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# Monotonicity of Kinetic Proofreading

**Christian Kallies, Monica Schliemann-Bullinger**
**Rolf Findeisen, Sergio Lucia, Eric Bullinger**

*Laboratory for Systems Theory and Automatic Control,*
*Otto-von-Guericke University, 39106 Magdeburg, Germany*
*({christian.kallies, monica.schliemann-bullinger, rolf.findeisen,*
*sergio.lucia, eric.bullinger}@ovgu.de)*

**Abstract:** This manuscript studies the monotonicity of multi-step ligand-receptor signalling motifs. Monotonicity with respect to parameters and state perturbations allow not only to exclude periodic solutions, but also to easily bound the responses in cases of bounded perturbations.
In classical coordinates, multi-step ligand-receptor signalling motifs are known not to be monotone. However, a generic coordinate transform allows for deriving conditions on the kinetic rate constants such that the signal is monotonously affected by perturbations to any one of the kinetic rate constants. The result is illustrated at the hand of a model of kinetic proofreading.



*Keywords:* biomedical applications, monotonicity, kinetic proofreading

## 1. INTRODUCTION

Signalling by ligands binding to their receptors are ubiquitous in cell biology. Examples are T cell activation (McKeithan, 1995), TNF-induced pro- and anti-apoptotic signalling (Schliemann et al., 2011), EPO-signalling (Lucia et al., 2016). Similar, though fully inside the cell are DNA repair of protein synthesis reaction (Hopfield, 1974). Multi-step activation was proposed as a mechanism to achieve high specificity even though the initial receptor-ligand binding step is rather unspecific.

In this paper, we study how the dynamic responses of the receptor-ligand complexes are affected by perturbations of the kinetic parameters. This could for example be achieved directly by targeting the production or degradation of the ligand, e.g. by injecting the drug as in hormone therapies, see e.g. Lucia et al. (2016). Alternatively, a drug could have an indirect effect, for example by modulating the concentration or the activity of phosphatases responsible for an activation step.

We will derive sufficient conditions such that an increase or decrease in a kinetic rate constant leads to a monotonous change of the signal induced by the last step of the signalling cascade. The main advantage of monotone system is that the state trajectories for parameters within a bounded set are bounded by the extreme values of the individual parameters, see Figure 1. This allows for efficient analysis and optimal control design approaches.

### 1.1 Receptor-ligand signalling

We analyse two variants of the initial signalling. First, the ligand $L$ binds to the receptor $R$ to form a complex $C_1$ and then this complex is activated in several, possibly reversible steps, e.g. via multiple phosphorylation, see Figure 2. Classical kinetic proofreading models assume this complex activation as being irreversible. However, as shown below, reversibility has no adverse effect on the here presented analysis. Later, a second messenger will be activated by the last step. Throughout this paper, all reactions are modelled as mass action kinetics.

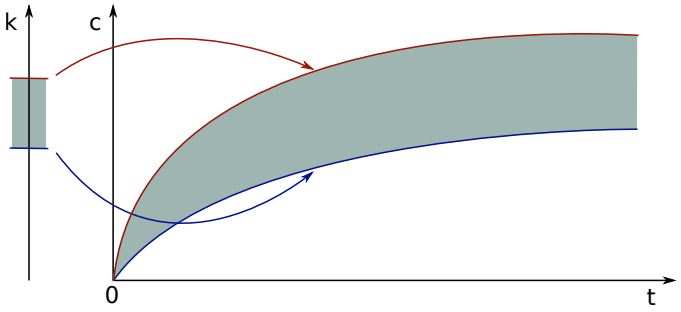


Figure 1. Sketch of a monotone system's response to parameter changes. In a monotone system, a bounded set of parameters is mapped to a bounded set of trajectories, where the extreme parameters correspond to extreme trajectories, see the blue and red cases.

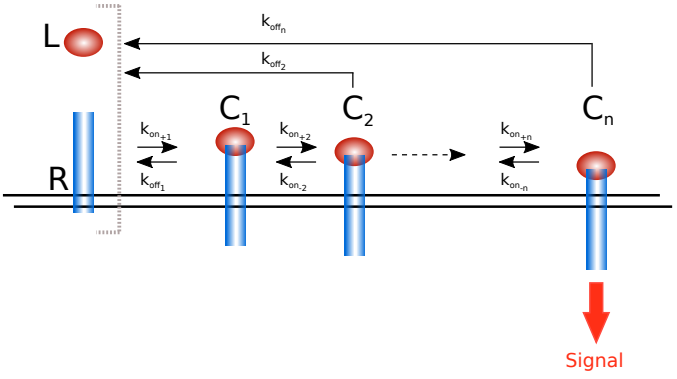


Figure 2. Kinetic proofreading reaction network


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### 1.2 Novelty and structure of the paper

The key novelty of this paper is to uncover monotonic behaviour of multi-step ligand-receptor signalling modules, using a special, though generic coordinate transform. The mathematical tools employed here are graph theoretical approaches that connect the Jacobian of the differential equations with monotonicity.

The paper is structured as follows. First, Section 2 discusses the smallest possible system to illustrate the concept and introduce the terminology and notation. Then, monotonicity conditions are derived for a multi-step signalling system without (Section 3) and with second messenger (Section 4). Finally, the results are applied to a kinetic proofreading example in Section 5.

## 2. MOTIVATING EXAMPLE

Let us first study the following reaction network of three species $R$, $L$ and $C$:

$$\mathrm{L} + \mathrm{R} \underset{\mathrm{k_{off_1}}}{\overset{\mathrm{k_{on_{+1}}}}{\rightleftharpoons}} \mathrm{C} \tag{1}$$

modelled by mass action kinetics. Let the state vector be

$$c = \begin{bmatrix} L \\ R \\ C \end{bmatrix}, \tag{2}$$

where each state (species) is non-negative. The reversible reaction (1) has the rate

$$v_1 = k_{\mathrm{on}_{+1}}\mathrm{LR} - k_{\mathrm{off}_1}\mathrm{C}, \tag{3}$$

where the reaction constants $k_{\mathrm{on}_{+1}}$ and $k_{\mathrm{off}_1}$ are positive constants. Then, the differential equation model corresponding to (1) is

$$\dot{c} = \frac{dc}{dt} = N v_1 \tag{4}$$

with $v_1$ as in (3) and

$$N = \begin{bmatrix} -1 \\ -1 \\ 1 \end{bmatrix}.$$

The Jacobian matrix of the right hand side of (4) is

$$\begin{aligned} J = \begin{bmatrix} -1 \\ -1 \\ 1 \end{bmatrix} \frac{\partial v_1}{\partial c} &= \begin{bmatrix} -k_{\mathrm{on}_{+1}}R & -k_{\mathrm{on}_{+1}}L & k_{\mathrm{off}_1} \\ -k_{\mathrm{on}_{+1}}R & -k_{\mathrm{on}_{+1}}L & k_{\mathrm{off}_1} \\ k_{\mathrm{on}_{+1}}R & k_{\mathrm{on}_{+1}}L & -k_{\mathrm{off}_1} \end{bmatrix} \\ &= \begin{bmatrix} -1 & -1 & 1 \\ -1 & -1 & 1 \\ 1 & 1 & -1 \end{bmatrix} \operatorname{diag} \begin{bmatrix} k_{\mathrm{on}_{+1}}R \\ k_{\mathrm{on}_{+1}}L \\ k_{\mathrm{off}_1} \end{bmatrix}. \end{aligned} \tag{5}$$

This Jacobian has the following sign pattern

$$\operatorname{sign} J = \begin{bmatrix} - & - & + \\ - & - & + \\ + & + & - \end{bmatrix}, \tag{6}$$

where the symbol "+" states that the entry is $\geq 0$, a "−" stands for $\leq 0$ while a "0" is an entry that is zero.

Following Sontag (2007), we now draw the corresponding species graph $G$. This has one node per species, and one signed edge per non-vanishing off-diagonal entry of the Jacobian, the sign of each edge is the sign of that entry in the Jacobian. More precisely, an edge from species $i$ to species $j$ corresponds to $J_{j,i}$. For the example (1), the species graph is shown in Figure 3. This graph has three

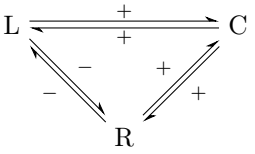


Figure 3. Species graph for Example (1)




Figure 4. Species graph for Example (1) in total coordinates. Arrows stand for an influence, not for a biochemical reaction. Blue arrow denote positive influence.

nodes ($L$, $R$ and $C$) and six edges, two of them negative, the ones connecting $L$ and $R$.

The sign of a path between two nodes is denoted as positive if the path contains an even number of negative edges, and negative for an odd number. If two nodes are connected by negative and positive paths, the graph is inconsistent. However, if a graph is consistent, then the system is monotone. Although graph consistency is only a sufficient condition, it is a condition that is relatively easy to check, thus can quickly give insights as will be seen in this paper.

From the species graph in Figure 3 it follows that a path from $L$ to $R$ has either a negative sign when using the direct connection, or a positive one if passing via $C$. Thus, this graph is inconsistent. As a consequence, no conclusion can be drawn, whether this system is monotone or not.

Lucia et al. (2016) however showed that the same network is monotone, when transformed into the following coordinates

$$\tilde{c} = \begin{bmatrix} L + C \\ R + C \\ C \end{bmatrix} = \begin{bmatrix} L_{\mathrm{tot}} \\ R_{\mathrm{tot}} \\ C \end{bmatrix} \tag{7}$$

as

$$\dot{\tilde{c}} = \begin{bmatrix} 1 & 0 & 1 \\ 0 & 1 & 1 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} -1 \\ -1 \\ 1 \end{bmatrix} v_1 = \begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix} v_1 \tag{8}$$

with

$$v_1 = k_{\mathrm{on}_{+1}}(L_{\mathrm{tot}} - C)(R_{\mathrm{tot}} - C) - k_{\mathrm{off}_1}C \tag{9}$$

and the Jacobian

$$\begin{aligned} \tilde{J} = \frac{\partial \dot{\tilde{c}}}{\partial \tilde{c}} &= \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ k_{\mathrm{on}_{+1}}R & k_{\mathrm{on}_{+1}}L & -k_{\mathrm{off}_1} \end{bmatrix} \\ &= \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 1 & 1 & -1 \end{bmatrix} \operatorname{diag} \left[ k_{\mathrm{on}_{+1}}R \;\; k_{\mathrm{on}_{+1}}L \;\; k_{\mathrm{off}_1} \right]. \end{aligned} \tag{10}$$

As the diagonal matrix has only non-negative entries, the Jacobian $\tilde{J}$ has the sign pattern

$$\operatorname{sign} \tilde{J} = \begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ + & + & - \end{bmatrix}. \tag{11}$$

This Jacobian is now sign consistent and the system is monotone, see Figure 4 for the corresponding species graph.

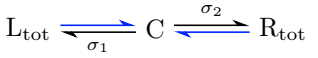


Figure 5. Species graph for Example (1) in total coordinates with turnover. Arrows stand for an influence, not for a biochemical reaction. Blue arrow denote positive influence.

Next, let us add turnover to all three species, i.e. each species is produced with constant rate $k_{p_i}$ and degraded with rate $k_{d_i} i$ where $i$ is $L$, $R$ or $C$. The differential equations for the model with turnover are then

$$\frac{dc}{dt} = N v_1 + \begin{bmatrix} k_{p_L} \\ k_{p_R} \\ k_{p_C} \end{bmatrix} - \begin{bmatrix} k_{d_L} L \\ k_{d_R} R \\ k_{d_C} C \end{bmatrix} \tag{12}$$

and in the transformed states (7)

$$\frac{d\tilde{c}}{dt} = \begin{bmatrix} 0 \\ 0 \\ -1 \end{bmatrix} v_1 + \begin{bmatrix} k_{p_L} + k_{p_C} \\ k_{p_R} + k_{p_C} \\ k_{p_C} \end{bmatrix} \tag{13}$$

$$- \begin{bmatrix} k_{d_L} L_{\text{tot}} + (k_{d_C} - k_{d_L}) C \\ k_{d_R} R_{\text{tot}} + (k_{d_C} - k_{d_R}) C \\ k_{d_C} C \end{bmatrix}. \tag{14}$$

Then, the Jacobian is

$$\tilde{J}_{\text{to}} = \begin{bmatrix} -k_{d_L} & 0 & k_{d_L} - k_{d_C} \\ 0 & -k_{d_R} & k_{d_R} - k_{d_C} \\ k_{\text{on}_{+1}} R & k_{\text{on}_{+1}} L & -k_{\text{off}_1} - k_{d_C} \end{bmatrix} \tag{15}$$

and its sign pattern

$$\operatorname{sign} \tilde{J}_{\text{to}} = \begin{bmatrix} - & 0 & \sigma_1 \\ 0 & - & \sigma_2 \\ + & + & - \end{bmatrix} \tag{16}$$

with

$$\sigma_1 = \operatorname{sign}(k_{d_L} - k_{d_C}) \tag{17}$$

$$\sigma_2 = \operatorname{sign}(k_{d_R} - k_{d_C}). \tag{18}$$

By abuse of notation, we write "+" for $+1$ and "−" for $-1$. Sign consistency of the Jacobian (15) is then obtained whenever $\sigma_1$ and $\sigma_2$ are non-negative, i.e. if

$$k_{d_L} \geq k_{d_C} \tag{19a}$$

$$k_{d_R} \geq k_{d_C}. \tag{19b}$$

From this Jacobian, we obtain the species graph in Figure 5, which contains only positive loops whenever $\sigma_1$ and $\sigma_2$ are non-negative.

### 2.1 Monotonicity with respect to kinetic parameters

The previous discussion concerned monotonicity with respect to initial conditions. In practical applications, also monotonicity with respect to parameters is of interest. For this we extend the state space to include both the species and the rate constants. For the Example (1) in the total coordinates, this means

$$x = \left[ \begin{array}{ccc|ccc} \mathrm{L_{tot}} & \mathrm{R_{tot}} & \mathrm{C} & k_{\text{on}_{+1}} & k_{\text{off}_1} & \\ & & & k_{p_\mathrm{L}} & k_{p_\mathrm{R}} & k_{p_\mathrm{C}} \end{array} \right. \left| \begin{array}{ccc} & & \\ k_{d_\mathrm{L}} & k_{d_\mathrm{R}} & k_{d_\mathrm{C}} \end{array} \right]^\top \tag{20}$$

with the Jacobian

$$\frac{\partial \dot{x}}{\partial x} = \begin{bmatrix} \frac{\partial \dot{\tilde{c}}}{\partial x} \\ 0 \dots 0 \\ \vdots \\ 0 \dots 0 \end{bmatrix}, \tag{21}$$

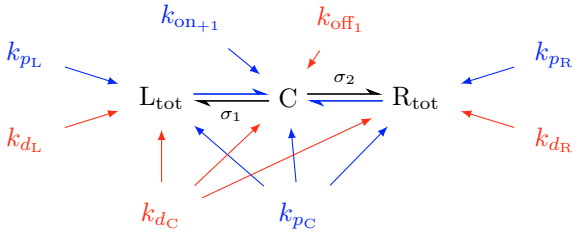


Figure 6. Generalised species graph for Example (1) in total coordinates, which shows the impact of kinetic parameters on the species, not the biochemical reactions. Blue arrows corresponds for positive, red for negative influence. Parameters in blue have an increasing impact on the states, in contrary to those in red.

where

$$\frac{\partial \dot{\tilde{c}}}{\partial x} = \left[ \begin{array}{ccc|ccc} -k_{d_\mathrm{L}} & 0 & k_{d_\mathrm{L}} - k_{d_\mathrm{C}} & 0 & 0 & \\ 0 & -k_{d_\mathrm{R}} & k_{d_\mathrm{R}} - k_{d_\mathrm{C}} & 0 & 0 & \\ k_{\text{on}_{+1}} \mathrm{R} & k_{\text{on}_{+1}} \mathrm{L} & -k_{\text{off}_1} & \mathrm{R} & \mathrm{L} & -\mathrm{C} \\ \hline 1 & 0 & 1 & -\mathrm{L} & 0 & -\mathrm{C} \\ 0 & 1 & 1 & 0 & -\mathrm{R} & -\mathrm{C} \\ 0 & 0 & 1 & 0 & 0 & -\mathrm{C} \end{array} \right]. \tag{22}$$

All remaining rows of the Jacobian are zero. From this Jacobian, we obtain the generalised species graph Figure 6. Both the Jacobian and the species graph imply that the system is monotone with respect to state and parameter perturbations. In particular, perturbation on $k_{d_\mathrm{L}}$

From Figure 6 follows that all loops are positive when conditions (19) are satisfied. Minus signs appear only on loop-less paths, which corresponds to parameters whose increase leads to decrease of the states. Thus, this reaction network is not only monotone in the species initial condition, but also in the reaction rate constants. Thus, for systems starting from a steady-state, changes in a parameter will cause each time response to monotonically increase or decrease.

## 3. MONOTONICITY OF THE MULTI-STEP LIGAND-RECEPTOR ACTIVATION SCHEME

We now study the reaction network depicted in Figure 2. Note that there is no $k_{-\text{on}_1}$ in this reaction scheme, as this can be incorporated into the kinetic parameter $k_{\text{off}_1}$.

Defining the state as

$$c = [\mathrm{L}\ \mathrm{R}\ \mathrm{C}_1\ \dots\ \mathrm{C_n}]^\top \tag{23}$$

a differential equation model is given by

$$\dot{c} = N v \tag{24}$$

with

$$v = \left[ \bar{v}_1\ \dots\ \bar{v}_n\ \tilde{v}_2\ \dots\ \tilde{v}_n\ \hat{v}_L\ \hat{v}_R\ \hat{v}_{\mathrm{C}_1}\ \dots \hat{v}_{\mathrm{C_n}} \right]^\top \tag{25}$$

and

$$
\begin{aligned}
\bar{v}_1 &= k_{\mathrm{on}_{+1}} \mathrm{L}\,\mathrm{R} \\
\bar{v}_i &= k_{\mathrm{on}_{+i}} \mathrm{C_{i\text{-}1}} - k_{\mathrm{on}_{-i}} \mathrm{C_i}, \quad i = 2, \ldots, n \\
\tilde{v}_i &= k_{\mathrm{off}_{-i}} \mathrm{C_i}, \quad i = 1, \ldots, n \\
\hat{v}_\mathrm{L} &= k_{p_\mathrm{L}} - k_{d_\mathrm{L}} \mathrm{L}, \\
\hat{v}_\mathrm{R} &= k_{p_\mathrm{R}} - k_{d_\mathrm{R}} \mathrm{R}, \\
\hat{v}_{\mathrm{C_i}} &= k_{p_{\mathrm{C_i}}} - k_{d_{\mathrm{C_i}}} \mathrm{C_i}, \quad i = 1, \ldots, n \\
N &= \begin{bmatrix} \bar{N} & \tilde{N} & I \end{bmatrix}
\end{aligned}
$$

$$
\bar{N} = \begin{bmatrix} -1 & 0 & \ldots & 0 \\ -1 & 0 & \ldots & 0 \\ 1 & -1 & & \\ 0 & 1 & \ddots & \\ & & \ddots & -1 \\ & & & 1 \end{bmatrix}, \quad \tilde{N} = \begin{bmatrix} 1 & \ldots & 1 \\ 1 & \ldots & 1 \\ -1 & & \\ & \ddots & \\ & & -1 \end{bmatrix}
$$

and $I$ is the identity matrix. Similarly to the network (1), the reaction network depicted in Figure 2 is not monotone in the states L, R and $\mathrm{C_i}$. It is however possible to find a similar transformation to (7):

$$
\tilde{c} = \begin{bmatrix} \mathrm{L_{tot}} \\ \mathrm{R_{tot}} \\ \mathrm{C_{1tot}} \\ \mathrm{C_{2tot}} \\ \vdots \\ \mathrm{C_{ntot}} \end{bmatrix} = \begin{bmatrix} \mathrm{L} + \mathrm{C_1} + \mathrm{C_2} + \ldots + \mathrm{C_n} \\ \mathrm{R} + \mathrm{C_1} + \mathrm{C_2} + \ldots + \mathrm{C_n} \\ \mathrm{C_1} + \mathrm{C_2} + \ldots + \mathrm{C_n} \\ \mathrm{C_2} + \ldots + \mathrm{C_n} \\ \ddots \quad \vdots \\ \mathrm{C_n} \end{bmatrix} \tag{26}
$$

$$
= \begin{bmatrix} 1 & 0 & 1 & \cdots & 1 \\ 0 & 1 & 1 & \cdots & \vdots \\ 0 & 0 & 1 & \cdots & \vdots \\ \vdots & & \ddots & \ddots & \vdots \\ 0 & \cdots & & 0 & 1 \end{bmatrix} \begin{bmatrix} \mathrm{L} \\ \mathrm{R} \\ \mathrm{C_1} \\ \vdots \\ \mathrm{C_n} \end{bmatrix} \tag{27}
$$

Note that this coordinate transform does not modify the last step $C_n$. As this is usually the one that triggers a signal, monotonicity in the new states imply that also the state triggering the signal responds monotonously.

$$
J = \begin{bmatrix} * & 0 & \delta_L & \delta_1 & \delta_2 & \cdots & \delta_{n-1} \\ 0 & * & \delta_R & \delta_1 & \delta_2 & \cdots & \delta_{n-1} \\ k_{\mathrm{on}_{+1}}\mathrm{R} & k_{\mathrm{on}_{+1}}\mathrm{L} & * & \Delta_1 & \Delta_2 & \cdots & \Delta_{n-1} \\ 0 & 0 & k_{\mathrm{on}_{+2}} & * & \tilde{\Delta}_2 & \Delta_3 & \cdots & \Delta_{n-1} \\ & & & \ddots & \ddots & \ddots & & \vdots \\ & & & & * & \tilde{\Delta}_{n-2} & \Delta_{n-1} \\ & & & & & * & \tilde{\Delta}_{n-1} \\ 0 & & & & & 0 & k_{\mathrm{on}_{+n}} & * \end{bmatrix}
$$

with

$$
\begin{aligned}
\delta_L &= k_{d_L} - k_{d_{C_1}}, \\
\delta_R &= k_{d_R} - k_{d_{C_1}}, \\
\delta_i &= k_{d_{C_i}} - k_{d_{C_{i+1}}}, \; i = 1, \ldots, n-1, \\
\Delta_i &= k_{\mathrm{off}_i} - k_{\mathrm{off}_{i+1}} + \delta_i, \; i = 1, \ldots, n-1, \\
\tilde{\Delta}_i &= k_{\mathrm{on}_{-i}} + \Delta_i, \; i = 2, \ldots, n-1.
\end{aligned}
$$

In addition, the Jacobian with respect to the parameters (not shown here) has no sign switch within any column.

Thus, the system of Figure (2) is monotone with respect to each one of its kinetic parameters if

$$
\begin{aligned}
k_{d_L} &\geq k_{d_{C_1}} && \text{(28a)} \\
k_{d_R} &\geq k_{d_{C_1}} && \text{(28b)} \\
k_{d_{C_i}} &\geq k_{d_{C_{i+1}}}, \; i = 1, \ldots, n-1, && \text{(28c)} \\
k_{\mathrm{off}_i} &\geq k_{\mathrm{off}_{i+1}}, \; i = 1, \ldots, n-1. && \text{(28d)}
\end{aligned}
$$

A particular case is if all degradation rate constants are equal, i.e.

$$
k_{d_L} = k_{d_R} = k_{d_{C_i}}, \; i = 1, \ldots, n. \tag{29}
$$

Then, monotonicity holds if

$$
k_{\mathrm{off}_i} \geq k_{\mathrm{off}_{i+1}}, \; i = 1, \ldots, n-1. \tag{30}
$$

In all cases, monotonicity is not influenced by the (reverse) activation rate constants $k_{\mathrm{on}_{+i}}$ and $k_{\mathrm{on}_{-i}}$ nor by the production rate constants $k_{p_L}$, $k_{p_R}$, $k_{p_{C_i}}$. In particular, the classical case of kinetic proofreading, which has irreversible activation steps ($k_{\mathrm{off}_i} = 0$), is monotone if Conditions (28) are satisfied. As for the simple reaction scheme (1), whether increasing a specific parameter has an increasing or decreasing effect on the states can be straightforwardly obtained by calculating the Jacobian with respect to this parameter and analysing the sign pattern.

## 4. MONOTONICITY OF THE MULTI-STEP LIGAND-RECEPTOR ACTIVATION SCHEME WITH SECOND MESSENGER

In addition to the reactions in Figure 2, the system now includes as additional states a second messenger $X$, the complex $\mathrm{C_nX}$ and the activated second messenger $\mathrm{X^*}$ as well as the following reactions:

$$
\begin{aligned}
\mathrm{C}_n + \mathrm{X} \underset{k_{\mathrm{on}_-X}}{\overset{k_{\mathrm{on}_+X}}{\rightleftharpoons}} \mathrm{C}_n\mathrm{X} &\xrightarrow{k_{\mathrm{act}}} \mathrm{C}_n + \mathrm{X^*} && \text{(31a)} \\
\mathrm{X} \underset{k_{\mathrm{deact}}}{\overset{k_{\mathrm{autoact}}}{\rightleftharpoons}} \mathrm{X^*} && && \text{(31b)} \\
\mathrm{C}_n\mathrm{X} &\xrightarrow{k_{\mathrm{off_X}}} \mathrm{R} + \mathrm{L} + \mathrm{X} && \text{(31c)} \\
\underset{k_{d_{C_nX}}}{\overset{k_{p_{C_nX}}}{\rightleftharpoons}} \mathrm{C}_n\mathrm{X} && && \text{(31d)} \\
\underset{k_{d_{C_nX}}}{\overset{k_{p_{C_nX}}}{\rightleftharpoons}} \mathrm{X} && && \text{(31e)} \\
\underset{k_{d_{C_nX}}}{\overset{k_{p_{C_nX}}}{\rightleftharpoons}} \mathrm{X^*}, && && \text{(31f)}
\end{aligned}
$$

see also Figure 7.

Similarly to the network (26), the reaction network cannot be shown to be monotone in the states L, R and $\mathrm{C}_i$ nor in the states $\mathrm{C}_n\mathrm{X}$, X or $\mathrm{X^*}$. However, monotonicity is achievable in the coordinates $\hat{c}$ defined below.

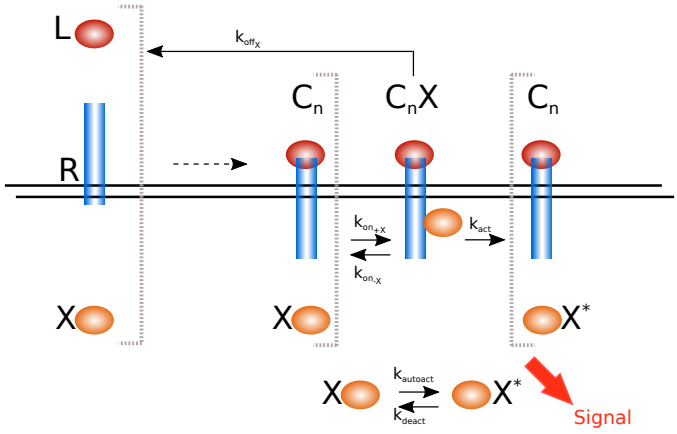


Figure 7. Kinetic proofreading with second messenger

$$\hat{c} = \begin{bmatrix} \mathrm{L}_{\mathrm{tot}} \\ \mathrm{R}_{\mathrm{tot}} \\ \mathrm{C}_{1_{\mathrm{tot}}} \\ \mathrm{C}_{2_{\mathrm{tot}}} \\ \vdots \\ \mathrm{C}_{n_{\mathrm{tot}}} \\ \mathrm{X}^*_{\mathrm{tot}} \\ \mathrm{X}_{\mathrm{tot}} \\ \mathrm{X}^* \end{bmatrix} = \begin{bmatrix} \mathrm{L}+\mathrm{C}_1+\mathrm{C}_2+\ldots+\mathrm{C}_\mathrm{n}+\mathrm{C}_\mathrm{n}\mathrm{X} & & \\ \mathrm{R}+\mathrm{C}_1+\mathrm{C}_2+\ldots+\mathrm{C}_\mathrm{n}+\mathrm{C}_\mathrm{n}\mathrm{X} & & \\ \mathrm{C}_1+\mathrm{C}_2+\ldots+\mathrm{C}_\mathrm{n}+\mathrm{C}_\mathrm{n}\mathrm{X} & & \\ \mathrm{C}_2+\ldots+\mathrm{C}_\mathrm{n}+\mathrm{C}_\mathrm{n}\mathrm{X} & & \\ \ddots\ \vdots & & \\ \mathrm{C}_\mathrm{n}+\mathrm{C}_\mathrm{n}\mathrm{X} & & \\ \mathrm{C}_\mathrm{n}\mathrm{X} & +\mathrm{X}^* & \\ \mathrm{C}_\mathrm{n}\mathrm{X}+\mathrm{X}+\mathrm{X}^* & & \\ \mathrm{X}^* & & \end{bmatrix}$$

$$= \begin{bmatrix} 1 & 0 & 1 & \ldots & 1 & 1 & 0 & 0 \\ 0 & 1 & 1 & \ldots & 1 & 1 & 0 & 0 \\ 0 & 0 & 1 & \ldots & 1 & 1 & 0 & 0 \\ \vdots & & \ddots & \ddots & \vdots & \vdots & \vdots & \vdots \\ 0 & \ldots & \ldots & 0 & 1 & 1 & 0 & 0 \\ 0 & \ldots & \ldots & 0 & 0 & 1 & 0 & 1 \\ 0 & \ldots & \ldots & 0 & 0 & 1 & 1 & 1 \\ 0 & \ldots & \ldots & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \mathrm{L} \\ \mathrm{R} \\ \mathrm{C}_1 \\ \vdots \\ \mathrm{C}_\mathrm{n} \\ \mathrm{C}_\mathrm{n}\mathrm{X} \\ \mathrm{X} \\ \mathrm{X}^* \end{bmatrix} \tag{32}$$

The Jacobian of this system is depicted in Figure 8. The constants are defined as before, with additionally

$$\begin{aligned} \delta_n &= k_{d_{C_n}} - k_{d_{\mathrm{C_nX}}} \\ \Delta_n &= k_{\mathrm{off}_n} - k_{\mathrm{off}_X} + \delta_n, \\ \tilde{\Delta}_n &= k_{\mathrm{on}_{-n}} + \Delta_n, \\ \hat{\Delta}_n &= k_{\mathrm{on}_{-X}} - k_{\mathrm{deact}} + k_{\mathrm{off}_X} - k_{d_{X^*}} + k_{d_{\mathrm{C_nX}}} + k_{\mathrm{on}_{+X}} X. \end{aligned}$$

The system with second messenger is monotone if conditions (28) hold as well as the following ones

$$\begin{aligned} k_{d_{\mathrm{C_nX}}} &= k_{d_{\mathrm{C_n}}} && \Leftrightarrow \delta_n = 0 && \text{(33a)} \\ k_{\mathrm{off}_n} &= k_{\mathrm{off}_X} && \Leftrightarrow \Delta_n = 0 && \text{(33b)} \\ k_{\mathrm{on}_{-n}} &= 0 && \Leftrightarrow \tilde{\Delta}_n = 0 && \text{(33c)} \\ k_{\mathrm{on}_{-X}} + k_{\mathrm{off}_X} + k_{d_{\mathrm{C_nX}}} &\geq k_{\mathrm{deact}} + k_{d_{X^*}} && \Rightarrow \hat{\Delta}_n = 0 && \text{(33d)} \\ k_{d_X} &\geq k_{d_{\mathrm{C_nX}}} && && \text{(33e)} \\ k_{d_{X^*}} &\leq k_{d_{\mathrm{C_nX}}} && && \text{(33f)} \\ k_{\mathrm{act}} &\geq k_{\mathrm{autoact}}. && && \text{(33g)} \end{aligned}$$

Conditions (33) are much more restrictive as several of them are equality constraints. However, they are too restrictive as they only require that the most activated complex, $\mathrm{C_n}$ and the complex with the second messenger $\mathrm{C_nX}$ break up as well as are degraded with equal probability.

Since the reaction rates constants are independent of other rates and the states, this can not produce a negative loop. Furthermore, since all edges between the states are positive and the derivatives $\partial \dot{c}_i / \partial k_j$ have the same sign for each $i$ also all paths from $k_j$ to $c_i$ have the same sign. So the graph shows complete monotonic behaviour (Ascensao et al. (2016)).

## 5. EXAMPLES

The monotonicity analysis is applied to two examples. First small example with two step activation, then an n-step activation with second messenger.

### 5.1 Application to small proofreading scheme

We first study the scheme of Section 3 with $n = 2$ and nominal parameter values

$$\begin{aligned} k_{\mathrm{on}+1} &= 1, & k_{\mathrm{on}+2} &= 0.1, & k_{\mathrm{off}1} &= 0.1, & k_{\mathrm{off}2} &= 0.1, \\ k_{p_\mathrm{L}} &= 0.7, & k_{p_\mathrm{R}} &= 3, & k_{p_{\mathrm{C}_1}} &= 1, & k_{p_{\mathrm{C}_2}} &= 2, \\ k_{d_\mathrm{L}} &= 0.1, & k_{d_\mathrm{R}} &= 0.2, & k_{d_{\mathrm{C}_1}} &= 0.1, & k_{d_{\mathrm{C}_2}} &= 0.1, \end{aligned}$$

and the system state in its steady state up to $t = 0$. Then, $k_{\mathrm{off}_1}$ is modified, taking values in the interval of 0.01 to 10 times its nominal value of 1. Figure 9 shows the response of $\mathrm{C}_1$ on the left and of $\mathrm{C}_{1\mathrm{tot}}$ on the right. While the system in original coordinates does not show monotonous responses, in the total coordinates it does, even for parameters that do not satisfy the conditions (33), illustrating that they are sufficient, not necessary conditions.

### 5.2 Application to kinetic proofreading

Now this result can be applied to the extended model for kinetic proofreading introduced in Goldstein et al. (2004). This corresponds to the reaction network depicted in Figure 7 without turnover, i.e. with all $k_p$ and $k_d$ zero, and with the following parameters

$$\begin{aligned} R(0) &= 900\,\text{molecules}, \quad L(0) = 30\,\text{molecules}, \\ C_1(0) &= \ldots = C_n(0) = C_{nX}(0) = X^*(0) = 0\,\text{molecules}, \\ X(0) &= 10{,}000\,\text{molecules}, \\ k_{\mathrm{on}+1} &= 0.0067\,\text{molecules}^{-1}\,\mathrm{s}^{-1}, \\ k_{\mathrm{on}+2} &= \ldots k_{\mathrm{on}+n} = 0.25\,\mathrm{s}^{-1}, \\ k_{\mathrm{on}-1} &= 0\,\text{molecules}^{-1}\,\mathrm{s}^{-1},\ k_{\mathrm{on}-2} = \ldots = k_{\mathrm{on}-n} = 0\,\mathrm{s}^{-1}, \\ k_{\mathrm{off}_1} &= \ldots = k_{\mathrm{off}_n} = k_{\mathrm{off}_X} = 0.5\,\mathrm{s}^{-1}, \\ k_{\mathrm{on}+X} &= 0.0012\,\text{molecules}^{-1}\,\mathrm{s}^{-1},\ k_{\mathrm{on}-X} = 0.01\,\mathrm{s}^{-1}, \\ k_{\mathrm{act}} &= 100\,\mathrm{s}^{-1},\ k_{\mathrm{deact}} = 0.002\,\mathrm{s}^{-1},\ k_{\mathrm{autoact}} = 0.0\,\mathrm{s}^{-1}. \end{aligned}$$

These values satisfy the monotonicity condition, as

$$\begin{aligned} \delta_n &= k_{d_{C_n}} - k_{d_{\mathrm{C_nX}}} = 0\,\mathrm{s}^{-1} - 0\,\mathrm{s}^{-1} = 0\,\mathrm{s}^{-1}. \\ \Delta_n &= k_{\mathrm{off}_n} - k_{\mathrm{off}_X} + \delta_n = (0.5 - 0.5 - 0)\,\mathrm{s}^{-1} = 0\,\mathrm{s}^{-1}, \\ \tilde{\Delta}_n &= k_{\mathrm{on}_{-n}} + \Delta_n = 0\,\mathrm{s}^{-1} + 0\,\mathrm{s}^{-1} = 0\,\mathrm{s}^{-1}, \\ \hat{\Delta}_n &= k_{\mathrm{on}_{-X}} - k_{\mathrm{deact}} + k_{\mathrm{off}_X} - k_{d_{X^*}} + k_{d_{\mathrm{C_nX}}} + k_{\mathrm{on}_{+X}} X \\ &\geq (0.01 - 0.002 + 0.5 - 0 + 0)\,\mathrm{s}^{-1} > 0\,\mathrm{s}^{-1}. \end{aligned}$$

Thus, the considered chemical reaction network shows in transformed coordinates a monotonic behaviour in each state trajectory with respect to state and parameter perturbations, see Figure 10 for an example.

$$
J = \left[\begin{array}{ccccccccc|ccc}
* & 0 & \delta_{\mathrm{L}} & \delta_1 & \delta_2 & \cdots & \delta_{n-2} & \delta_{n-1} & \delta_n & 0 & -\delta_n \\
0 & * & \delta_{\mathrm{R}} & \delta_1 & \delta_2 & \cdots & \delta_{n-2} & \delta_{n-1} & \delta_n & 0 & -\delta_n \\
k_{\mathrm{on}_{+1}}\mathrm{L} & k_{\mathrm{on}_{+1}}\mathrm{R} & * & \Delta_1 & \Delta_2 & \cdots & \Delta_{n-2} & \Delta_{n-1} & \Delta_n & 0 & -\Delta_n \\
0 & 0 & k_{\mathrm{on}_{+2}} & * & \tilde{\Delta}_2 & \Delta_3 & & & \Delta_n & 0 & -\Delta_n \\
0 & 0 & 0 & k_{\mathrm{on}_{+3}} & * & & & & \Delta_n & 0 & -\Delta_n \\
\vdots & \vdots & \vdots & \vdots & & \ddots & & \Delta_{n-1} & \vdots & \vdots & \vdots \\
\vdots & \vdots & \vdots & \vdots & \vdots & & * & \tilde{\Delta}_{n-1} & \Delta_n & \vdots & -\Delta_n \\
0 & 0 & 0 & 0 & 0 & \cdots & k_{\mathrm{on}_{+n}} & * & \tilde{\Delta}_n & 0 & -\tilde{\Delta}_n \\
\hline
0 & 0 & 0 & 0 & 0 & \cdots & 0 & k_{\mathrm{on}_{+\mathrm{X}}}\mathrm{X} & * & k_{\mathrm{autoact}} + k_{\mathrm{on}_{+\mathrm{X}}}\mathrm{C_n} & \hat{\Delta}_n \\
0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & k_{d_{\mathrm{X}}} - k_{d_{\mathrm{C_nX}}} & * & k_{d_{\mathrm{C_nX}}} - k_{d_{\mathrm{X}^*}} \\
0 & 0 & 0 & 0 & 0 & \cdots & 0 & 0 & k_{\mathrm{act}} - k_{\mathrm{autoact}} & k_{\mathrm{autoact}} & * \\
\mathrm{L_{tot}} & \mathrm{R_{tot}} & \mathrm{C_{1tot}} & \mathrm{C_{2tot}} & \mathrm{C_{3tot}} & \cdots & \mathrm{C_{(n-1)tot}} & \mathrm{C_{ntot}} & \mathrm{X^*_{tot}} & \mathrm{X_{tot}} & \mathrm{X^*}
\end{array}\right]
$$

Figure 8. Jacobian of the system with second messenger in total coordinates

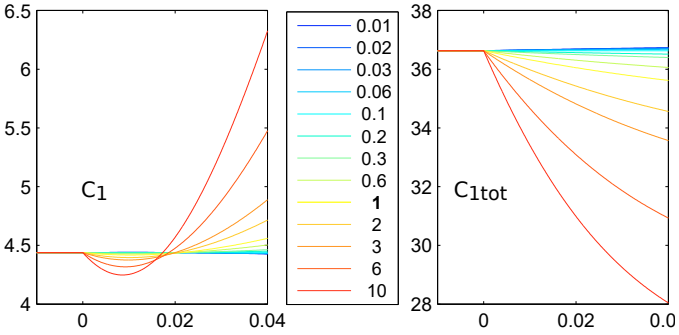


Figure 9. Response of the model of Section 5.1. Left: $\mathrm{C}_1$ and right: $\mathrm{C}_{1\mathrm{tot}}$ initialised in steady-state and responding to a step perturbation at time zero of $k_{\mathrm{off}_1}$ from its nominal value 1 to a value in the interval of 0.01 to 10. For $\mathrm{C}_1$, the state trajectories intersect, thus the response is not monotone. For $\mathrm{C}_{1\mathrm{tot}}$ the response is monotone.

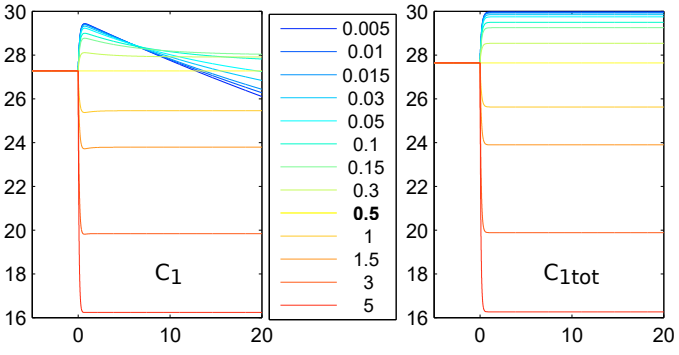


Figure 10. Response of the model of Section 5.2. Left: $\mathrm{C}_1$ and right: $\mathrm{C}_{1\mathrm{tot}}$ initialised in steady-state and responding to a step perturbation at time zero of $k_{\mathrm{off}_1}$, whose nominal value is 0.5. For $\mathrm{C}_1$, the state trajectories intersect, thus the response is not monotone. For $\mathrm{C}_{1\mathrm{tot}}$ the response is monotone.

## 6. CONCLUSIONS

The here presented analysis sheds light on the monotonicity property of ligand-receptor signalling network motifs such as the kinetic proofreading one. The novelty of this paper is a generic coordinate transformation that does not modify the last stage of the receptor complex, which is usually responsible for intracellular signalling. Monotonicity is proven for a class of signalling networks that encompasses those of kinetic proofreading. For the case without second messenger, sufficient conditions for monotonicity are inequality constraints on the parameters. With second messenger, two parametric equality constraints are required in addition to inequality ones. As shown in the applications examples, these equality constraints are satisfied in some published models.

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