding the concentration of L_T that satisfies the expression $R = E_{\max}/2$. From equations (65) and (67), we obtain that this condition is satisfied for:

$$\widehat{C}_T = \frac{T_T}{2}. \tag{68}$$

Inserting Eqn. (61) into Eqn. (64) yields:

$$\widehat{C}_T = \widehat{C}_0 \cdot \frac{1}{F_N} \cdot \prod_{i=1}^{N-1} \frac{1}{F_i} \left[1 + F_N \left(1 + \prod_{i=1}^{N-1} F_i + \sum_{i=1}^{N-2} \left(\prod_{j=1}^{i} F_{N-j} \right) \right) \right].$$

Let us simplify notation again by defining:

$$G = \frac{1}{F_N} \cdot \prod_{i=1}^{N-1} \frac{1}{F_i} \left[1 + F_N \left(1 + \prod_{i=1}^{N-1} F_i + \sum_{i=1}^{N-2} \left(\prod_{j=1}^{i} F_{N-j} \right) \right) \right]. \tag{69}$$

Then,

$$\widehat{C}_T = G \cdot \widehat{C}_0. \tag{70}$$

From the equation for $dC_0 = /dt$ at steady-state, and using the above equation, we have:

$$\left(\frac{k_{\text{off}}(0) + k_p(0)}{k_{\text{on}}} \right) \widehat{C}_0 = \left(\frac{k_{\text{off}} + k_p}{k_{\text{on}}} \right) \widehat{C}_0 = (L_T - G\,\widehat{C}_0)(T_T - G\,\widehat{C}_0).$$

Hence,

$$\widehat{C}_0^2\, G^2 - \widehat{C}_0 \left(G(L_T + T_T) + \frac{k_{\text{off}} + k_p}{k_{\text{on}}} \right) + L_T\, T_T = 0.$$

Therefore,

$$\widehat{C}_0 = \frac{\left(G(L_T + T_T) + \frac{k_{\text{off}}+k_p}{k_{\text{on}}} \right) - \sqrt{\left(G(L_T + T_T) + \frac{k_{\text{off}}+k_p}{k_{\text{on}}} \right)^2 - 4\,G^2\,L_T\,T_T}}{2\,G^2}. \tag{71}$$

Inserting Eqns. (70) and (71) into Eqn. (68) yields:

$$\frac{\left(G(L_T + T_T) + \frac{k_{\text{off}}+k_p}{k_{\text{on}}} \right) - \sqrt{\left(G(L_T + T_T) + \frac{k_{\text{off}}+k_p}{k_{\text{on}}} \right)^2 - 4\,G^2\,L_T\,T_T}}{G} = T_T.$$

Isolating now L_T from the above equation gives:

$$\left(\left(G(L_T + T_T) + \frac{k_{\text{off}} + k_p}{k_{\text{on}}} \right) - G\,T_T \right)^2 = \left(G(L_T + T_T) + \frac{k_{\text{off}} + k_p}{k_{\text{on}}} \right)^2 - 4\,G^2\,L_T\,T_T,$$

and, after some algebraic rearrangements, we get:

$$EC_{50} = L_{T_{50}} = \frac{T_T}{2} + \frac{k_{\text{off}} + k_p}{G\,k_{\text{on}}}. \tag{72}$$

□

Zero-order ultrasensitivity model (ZU)

Proof. **Step 1:** The ODE system at steady-state is:

$$\frac{dT}{dt} = 0 = -k_{\text{on}}TL + \lambda D + k_{\text{off}}C_0$$

$$\frac{dL}{dt} = 0 = -k_{\text{on}}TL + (k_p + k_{\text{off}})C_0$$

$$\frac{dC_0}{dt} = 0 = k_{\text{on}}TL - (k_{\text{off}} + k_p)C_0$$

$$\frac{dD}{dt} = 0 = k_+T_pP - (k_- + \lambda)D$$

$$\frac{dT_p}{dt} = 0 = -k_+T_pP + k_-D + k_pC_0$$

$$\frac{dP}{dt} = 0 = -k_+T_pP + (k_- + \lambda)D$$

The conservation equations are:

$$L_T = L + C_0$$

$$T_T = T + C_0 + T_p + D$$

$$P_T = P + D$$

Step 2: In this model the response is defined as $R(t) = T_p(t)$. Thus, obtaining an expression for R is equivalent to find an expression for T_p. For that we first notice that from the conservation equation for L we have:

$$C_0 = L_T - L, \tag{73}$$

and from the equations for dT/dt and dC_0/dt at steady-state we have, respectively:

$$k_{\text{on}}\,\widehat{T}\widehat{L} = \lambda\widehat{D} + k_{\text{off}}\widehat{C}_0 \qquad \text{and} \qquad k_{\text{on}}\,\widehat{T}\widehat{L} = (k_{\text{off}} + k_p)\widehat{C}_0.$$

Combining them and using Eqn. (73) yields:

$$\widehat{D} = \frac{k_p}{\lambda}\widehat{C}_0 = \frac{k_p}{\lambda}(L_T - \widehat{L}). \tag{74}$$

On the other hand, from Eqn. (74) and the conservation equation for P it follows,

$$\widehat{P} = P_T - \widehat{D} = P_T - \frac{k_p}{\lambda}(L_T - \widehat{L}),$$

Finally, using this expression in the equation for dP/dt at steady-state, we are lead to:

$$\widehat{T}_pk_+\left(P_T - \frac{k_p}{\lambda}(L_T - \widehat{L})\right) = (k_- + \lambda)\frac{k_p}{\lambda}(L_T - \widehat{L}),$$

and hence,

$$\widehat{T}_p = \frac{(k_- + \lambda)\,k_p\,(L_T - \widehat{L})}{k_+\,\lambda\,P_T - k_+\,k_p\,(L_T - \widehat{L})}. \tag{75}$$

Step 3: To calculate $E_{\max} = \lim_{L_T\to\infty}\widehat{R}$ we note that $C_0(t) \leq T_T$ and, therefore, $\widehat{C}_0 \leq T_T$. Now, from the equation for dL/dt at steady-state, and using the conservation equations, we obtain:

$$\frac{\widehat{C}_0}{L_T - \widehat{C}_0} = \frac{k_{\text{on}}}{k_{\text{off}} + k_p}(T_T - \widehat{C}_0 - \widehat{T}_p - \widehat{D}),$$

and in the limit $L_T \to \infty$ it verifies:

$$\lim_{L_T\to\infty} \frac{k_{\text{on}}}{k_{\text{off}}+k_p}(T_T - \widehat{C}_0 - \widehat{T}_p - \widehat{D}) = \lim_{L_T\to\infty} \frac{\widehat{C}_0}{L_T - \widehat{C}_0} = 0.$$

Consequently, $T_T - \widehat{C}_0 - \widehat{T}_p - \widehat{D} = 0$, and using the first result from Eqn. (74), one has in that limit: $\dfrac{\lambda}{k_p+\lambda}(T_T - \widehat{T}_p) = \widehat{C}_0$. Combining with the conservation equation, $\dfrac{\lambda}{k_p+\lambda}(T_T - \widehat{T}_p) = L_T - \widehat{L}$.

Using this result within Eqn. (75) yields,

$$k_+\,\lambda\,P_T\widehat{T}_p - \left(\frac{k_p\,k_+\,\lambda}{k_p+\lambda}\right)(T_T - \widehat{T}_p)\,\widehat{T}_p = \frac{k_p\,(k_- + \lambda)\,\lambda}{k_p+\lambda}(T_T - \widehat{T}_p).$$

Rearranging terms, this expression can be written as:

$$k_p\,k_+\,\lambda\,\widehat{T}_p^2 - \big(k_p\,k_+\,\lambda\,T_T - k_+\,\lambda\,(\lambda+k_p)\,P_T - k_p\,\lambda\,(k_- + \lambda)\big)\widehat{T}_p - k_p\,\lambda\,(k_- + \lambda)T_T = 0.$$

Finally, denoting

$$\alpha = \big(k_p\,k_+\,\lambda\,T_T - k_+\,\lambda\,(\lambda+k_p)\,P_T - k_p\,\lambda\,(k_- + \lambda)\big) \tag{76}$$

it is obtained:

$$E_{\max} = \lim_{L_T\to\infty} \widehat{R} = \lim_{L_T\to\infty} \widehat{T}_p = \frac{\alpha + \sqrt{\alpha^2 + 4\,k_p^2\,\lambda^2\,k_+\,(k_- + \lambda)\,T_T}}{2\,k_p\,k_+\,\lambda}.$$

Step 4: To derive an expression for EC_{50} it is necessary to first express $\widehat{R}$, and hence $\widehat{T}_p$, in terms of L_T. For that, we start from the equation for dL/dt at steady-state together with the conservation equations and Eqns. (74) and (75):

$$k_{\text{on}}\left(T_T - (L_T - \widehat{L}) - \frac{(k_- + \lambda)\,k_p\,(L_T - \widehat{L})}{k_+\,\lambda\,P_T - k_+\,k_p\,(L_T - \widehat{L})} - \frac{k_p}{\lambda}(L_T - \widehat{L})\right)\widehat{L} = (k_{\text{off}} + k_p)\,(L_T - \widehat{L}).$$

This gives the following cubic polynomial in $\widehat{L}$:

$$a\,\widehat{L}^3 + b\,\widehat{L}^2 + c\,\widehat{L} + d = 0, \tag{77}$$

where

$$\begin{aligned}
a &= k_+\,k_{\text{on}}\,k_p\,(\lambda + k_p)\\
b &= k_+\,\Big(\lambda^2\,k_{\text{on}}\,P_T + \lambda\,k_p\,\big(k_{\text{off}} + k_p + k_{\text{on}}\,(P_T + T_T - 2L_T)\big) - 2k_{\text{on}}\,L_T\,k_p^2\Big)\\
&\quad + \lambda\,k_{\text{on}}\,k_p\,(k_- + \lambda)\\
c &= k_+\,\lambda^2\,P_T\big(k_{\text{off}} + k_p + k_{\text{on}}\,(T_T - L_T)\big)\\
&\quad - k_+\,\lambda\,L_T\,k_p\big(2(k_{\text{off}} + k_p) + k_{\text{on}}\,(P_T + T_T - L_T)\big) + k_+\,k_{\text{on}}\,L_T^2\,k_p^2\\
&\quad - \lambda\,k_{\text{on}}\,L_T\,k_p\,(k_- + \lambda)\\
d &= k_+\,\lambda\,L_T(k_{\text{off}} + k_p)(k_p\,L_T - \lambda P_T).
\end{aligned}$$

Of the three solutions to this polynomial we are interested in the one that fulfils the following two conditions: 1) it is positive real, and 2) it is lower than L_T. Inserting this solution into Eqn. (75) gives $\widehat{T}_p$, and hence $\widehat{R}$, in terms of L_T.

Finally, isolating L_T from the equation $\widehat{R} = E_{\max}/2$ gives $L_{T_{50}} = EC_{50}$. Nevertheless, we cannot provide a general formula because the results for both $\widehat{R}$ and EC_{50} involve quite big expressions. Therefore, in order to obtain values for these quantities, one has to resort to numerical calculations using specific sets of parameter values.

□

KPR with zero-order ultraspecificity (*KPZU*)

Proof. **Step 1:** The ODE system at steady-state, with $C_T = \sum_{i=0}^{N} C_i$, is:

$$\frac{dT}{dt} = 0 = -k_{\text{on}}TL + \lambda D + k_{\text{off}}C_T$$

$$\frac{dL}{dt} = 0 = -k_{\text{on}}TL + k_p C_N + k_{\text{off}}C_T$$

$$\frac{dT_p}{dt} = 0 = -k_+ T_p P + k_p C_N + k_- D$$

$$\frac{dC_0}{dt} = 0 = -(k_{\text{off}} + k_p)C_0 + k_{\text{on}}TL$$

$$\frac{dC_i}{dt} = 0 = -(k_{\text{off}} + k_p)C_i + k_p C_{i-1}$$

$$\frac{dP}{dt} = 0 = -k_+ T_p P + (k_- + \lambda)D$$

$$\frac{dD}{dt} = 0 = k_+ T_p P - (k_- + \lambda)D$$

The conservation equations are:

$$L_T = L + C_T$$

$$T_T = T + C_T + T_p + D$$

$$P_T = P + D$$

Step 2: Like in th ZU model, in this model the response is also defined as $R(t) = T_p(t)$. Thus, in order to obtain an expression for R it is enough to find an expression for T_p. This model includes a simple kinetic proofreading chain in addition to the processes of the ZU model, such that in the steady-state there is the following simple relationship between complexes C_i and C_{i-1}:

$$\widehat{C}_i = \frac{k_p}{k_{\text{off}} + k_p}\widehat{C}_{i-1} \qquad \text{for} \quad i = 1, \ldots, N.$$

Thus, applying it recursively, one has:

$$\widehat{C}_i = \psi^i \widehat{C}_0 \qquad \text{with} \quad \psi = \frac{k_p}{k_{\text{off}} + k_p},$$

and, in particular:

$$\widehat{C}_N = \psi^N \widehat{C}_0.$$

From here it is obtained:

$$\widehat{C}_T = \sum_{i=0}^{N} \psi^i \widehat{C}_0 = \widehat{C}_0 \left(\frac{1 - \psi^{N+1}}{1 - \psi} \right),$$

and, therefore:

$$\widehat{C}_0 = \widehat{C}_T \left(\frac{1 - \psi}{1 - \psi^{N+1}} \right),$$

and

$$\widehat{C}_N = \psi^N \left(\frac{1 - \psi}{1 - \psi^{N+1}} \right) \widehat{C}_T.$$

Summing the equations for dT/dt and dD/dt at steady-state yields:

$$\widehat{D} = \frac{k_p}{\lambda}\widehat{C}_N = \frac{\Phi}{\lambda}\widehat{C}_T, \qquad \text{with} \quad \Phi = k_p\, \psi^N \left(\frac{1 - \psi}{1 - \psi^{N+1}} \right). \tag{78}$$

and using this result in the conservation equation for $\widehat{P}$ one has:

$$\widehat{P} = P_T - \widehat{D} = P_T - \frac{\Phi}{\lambda}\widehat{C}_T. \tag{79}$$

On the other hand, from the equation for dD/dt at steady-state one has:

$$k_2\,\widehat{T}_p\,\widehat{P} = (k_- + \lambda)\widehat{D}.$$

Substituting here $\widehat{D}$ and $\widehat{P}$ as given by Eqns. (78) and (79) yields:

$$k_2\,\widehat{T}_p\left(P_T - \frac{\Phi}{\lambda}\widehat{C}_T\right) = (k_- + \lambda)\frac{\Phi}{\lambda}\widehat{C}_T$$

Therefore,

$$\widehat{R} = \widehat{T}_p = \frac{(k_- + \lambda)\Phi\,\widehat{C}_T}{k_2(\lambda\,P_T - \Phi\,\widehat{C}_T)}. \tag{80}$$

Notice that in Eqns. (78)–(80) and in the following calculations the complex parameter Φ plays the same role as k_p in the ZU model.

Step 3: To calculate $E_{\max} = \lim\limits_{L_T\to\infty}\widehat{R}$ we note that $C_0(t) \le C_T$ and, therefore, $\widehat{C}_0 \le \widehat{C}_T$. Now, from the equation for dC_0/dt at steady-state, and using the conservation equations, we obtain:

$$\frac{k_{\text{on}}}{k_{\text{off}} + k_p}(T_T - \widehat{C}_T - \widehat{T}_p - \widehat{D}) = \frac{\widehat{C}_0}{L_T - \widehat{C}_0},$$

and in the limit $L_T \to \infty$ it verifies:

$$\lim_{L_T\to\infty}\frac{k_{\text{on}}}{k_{\text{off}} + k_p}(T_T - \widehat{C}_T - \widehat{T}_p - \widehat{D}) = \lim_{L_T\to\infty}\frac{\widehat{C}_0}{L_T - \widehat{C}_T} \le \lim_{L_T\to\infty}\frac{\widehat{C}_T}{L_T - \widehat{C}_T} = 0.$$

Consequently, in that limit $T_T - \widehat{C}_T - \widehat{T}_p - \widehat{D} = 0$, and using Eqn. (78), one has:

$$\frac{\lambda}{\Phi + \lambda}(T_T - \widehat{T}_p) = \widehat{C}_T = L_T - \widehat{L}.$$

Using this result within Eqn. (80) yields,

$$k_+\,\lambda\,P_T\widehat{T}_p - \left(\frac{\Phi\,k_+\,\lambda}{\Phi + \lambda}\right)(T_T - \widehat{T}_p)\,\widehat{T}_p = \frac{\Phi\,(k_- + \lambda)\,\lambda}{\Phi + \lambda}(T_T - \widehat{T}_p).$$

Rearranging terms, this expression can be written as:

$$\Phi\,k_+\,\lambda\,\widehat{T}_p^2 - \big(\Phi\,k_+\,\lambda\,T_T - k_+\,\lambda\,(\lambda + \Phi)\,P_T - \Phi\,\lambda\,(k_- + \lambda)\big)\widehat{T}_p - \Phi\,\lambda\,(k_- + \lambda)T_T = 0.$$

Finally, denoting $\alpha = \big(\Phi\,k_+\,\lambda\,T_T - k_+\,\lambda\,(\lambda + \Phi)\,P_T - \Phi\,\lambda\,(k_- + \lambda)\big)$ it is obtained:

$$E_{\max} = \lim_{L_T\to\infty}\widehat{R} = \lim_{L_T\to\infty}\widehat{T}_p = \frac{\alpha + \sqrt{\alpha^2 + 4\,\Phi^2\,\lambda^2\,k_+\,(k_- + \lambda)\,T_T}}{2\,\Phi\,k_+\,\lambda}.$$

Step 4: To derive an expression for EC_{50} it is necessary to first express $\widehat{R}$, and hence $\widehat{T}_p$, in terms of L_T. For that, we start from the equation for dT/dt at steady-state together with the conservation equations and Eqns. (78) and (80):

$$k_{\text{on}}\left(T_T - (L_T - \widehat{L}) - \frac{(k_- + \lambda)\,\Phi\,(L_T - \widehat{L})}{k_+\,\lambda\,P_T - k_+\,\Phi\,(L_T - \widehat{L})} - \frac{\Phi}{\lambda}(L_T - \widehat{L})\right)\widehat{L} = (k_{\text{off}} + \Phi)\,(L_T - \widehat{L}).$$

This gives the following cubic polynomial in $\widehat{L}$:

$$a\,\widehat{L}^3 + b\,\widehat{L}^2 + c\,\widehat{L} + d = 0, \tag{81}$$

where:

$$
\begin{aligned}
a &= k_+\, k_{\text{on}}\, \Phi\, (\lambda + \Phi) \\
b &= k_+ \left(\lambda^2\, k_{\text{on}}\, P_T + \lambda\, \Phi \left(k_{\text{off}} + \Phi + k_{\text{on}}\, (P_T + T_T - 2L_T)\right) - 2k_{\text{on}}\, L_T\, \Phi^2\right) \\
&\quad + \lambda\, k_{\text{on}}\, \theta\, (k_- + \lambda) \\
c &= k_+\, \lambda^2\, P_T \big(k_{\text{off}} + \Phi + k_{\text{on}}\, (T_T - L_T)\big) \\
&\quad - k_+\, \lambda\, L_T\, \Phi \big(2(k_{\text{off}} + \Phi) + k_{\text{on}}\, (P_T + T_T - L_T)\big) + k_+\, k_{\text{on}}\, L_T^2\, \Phi^2 \\
&\quad - \lambda\, k_{\text{on}}\, L_T\, \Phi\, (k_- + \lambda) \\
d &= k_+\, \lambda\, L_T (k_{\text{off}} + \Phi)(\Phi\, L_T - \lambda P_T).
\end{aligned}
$$

Of the three solutions to this polynomial we are interested in the one that fulfills the following two conditions: 1) it is positive real, and 2) it is lower than L_T. Then, substituting $\widehat{C}_T$ for $L_T - \widehat{L}$ in Eqn. (80) and inserting there the above solution for $\widehat{L}$ gives $\widehat{T}_p$, and hence $\widehat{R}$, in terms of L_T.

Finally, isolating L_T from the equation $\widehat{R} = E_{\max}/2$ gives $L_{T_{50}} = EC_{50}$. Nevertheless, like in the ZU model, we cannot provide here a general formula because the results for both $\widehat{R}$ and EC_{50} involve quite big expressions. Therefore, in order to obtain values for these quantities, one has to resort to numerical calculations using specific sets of parameter values.

□

KPR with concentration compensation (*KPC*)

Proof. **Step 1:** The ODE system at steady-state, with $C_T = \sum_{i=0}^{N} C_i$, is:

$$
\begin{aligned}
\frac{dT}{dt} &= 0 = -k_{\text{on}} T X + k_3 D + k_{\text{off}} C_T \\
\frac{dX}{dt} &= 0 = -(k_{\text{on}} T X + k_+ T_p X) + k_p C_N + k_{\text{off}} C_T + (k_- + \lambda) D \\
\frac{dC_0}{dt} &= 0 = -(k_{\text{off}} + k_p) C_0 + k_{\text{on}} T X \\
\frac{dC_i}{dt} &= 0 = -(k_{\text{off}} + k_p) C_i + k_p C_{i-1} \\
\frac{dT_p}{dt} &= 0 = k_p C_N - k_+ T_p X + k_- D \\
\frac{dD}{dt} &= 0 = k_+ T_p X - (k_- + \lambda) D
\end{aligned}
$$

The conservation equations are:

$$
\begin{aligned}
X_T &= X + C_T + D \\
T_T &= T + C_T + T_P + D
\end{aligned}
$$

Step 2: Similarly to the ZU and KPZU models, in this model the response is also defined as $R(t) = T_p(t)$. We are, therefore, interested in finding an expression for T_p in terms of L_T. For that we will proceed in a way similar to the KPZU model, noticing that this model also includes a kinetic proofreading chain. Thus, from the equation for dC_i/dt at steady-state one has:

$$
\widehat{C}_i = \frac{k_p}{k_{\text{off}} + k_p} \widehat{C}_{i-1} \qquad \text{for} \quad i = 1, \ldots, N,
$$

and applying it recursively, it yields:

$$
\widehat{C}_i = \psi^i \widehat{C}_0 \qquad \text{with} \quad \psi = \frac{k_p}{k_{\text{off}} + k_p},
$$

and:

$$\widehat{C}_T = \sum_{i=0}^{N} \psi^i \widehat{C}_0 = \widehat{C}_0 \left(\frac{1-\psi^{N+1}}{1-\psi} \right).$$

Therefore:

$$\widehat{C}_0 = \widehat{C}_T \left(\frac{1-\psi}{1-\psi^{N+1}} \right),$$

and

$$\widehat{C}_N = \frac{\psi^N - \psi^{N+1}}{1-\psi^{N+1}} \widehat{C}_T \tag{82}$$

Summing the steady-state equations for dT/dt and dD/dt and using the above equation for $\widehat{C}_N$ yields:

$$\widehat{D} = \Phi \cdot \widehat{C}_T \tag{83}$$

where $\Phi = \dfrac{k_p}{\lambda} \cdot \dfrac{\psi^N - \psi^{N+1}}{1-\psi^{N+1}}$.

Inserting the conservation equations and Eqn. (83) into the equations for dT/dt and dD/dt at steady-state yields, respectively,

$$k_{\text{on}}(T_T - \widehat{T}_p - (1+\Phi)\widehat{C}_T)(X_T - (1+\Phi)\widehat{C}_T) = (\lambda\,\Phi + k_{\text{off}})\widehat{C}_T \tag{84}$$

$$k_+ \widehat{T}_p (X_T - (1+\Phi)\widehat{C}_T) = (k_- + \lambda)\Phi\widehat{C}_T \tag{85}$$

Finally, solving the system formed by Eqns. (84) and (85) for $\widehat{T}_p$ and $\widehat{C}_T$, we get:

$$\widehat{R} = \widehat{T}_p = -\frac{(k_- + \lambda)\left(\beta_1 - \sqrt{\beta_2^2 - 4\,k_+^2\,k_{\text{on}}^2\,T_T\,X_T(1+\Phi)^2\psi^{2(N+1)}}\right)}{2\,k_+\,(1+\Phi)\Big(k_+\,\lambda + k_{\text{on}}\,(k_- + \lambda)\,\psi^{N+1}\Big)}, \tag{86}$$

where:

$$\beta_1 = k_+\,\lambda\,\Phi + \Big((k_- + \lambda)\,\Phi + k_+\,(X_T - T_T)(1+\Phi)\Big)\,k_{\text{on}}\,\psi^{N+1},$$

and

$$\beta_2 = k_+\,\lambda\,\Phi + \Big((k_- + \lambda)\,\Phi + k_+\,(X_T + T_T)(1+\Phi)\Big)\,k_{\text{on}}\,\psi^{N+1}.$$

Step 3: To calculate $E_{\max} = \lim\limits_{L_T\to\infty} \widehat{R}$ we note that, as in previous models, $C_0(t) \le C_T(t)$ and, therefore, $\widehat{C}_0 \le \widehat{C}_T \le (1+\Phi)\,\widehat{C}_T$. Now, from the equation for dC_0/dt at steady-state, and using the conservation equations, we obtain:

$$\frac{k_{\text{on}}}{k_{\text{off}} + k_p}(T_T - \widehat{T}_p - (1+\Phi)\,\widehat{C}_T) = \frac{\widehat{C}_0}{X_T - (1+\Phi)\,\widehat{C}_T} \le \frac{(1+\Phi)\,\widehat{C}_T}{X_T - (1+\Phi)\,\widehat{C}_T}$$

In the limit when there is ligand saturation,

$$\lim_{X_T\to\infty} \left(T_T - \widehat{T}_p - (1+\Phi)\widehat{C}_T\right) = \lim_{X_T\to\infty} \frac{(1+\Phi)\,\widehat{C}_T}{X_T - (1+\Phi)\,\widehat{C}_T} = 0 \tag{87}$$

On the other hand, from the equation for dD/dt at steady-state, and using the conservation equations and Eq. (83) one has,

$$\frac{k_+}{k_- + \lambda}\widehat{T}_p = \frac{\Phi\widehat{C}_T}{X_T - (1+\Phi)\widehat{C}_T}$$

Taking now the limit given when there is ligand saturation, we find:

$$\lim_{X_T\to\infty} \frac{k_+}{k_- + \lambda}\widehat{T}_p = \lim_{X_T\to\infty} \frac{\Phi\widehat{C}_T}{X_T - (1+\Phi)\widehat{C}_T} = 0.$$

Therefore, $\lim\limits_{X_T\to\infty} \widehat{T}_p = 0$. Notice that this result is at odds compared to most of the other models.

Introducing this last result into Eqn. (87) we get,

$$\lim_{X_T\to\infty}\left[T_T-(1+\Phi)\widehat{C}_T\right]-\lim_{X_T\to\infty}\widehat{T}_p=\lim_{X_T\to\infty}\left[T_T-(1+\Phi)\widehat{C}_T\right]=0, \tag{88}$$

therefore,

$$\lim_{X_T\to\infty}\widehat{C}_T=\frac{1}{1+\Phi}T_T.$$

This conclusion is validated numerically as shown in Fig. 5 in the main text. In addition, Eqn. (88) indicates that, under conditions of ligand saturation, $\widehat{D}=\dfrac{\Phi}{1+\Phi}T_T$.

Analysing Eqn. (86) it can be shown that $\widehat{R}$ increases as L_T decreases. This indicates that $E_{\max}$ is obtained in the limit $\lim\limits_{X_T\to 0}\widehat{R}$. Therefore, taking $X_T=0$ in Eqn. (86) (that is, in the auxiliary parameters β_1 and β_2) one has:

$$E_{\max}=\frac{k_{\text{on}}\,(k_-+\lambda)\,\psi^{N+1}\,T_T}{k_+\,\lambda+k_{\text{on}}\,(k_-+\lambda)\,\psi^{N+1}}=\frac{k_{\text{on}}\,(k_-+\lambda)\,k_p^{N+1}\,T_T}{k_+\,\lambda\,(k_{\text{off}}+k_p)^{N+1}+k_{\text{on}}\,(k_-+\lambda)\,k_p^{N+1}}. \tag{89}$$

Step 4: EC_{50} is the ligand concentration that satisfies the relation: $\widehat{R}=E_{\max}/2$, with $\widehat{R}$ given by Eqn. (86) and $E_{\max}$ by Eqn. (89). Thus, according to those equations, EC_{50} is the ligand concentration that satisfies:

$$\beta_2^2-4\,k_+^2\,k_{\text{on}}^2\,T_T\,X_T(1+\Phi)^2\psi^{2(N+1)}=\left(\beta_1+k_+\,k_{\text{on}}\,T_T\,\psi^{N+1}(1+\Phi)\right)^2.$$

Then, after after substituting there the auxiliary parameters β_1 and β_2, and solving the resulting equation for X_T and simplifying it, it is obtained:

$$EC_{50}=\frac{T_T}{2}+\frac{\Phi\left(\frac{k_-+\lambda}{k_+}+\frac{\lambda}{k_{\text{on}}\,\psi^{N+1}}\right)}{1+\Phi},$$

which, after substituting ψ and Φ, and after some algebraic rearrangements, yields:

$$EC_{50}=\frac{T_T}{2}+\frac{k_{\text{off}}\Big(k_-\,k_{\text{on}}\,k_p^{N+1}+\left(k_{\text{on}}\,k_p^{N+1}+k_+\,\lambda\,(k_{\text{off}}+k_p)^{N+1}\right)\Big)}{k_+\,k_{\text{on}}\left(k_{\text{off}}\left(k_p^{N+1}+\lambda\,(k_{\text{off}}+k_p)^N\right)-\lambda\,k_p\left(k_p^N-(k_{\text{off}}+k_p)^N\right)\right)}. \tag{90}$$

□

Modified serial triggering (ST-mod)

Proof. **Step 1:** The ODE system at steady-state is:

$$\frac{dS}{dt}=0=-\phi\left(S-\frac{1}{\lambda}T\right)+\sigma\left(\frac{1}{1+\lambda}-S\right)$$

$$\frac{dT}{dt}=0=\phi\left(S-\frac{1}{\lambda}T\right)+\sigma\left(\frac{\lambda}{1+\lambda}-T\right)-k_{\text{eff}}T^hL^h$$

$$\frac{dA}{dt}=0=k_{\text{eff}}T^hL^h-k_iA$$

This model is an extension of a previous one proposed by Bachmann *and col* [27] and inherits from it the following assumptions: (1) ligand-receptor complexes and complex multimers are in quasi steady-state relative to the kinetics of TCRs, and (2) the concentration of ligand-receptor complexes is much smaller than that of TCRs. Therefore, in this model the ligand concentration or density is considered a parameter, denoted here L. The three state variables of this model, S, T and A, denote the density of TCRs in three different compartments in a T lymphocyte membrane, respectively, TCRs in the spare pool, free TCRs in the interphase pool, and triggered or activated TCRs, which are marked for internalization and degradation.

Step 2: The response is quantified here in terms of activated TCRs, that is, $\widehat{R}=\widehat{A}$.

From the steady-state equation for dA/dt one has:

$$\widehat{A} = \frac{k_{\text{eff}}}{k_i}\,\widehat{T}^h L^h. \tag{91}$$

And from the steady-state equation for dS/dt,

$$\phi\left(\widehat{S} - \frac{1}{\lambda}\widehat{T}\right) = \sigma\left(\frac{1}{1+\lambda} - \widehat{S}\right) \tag{92}$$

Therefore,

$$\widehat{S} = \frac{\sigma\lambda + \phi(1+\lambda)\widehat{T}}{\lambda(1+\lambda)(\sigma+\phi)} \tag{93}$$

Inserting Eqn. (92) into the steady-state equation for dT/dt yields:

$$k_{\text{eff}}\widehat{T}^h L^h = \sigma\left(\frac{1}{1+\lambda} - \widehat{S}\right) + \sigma\left(\frac{\lambda}{1+\lambda} - \widehat{T}\right) = \sigma(1 - \widehat{S} - \widehat{T}),$$

and inserting there Eqn. (93) gives,

$$k_{\text{eff}}\widehat{T}^h \widehat{L}^h = \sigma\left(1 - \frac{\left(\sigma\,\lambda + (1+\lambda)\big(\phi + \lambda\,(\sigma+\phi)\big)\,\widehat{T}\right)}{\lambda\,(1+\lambda)(\sigma+\phi)}\right) \tag{94}$$

Finally, inserting Eqn. (94) into Eqn. (91) one has:

$$\widehat{R} = \widehat{A} = \frac{\sigma}{k_i}\left(1 - \frac{\left(\sigma\,\lambda + (1+\lambda)\big(\phi + \lambda\,(\sigma+\phi)\big)\,\widehat{T}\right)}{\lambda\,(1+\lambda)(\sigma+\phi)}\right) \tag{95}$$

Step 3: To calculate $E_{\max}$ we will proceed in two steps.

First, from Eqn. (94) one has:

$$1 = \frac{\sigma\,\left(\lambda\,(1+\lambda)(\sigma+\phi) - \sigma\,\lambda - (1+\lambda)\big(\phi + \lambda\,(\sigma+\phi)\big)\,\widehat{T}\right)}{\lambda\,(1+\lambda)(\sigma+\phi)\,k_{\text{eff}}\,\widehat{T}^h\widehat{L}^h}.$$

Let's now assume that $\lim\limits_{L\to\infty}\widehat{T} > 0$. Then, taking the same limit of the above equation one has:

$$1 = \lim_{L\to\infty} 1 = \lim_{L\to\infty}\frac{\sigma\,\left(\lambda\,(1+\lambda)(\sigma+\phi) - \sigma\,\lambda - (1+\lambda)\big(\phi + \lambda\,(\sigma+\phi)\big)\,\widehat{T}\right)}{\lambda\,(1+\lambda)(\sigma+\phi)\,k_{\text{eff}}\,\widehat{T}^h\widehat{L}^h} = 0,$$

which is a contradiction. Since $\widehat{T} \geq 0$, it follows then that $\lim\limits_{L\to\infty}\widehat{T} = 0$.

Second, applying this result to Eqn. (95) we have:

$$\begin{aligned}
\lim_{L\to\infty}\widehat{R} &= \lim_{L\to\infty}\frac{\sigma}{k_i}\left(\frac{\lambda\,(1+\lambda)(\sigma+\phi) - \sigma\,\lambda - (1+\lambda)\big(\phi + \lambda\,(\sigma+\phi)\big)\,\widehat{T}}{\lambda\,(1+\lambda)(\sigma+\phi)}\right)\\
&= \lim_{L\to\infty}\frac{\sigma}{k_i}\left(\frac{(1+\lambda)(\sigma+\phi) - \sigma}{(1+\lambda)(\sigma+\phi)}\right) - \lim_{L\to\infty}\frac{\sigma}{k_i}\left(\frac{\big(\phi + \lambda\,(\sigma+\phi)\big)\,\widehat{T}}{\lambda\,(\sigma+\phi)}\right)\\
&= \frac{\sigma}{k_i}\left(\frac{(1+\lambda)(\sigma+\phi) - \sigma}{(1+\lambda)(\sigma+\phi)}\right) - 0\\
&= \frac{\sigma\,\big((\sigma\,\lambda + \phi\,(1+\lambda)\big)}{k_i\,(1+\lambda)\,(\sigma+\phi)}.
\end{aligned}$$

Therefore:

$$E_{\max} = \lim_{L\to\infty}\widehat{R} = \frac{\sigma\,\big(\sigma\,\lambda + \phi\,(1+\lambda)\big)}{k_i\,(1+\lambda)\,(\sigma+\phi)}.$$

Step 4: EC_{50} is the value of L that satisfies the equation $\widehat{R} = E_{\max}/2$. To solve it requires deriving previously $\widehat{T}$ in Eqn. (94) in terms of L. However, that equation can be solved analytically only up to $h = 4$. Moreover, after substituting $\widehat{T}$ into the generic formula $\widehat{R} = E_{\max}/2$, the resulting equation become exceedingly complicated even for $h = 3$. Therefore, in what follows, we present general calculations of EC_{50} only for $h = 1,\ 2$.

$\mathbf{h = 1}$

From Eqn. (94) one has,

$$\widehat{T} = \frac{\sigma\,\lambda\,\big(\phi + \lambda\,(\sigma+\phi)\big)}{(1+\lambda)\Big(\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big) + k_{\text{eff}} L\lambda(\sigma+\phi)\Big)},$$

and applying this result to Eqn. (95) yields,

$$\widehat{R} = \widehat{A} = \frac{\lambda\,\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big)\,k_{\text{eff}}\,L}{k_i\,(1+\lambda)\,\Big(\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big) + \lambda\,(\sigma+\phi)\,k_{\text{eff}} L\Big)},$$

EC_{50} is the value of L that satisfies the equation:

$$\frac{\lambda\,\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big)\,k_{\text{eff}}\,L}{k_i\,(1+\lambda)\,\Big(\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big) + \lambda\,(\sigma+\phi)\,k_{\text{eff}} L\Big)} = \frac{\sigma\,\big((\sigma\,\lambda + \phi\,(1+\lambda)\big)}{2\,k_i\,(1+\lambda)\,(\sigma+\phi)}.$$

Solving this equation gives:

$$EC_{50} = L_{50} = \frac{\sigma\,\big(\sigma\,\lambda + \phi\,(1+\lambda)\big)}{\lambda\,k_{\text{eff}}\,(\sigma+\phi)}$$

$\mathbf{h = 2}$

From Eqn. (94),

$$k_{\text{eff}}\widehat{T}^2 L^2 = \sigma\left(1 - \frac{\Big(\sigma\,\lambda + (1+\lambda)\big(\phi + \lambda\,(\sigma+\phi)\big)\,\widehat{T}\Big)}{\lambda\,(1+\lambda)(\sigma+\phi)}\right).$$

Solving this quadratic equation for T gives:

$$\widehat{T} = \frac{2\,\lambda\,\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big)}{\sigma\,\Omega + \sqrt{\sigma\,\Omega\,\big(4\,k_{\text{eff}}\,L^2\lambda^2(\sigma+\phi) + \sigma\,\Omega\big)}},$$

with: $\Omega = (1+\lambda)\big(\phi + \lambda\,(\sigma+\phi)\big)$; and substituting this result into Eqn. (95) yields:

$$\widehat{R} = \widehat{A} = \frac{\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big)\,\Big(\sigma\,\Omega - \sqrt{\sigma\,\Omega\,\big(4\,k_{\text{eff}}\,L^2\lambda^2(\sigma+\phi) + \sigma\,\Omega\big)}\Big)}{k_i\,(1+\lambda)(\sigma+\phi)\,\Big(\sigma\,\Omega + \sqrt{\sigma\,\Omega\,\big(4\,k_{\text{eff}}\,L^2\lambda^2(\sigma+\phi) + \sigma\,\Omega\big)}\Big)}.$$

EC_{50} is the value of L that satisfies the equation:

$$\frac{\sigma\,\big(\phi + \lambda\,(\sigma+\phi)\big)\,\Big(\sigma\,\Omega - \sqrt{\sigma\,\Omega\,\big(4\,k_{\text{eff}}\,L^2\lambda^2(\sigma+\phi) + \sigma\,\Omega\big)}\Big)}{k_i\,(1+\lambda)(\sigma+\phi)\,\Big(\sigma\,\Omega + \sqrt{\sigma\,\Omega\,\big(4\,k_{\text{eff}}\,L^2\lambda^2(\sigma+\phi) + \sigma\,\Omega\big)}\Big)} = \frac{\sigma\,\big((\sigma\,\lambda + \phi\,(1+\lambda)\big)}{2\,k_i\,(1+\lambda)\,(\sigma+\phi)}.$$

Solving this equation for L gives:

$$EC_{50} = L_{50} = \frac{\sqrt{2\,\sigma\,(1+\lambda)\,\big(\phi + \lambda\,(\sigma+\phi)\big)}}{\lambda\,\sqrt{k_{\text{eff}}\,(\sigma+\phi)}}.$$

□

Table 2: Parameter values used in the analyses.

Parameter	Value (units)	Description	Reference
k_{on}	5.0×10^{-5}	Binding rate between TCR and ligand	[4]
k_{off}	1.0×10^{-2}	Unbinding rate between TCR and ligand	[4]
T_T	3.0×10^{4}	Number of TCRs	[4]
k_p	1.0×10^{0}	Forward rate in proofreading	[4]
P_T	6.0×10^{5}	Total number of SHP-1	[5]
β/α	5.0×10^{2}	SHP-1 deactivation-activation ratio	[5]
b	4.0×10^{-2}	Spontaneous dephosphorylation rate by SHP-1	[2]
γ	1×10^{-6}	Dephosphorylation rate by SHP-1	[5]
γ	5.0×10^{2}	Ratio of background inhibition and activation (*KPR-LS-IFF*)	[7]
ϕ	9.0×10^{-2}	Forward rate from signalling to non-signalling	[8]
ρ	1.0×10^{3}	Rebinding rate between TCR and ligand	[1]
r	1.5×10^{0}	Factor for increasing dephosphorylation rate	[3]
r_p	1.03×10^{0}	Factor for increasing forward rate	[3]
Y_T	1.0×10^{2}	Total number of Y nodes	[7]
P_T^*	2.5×10^{0}	Total number of P nodes	[7]
δ	1.0×10^{2}	Activation parameter	[7]
μ	5.0×10^{2}	Inhibition parameter	[7]
λ	1.0×10^{2}	Amplification parameter for *KPR-LS-IFF*	[7]
λ	4.0×10^{-2}	Reaction constant	[6]
k_+	1.0×10^{-1}	Reaction constant	[6]
k_-	5.0×10^{-2}	Reaction constant	[6]

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