

Approximations and their Consequences for Dynamic Modelling of Signal Transduction Pathways

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(Dated: August 14, 2006, accepted for publication in *Mathematical Biosciences*)

Signal transduction is the process by which the cell converts one kind of signal or stimulus into another. This involves a sequence of biochemical reactions, carried out by proteins. The dynamic response of complex cell signalling networks can be modelled and simulated in the framework of chemical kinetics. The mathematical formulation of chemical kinetics results in a system of coupled differential equations. Simplifications can arise through assumptions and approximations. The paper provides a critical discussion of frequently employed approximations in dynamic modelling of signal transduction pathways. We discuss the requirements for conservation laws, steady state approximations, and the neglect of components. We show how these approximations simplify the mathematical treatment of biochemical networks but we also demonstrate differences between the complete system and its approximations with respect to the transient and steady state behavior.

I. INTRODUCTION

The processing of information in living cells is carried out by signalling networks [1]. The character of information and the corresponding responses include a wide range of physical and chemical quantities, including changes in temperature, pressure, water balance, concentration gradients, pH-level. Within these networks information is carried by dynamic changes in protein concentrations.

While there exist considerable experience in mathematical modelling of metabolic systems [2–5], cell cycle [6, 7], cellular rhythms [8, 9] and transcriptional networks (see [10, 11] for recent surveys and [12] for a recent textbook), the mathematical analysis of signal transduction pathways is a younger field [13–19]. An important difference between modelling metabolic systems and signal transduction pathways is that in cell signalling one is primarily interested in the analysis of rapid responses to stimuli, oscillatory dynamics, and transient changes. A number of simplifying mathematical assumptions related to steady-state analysis are therefore not available in the analysis of signal transduction pathways.

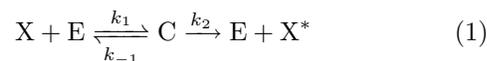
To allow a meaningful mathematical analysis, simplifications are of vital importance. It is only natural that one considers well established approximations for metabolic systems to be applicable in cell signalling. The present paper is to discuss a number of assumptions and approxi-

mations that are frequently used in dynamic modelling of signal transduction pathways. We show how these simplify the mathematical treatment but also illustrate what happens if they are not reasonable. We demonstrate that assumptions can lead to deceptive changes in the model's behavior. In cell signalling the choice and justification of assumptions is particularly important and relies on the experimental set-up, biological context and purpose of the model.

The outline of the paper is as follows. We begin with a description of the assumptions underlying our study, before introducing activation/deactivation modules which form the basic elements of cell signalling networks. In Sections III to VI, we introduce commonly used approximations and simplifications, including assumptions about stationarity assumptions, conservation and moiety laws, and the neglect of components. In Section VII, the application and consequences of assumptions and approximations are demonstrated for a MAPK cascade. This leads us to a discussion of simplifications in the description of feedback mechanisms.

II. ASSUMPTIONS OF THE STUDY

For the present paper we treat a signal transduction pathway as a network of coupled modules, as illustrated in Figure 1 [20, 21]. For each activation/deactivation cycle an enzyme kinetic reaction serves as a template:



Eq. (1) describes the modification of protein X into form X^* , facilitated by an enzyme E . During the modification of X an intermediate enzyme-substrate complex C is formed. The enzyme is considered to be an ideal catalyst, that is, the enzyme is released unchanged from the complex. If not stated otherwise, we assume that the en-

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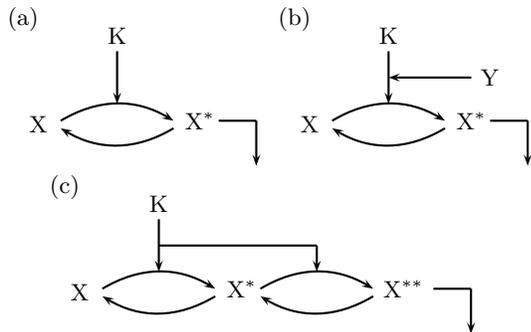


FIG. 1: Modules (of activation/deactivation cycles) of which signal transduction pathways are composed.

zyme in (1) is a kinase for activation and a phosphatase for deactivation.

A standard approach to the dynamics of cell signalling networks is the framework of chemical kinetics. Within this framework enzyme kinetic reaction (1) is represented as a system of four coupled ordinary differential equations [11, 22]

$$\frac{dX}{dt} = -k_1 EX + k_{-1} C, \quad (2)$$

$$\frac{dE}{dt} = -k_1 EX + (k_{-1} + k_2) C, \quad (3)$$

$$\frac{dC}{dt} = k_1 EX - (k_{-1} + k_2) C, \quad (4)$$

$$\frac{dX^*}{dt} = k_2 C. \quad (5)$$

Such a system of rate equations can be generalized to the following form [2]

$$\frac{dS_i}{dt} = \sum_{\mu=1}^m \nu_{\mu i} k_{\mu} \prod_j S_j^{l_{\mu j}}, \quad i = 1, \dots, n, \quad (6)$$

where $\nu_{\mu i}$ is the stoichiometric coefficient, which is positive for synthesis and negative for degradation, and $l_{\mu i}$ is the reaction order. The reaction rate is proportional to the concentration of reaction partners and rate coefficient k_{μ} .

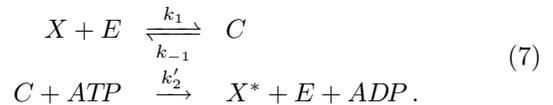
There is a no such thing as a hypothesis-free model and before we discuss various approximations that derive from the basic modification scheme (1), we ought to mention the assumptions that are implicitly made to this point. The approach has been originally derived from microscopic properties in statistical physics [23]. It links the rate of change of a component S_i to an average number of reactive collisions in a reaction volume. Eq. (6) therefore implies the assumption that a mean value is an appropriate description for changes in protein concentrations; random fluctuations (noise) are considered small in comparison to this mean [23]. In that respect, Eq. (6) describes ‘macroscopic’ changes of protein concentrations. A fundamental assumption of this approach is a homogeneous distribution of all components in the

reaction volume. Diffusion processes are assumed to be much faster than the chemical reaction made responsible for the signalling mechanism. We stay therefore in the realm of ordinary differential equations, not partial differential equations. Assuming that environmental variables (e.g. temperature and volume) do not change, k_{μ} is a reaction constant.

III. STATIONARY COMPONENTS

In case of (de)phosphorylations, the reaction schema (1) ignores the involvement of ATP. This simplification is made possibly by assuming that ATP is available either in great excess or can be supplied without significant changes in concentration. This assumption is frequently used for ATP in the activation cycles of Figure 1 [26].

We can illustrate this with a more detailed model



In a first reaction step, enzyme and protein X form a complex C, which is the target for the ATP molecule. The reaction between the complex and ATP activates the protein and release the unchanged enzyme E and ADP. The corresponding differential equation for the rate of change of the active protein is now

$$\frac{dX^*}{dt} = k'_2 ATP C.$$

If we assume a stationary ATP concentration we can introduce an effective or apparent rate constant

$$k_2 = k'_2 ATP$$

leading to a differential equation, equivalent to (5). In the same manner the other differential equations of reaction scheme (7) can be reduced to a formally equivalent system of (2)-(5). The effective rate constant k_2 depends on the ATP concentration. Experiments with different ATP levels will subsequently lead to a different dissociation constants k_2 . This underlines the importance for monitoring the concentration of stationary components. The identification of a parametric dependence of a rate coefficient on other species is an indication for a more complex reaction mechanism.

IV. CONSERVATION LAWS, MOIETY LAWS

A further simplification in the treatment of signalling pathways arises if some components obey conservation relations with respect to the number of molecules involved. Moving from molecule numbers to concentrations, so-called moiety laws are defined. For example, in case of the reaction scheme (1) we can assume

$$E^T = E(t) + C(t), \quad (8)$$

where E^T is the total enzyme concentration, composed of the free enzyme concentration $E(t)$ and the enzymes bound in the intermediate complex C . During the course of the reaction, the ratio of enzyme and enzyme-substrate complex is changing, but their sum remains constant over time. Conservation relation (8) allows us to replace $E(t)$ in other equations by $E^T - C(t)$ and thereby to reduce the overall number of differential equations. This idea is frequently used [13, 26–28].

A notable difference between metabolic networks and signalling pathways is the fact that the latter can often be considered a closed system, restricted by the cell membrane. In closed systems the conservation relation is an exact relation and has no consequences on the dynamic behavior. Even if the system as a whole is open, it can be closed with respect to some components.

V. QUASI-STEADY STATE APPROXIMATION

Within the modules of Figure 1 elementary reactions produce short-lived intermediates, like the enzyme-substrate complex in Eq. (1). This is then referred to as a pre-equilibrium [24], in which an intermediate is in equilibrium with the reactants. This is only possible if the rate of formation for an intermediate and its dissociation back into the reactants is much faster than its conversion into products [24]. For complex C this quasi-steady state assumption leads to a simplification in Eqs. (2)-(5)

$$\frac{dX^*}{dt} = -\frac{dX}{dt} = k_2 C(t) = k_2 \frac{X(t)E(t)}{K_M}. \quad (9)$$

The Michaelis-Menten scheme now formally follows a pseudo-bimolecular rate law. Such a reduction of the underlying mechanism is a characteristic property of the quasi-steady state approximation.

If we further use conservation relation (8) we obtain the well-known Michaelis-Menten equation [3, 27, 29, 30]

$$\frac{dX^*}{dt} = -\frac{dX}{dt} = \frac{V_{\max} X}{K_M + X}, \quad (10)$$

with the limiting rate $V_{\max} = k_2 E^T$ and Michaelis constant $K_M = (k_{-1} + k_2)/k_1$. The conditions for the application of the quasi-steady state assumption were generalized in [31, 32]. A generalization to reversible enzyme kinetics is given in [33].

Instead of the three rate coefficients k_1 , k_{-1} , and k_2 of complete model (2)-(5) the Michaelis-Menten equation (10) is parametrically dependent on two new coefficients, which can often be measured in experiments. Because the original rate coefficients cannot be uniquely determined from the limiting rate V_{\max} and Michaelis constant K_M , the quasi-steady state assumption leads to a loss of information about the reaction mechanism. Note, that the rational form of the Michaelis-Menten equation is a consequence of (8).

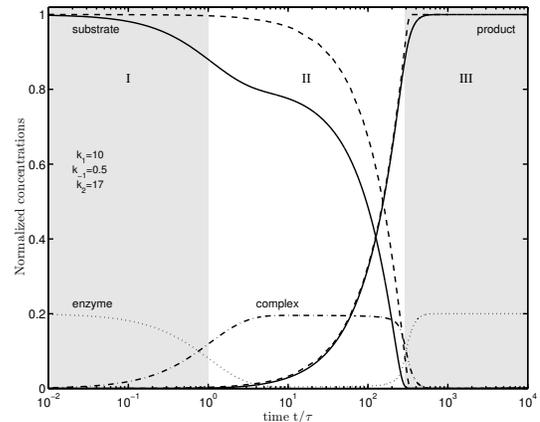


FIG. 2: Comparison of the integrated Michaelis-Menten equation (10) (dashed) and the numerical solution of the system of coupled ODE's (2)-(5) in a semilogarithmic representation. In addition to the evolution of protein X and its modified form (solid lines) proteins, the enzyme and complex concentrations are shown as a function of time. The concentrations are normalized to the initial protein concentration X_0 . The time axis is scaled with the characteristic time τ (11). The dynamics are separated into three parts: I - initial period, II - period of quasi-steady states, III - final stage.

In Figure 2, we compare the solution for the full system of coupled differential equations with its approximate description (10). In order to illustrate all three phases of the reaction we choose a normalized logarithmic time scale. As the scale factor we use the characteristic time needed to establish the quasi-steady state [34]

$$\tau = (k_1 X_0 + k_{-1} + k_2)^{-1}. \quad (11)$$

Apart from dissociation constants k_{-1} and k_2 , τ depends on the formation of complex C described by the association rate k_1 and the initial substrate concentration X_0 . During this initial period the Michaelis-Menten equation deviates from a detailed model, especially for the temporal evolution of the substrate. During phase (II), $1 < t/\tau < 300$, the enzyme complex is saturated due to the limited amount of enzyme. This is the region, where the quasi-steady state assumption is valid. Due to the ratio of enzyme and substrate in Figure 2, the temporal evolution of the substrate is not well described by the Michaelis-Menten equation, although in period (II) a quasi-steady state of the enzyme-substrate complex C is established. If the ratio becomes small ($X_0 \gg E_0$), deviations occurring from the initial period can be neglected.

Towards the end, in phase (III), the enzyme complex is degrading because of the lack of substrate leading to a decrease of the formation rate. The assumption of an equilibrium between complex and reactants is no longer valid and the two models differ in their transient behavior.

Note also that the enzyme kinetic reaction reaches a period during which the enzyme complex is saturated and shows no net change. However, this is not a necessary

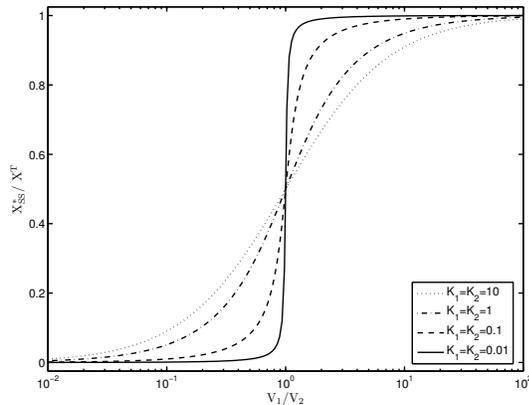


FIG. 3: The Goldbeter-Koshland function $G(V_1, V_2, K_1, K_2)$ (16) as function of the ratio of limiting rates V_1 and V_2 . The normalized steady state concentration X^*/X^T is plotted for the symmetrical modification cycle ($K_1 = K_2$). Depending on the values of the Michaelis constants, the Goldbeter-Koshland function can show sigmoidal or ultrasensitive behavior.

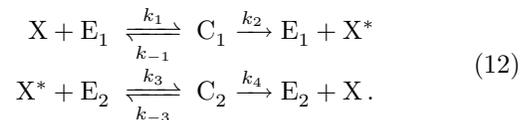
condition for the approximation of a quasi-steady state.

The Michaelis-Menten equation (10) and the enzyme kinetic reaction Eqs. (2)-(5) have a similar long-term behavior, but they deviate in the short-term behavior. Thus, the underlying quasi-steady state approximation may be applicable in metabolism, but can be problematic in signalling, where we are interested in the description of fast transient changes beyond the steady state. We are going to further discuss Michaelis-Menten modelling below. For a discussion of this modelling approach with applications in cell signalling see [36].

VI. NEGLECTING COMPONENTS

In order to simplify the mathematical treatment of (de)activation cycles (Figure 1), one often uses a combi-

nation of the previous discussed approximations. First, one can decompose the modification and the reverse process into two separate Michaelis-Menten-type reactions, where we assume, that additional participants are constant (See Section III) and obtain the reaction scheme



With the conservation laws for the kinase E_1 and the phosphatase E_2 , analogous to (8), for the protein

$$X^T = X(t) + X^*(t) + C_1(t) + C_2(t), \quad (13)$$

and the quasi-steady state approximation for the formed complexes C_1 and C_2 we obtain an expression which involves the complex concentrations. If we assume, that the concentration of the complexes C_1 and C_2 are much smaller than the concentration of the modified protein X^* and its original form X

$$X + X^* \gg C_1 + C_2. \quad (14)$$

we can neglect their contributions in conservation law (13). Because the maximal concentration of complexes is determined by the enzyme, from (14) the enzyme concentration must be small. We thus obtain the following rate equation [26]

$$\frac{dX^*/X^T}{dt} = \frac{1}{X^T} \left[\frac{V_1(1 - X^*/X^T)}{K_1 + 1 - X^*/X^T} - \frac{V_2 X^*/X^T}{K_2 + X^*/X^T} \right] \quad (15)$$

with the limiting rates $V_1 = k_2 E_1^T$ and $V_2 = k_4 E_2^T$ and the dimensionless Michaelis constants $K_1 = (k_{-1} + k_2)/(k_1 X^T)$ and $K_2 = (k_{-3} + k_4)/(k_3 X^T)$. The corresponding steady state is described by the Goldbeter-Koshland function [26]

$$G(V_1, V_2, K_1, K_2) = \frac{X_{ss}^*}{X^T} = \frac{V_1(1 - K_2) - V_2(1 + K_1) + \sqrt{[V_1(1 - K_2) - V_2(1 + K_1)]^2 + 4V_1K_2(V_1 - V_2)}}{2(V_1 - V_2)}, \quad (16)$$

having a characteristic sigmoidal shape as a function of the ratio of the limiting rates, see Figure 3. Roughly speaking, the ratio of kinase to phosphatase defines a switch-like behavior which depends on the Michaelis constants. The steady state concentration of X^* increases abruptly from a low (nearly zero) concentration to a high level (nearly X^T). Such behavior is called ‘ultrasensitivity’ [26, 28]. Apart from the described covalent mechanism, referred to as zero-order ultrasensitivity, other mechanisms leading to ultrasensitive behavior have been

described [27, 29, 34]. As noted in [26], the consideration of a non-negligible complex concentration moves the steady state concentration of both protein forms to lower concentrations. As a consequence, the activity X^*/X^T lies now in the range of $0 \leq A \leq A_{\max} < 1$. In contrast, Eq. (16) has a range of $[0, 1]$. This behavior is shown in Figure 4, illustrating the parametric dependencies on the ratio of enzyme and protein concentration. Whereas the dynamic behavior remains qualitatively unaffected, only the relaxation time increases. On the other hand, the

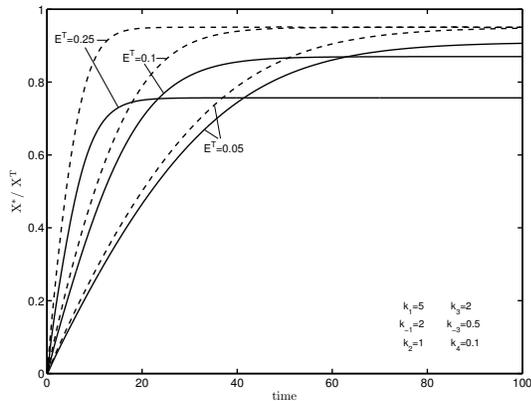


FIG. 4: Temporal evolution of modified protein X^* to its steady state for different enzyme concentrations. The solid lines are the solution for finite enzyme concentrations and the dashed lines the corresponding solution of (16).

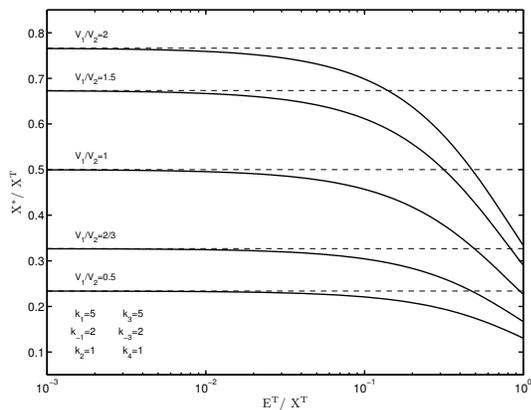


FIG. 5: Steady state concentration of modified protein X^* as function of the ratio of catalyzing enzyme concentration and total protein concentration X^T for different ratios of the limiting rates V_1 and V_2 . For small ratios the enzyme-substrate complex can be neglected in the protein balance and the steady state tends asymptotically to result of Goldbeter-Koshland [26] (dashed lines).

steady state for the modified form deviates from Eq. (16). The differences in the steady state values increases with the ratio of enzyme and protein concentration, as one could expect. Additionally, a further increase comes with the ratio of the limiting rates V_1/V_2 , as demonstrated in Figure 5.

Whereas assumption (14) holds true for most *in-vitro* enzyme reactions, it often fails in phosphorylation/dephosphorylation reactions in signalling pathways. In signal transduction the concentration of substrate, kinase and phosphatase can be comparable [15, 37, 38]. As a consequence of the decreased steady state, the sigmoidal shape is stretched and thereby the ultrasensitivity. With an increased enzyme concentration, the switch-like ultrasensitive behavior can disappear [36].

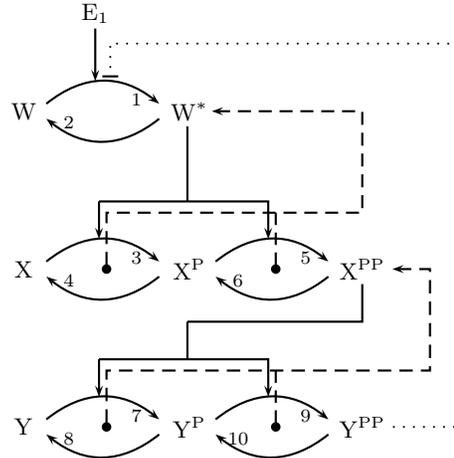


FIG. 6: Schematic representation of the basic MAPK cascade. The activated protein of the previous level acts as a kinase of the subsequent one. The dashed lines denote an implicit feedback from the upstream level back to their precursor through the released activated proteins if the complexes dissociate. A possible negative feedback loop (e.g. [28]) is denoted by a dotted line. There is a specialized phosphatase for each protein. As for the phosphorylation we assume for the dephosphorylation that the kinetic parameters are independent on the status of protein activation (non-, single- or double-phosphorylated). Each (de)activation step i is determined by the rate constants a_i describing the formation of complexes, d_i describing the reverse reaction, and k_i describing the dissociation into product and enzyme.

VII. SIGNALLING CASCADES

An example for a sequence of (de)activation cycles is the MAPK cascade [20] (Figure 6). MAPK cascades have been modelled by various authors [13–17, 28, 37–40, 44]. We will show in this section that these models result from different levels of approximation within a common fundamental reaction scheme. Considering some components of the fundamental model we will discuss a step-by-step reduction of the model and its consequences on dynamics. The starting point is the mechanistic model of Huang & Ferrell [13] which was also used in [14, 15, 39, 40]. Note, that the first assumption in all cited papers was that the concentrations of additional participants (ATP, H_2O , ...) are constant. As described in Section III, we can then use the enzyme kinetic reaction as a template for the (de)phosphorylation processes in the cascade. A model reduction occurs if we use the conservation relations for the phosphatases and the kinase E_1 [13] and for the proteins which contain the different protein forms and all related complexes. In [28] these complexes are neglected.

However, the conservation of phosphatases of proteins X and Y do not follow the conservation relation (8). Instead of we have

$$E^T = E(t) + C^P(t) + C^{PP}(t), \quad (17)$$

where the superscripts denote the complex of phos-

phatase and the different phosphorylated protein forms.

In contrast to the single activation/deactivation cycle, the rate equations of the cascade proteins are more complex as shown in Eq. (18) for the double phosphorylated protein X^{PP} . The active protein W^* acts as a kinase and the enzyme E_4 is the corresponding phosphatase. The formation of complexes is proportional to the rate constants a_i , the reverse reaction to d_i , and the dissociation into product and enzyme to the constants k_i . The underlined terms in (18) correspond to the ‘implicit feedback’, which describes the freed kinases in the modification process. Due to this ‘implicit feedback’ downstream activation steps also influence the dynamics of the previous steps. In Figure 6 this is shown with dashed lines.

$$\frac{dX^{PP}}{dt} = k_5 C_{W^*}^{X^P} - [a_6 E_4 + a_7 Y + a_9 Y^P] X^{PP} \quad (18)$$

$$+ \underline{d_6 C_{E_4}^{X^{PP}}} + \underline{[d_7 + k_7] C_Y^{X^{PP}}} + \underline{[d_9 + k_9] C_{Y^P}^{X^{PP}}},$$

where C denotes the formed complex with the protein as superscript with kinase. Besides the ‘implicit feedback’, see also [41], the enzyme involved in the (de)activation leads to an ‘implicit inhibition’ [42, 43]. For instance, the single-phosphorylated protein X^P inhibits the phosphorylation of X and X inhibits the phosphorylation of X^P through the competition for W^* . An analogous inhibition mechanism exists for the dephosphorylation of the proteins in the cascade.

A further simplification arises if one uses the quasi-steady state approximation for the formed complexes. The system of 30 coupled differential equations can then be reduced to eight equations involving only the proteins. Not only the number of equations is reduced, the structure of the equations is simplified as well. For instance rate equation (18) reduces to

$$\frac{dX^{PP}}{dt} = k_5 C_{W^*}^{X^P} - k_6 C_{E_4}^{X^{PP}}, \quad (19)$$

where the rate is now determined by the slowest step of phosphorylation and dephosphorylation. From the discussion in Section V, these slowest steps have to be the dissociation of the formed enzyme-substrate complex. As shown in (9), Eq. (19) can also be expressed in terms of pseudo-bimolecular reactions, leading to the representation of Heinrich et al. [17]. A comparison of Eqs. (18) and (19) shows that the reduced rate equation does not contain any implicit feedback. Therefore the dynamics of both representations will differ if the implicit feedback becomes important (i.e. whenever the steady state assumption is not satisfied).

Due to conservation relations, Eq. (19) can be transformed into rational expressions of the rate laws. On the other hand, the phosphatases of proteins X and Y support conservation relation (17), which does not allow an analogue transformation as discussed in Section V. In order to further simplify the rate laws of these phosphatases

we assume

$$E^T + C^P(t) \gg C^{PP}(t) \text{ single phosphorylated,}$$

$$E^T + C^{PP}(t) \gg C^P(t) \text{ double phosphorylated.}$$

These conditions can be fulfilled simultaneously only if

$$E^T \gg C^P(t) + C^{PP}(t) \quad (20)$$

for the phosphatases. In contrast to the treatment of the enzyme kinetic reaction, the phosphatases are not saturated during the quasi-steady state phase. The quasi-steady state expression of the protein-protein complexes are obtained if we assume that the complex concentrations are negligible. Only the current complex remains in the expression. After some transformation we obtain Goldbeter-Koshland like rate laws for the proteins in the cascade. For example, we have for the double phosphorylated signalling protein X^{PP}

$$\frac{dX^{PP}}{dt} = \frac{k_5 X^P W^*}{K_{M5} + X^P} - \frac{k_6 E_4^T X^{PP}}{K_{M6} + X^{PP}},$$

where $K_{M5,6}$ are the corresponding Michaelis constants. Note, that we substitute the conservation law term in the above equation (see for example the dynamic Goldbeter-Koshland function (15)). Such a representation was used for instance in [28, 44].

In the course of the derivation we assume that all complexes are simultaneously in a quasi-steady state. But due to the consecutive nature of the cascade there are delays between the different complexes. Furthermore we neglect the contributions of the complex concentrations to the dynamics and more important to the steady state concentrations of the proteins. As discussed in the previous section, one has to assume that the protein concentration of each consecutive activation cycle is much greater than in the previous step. From Figure 5 we can estimate that a magnitude of at least hundred is needed. For a three step cascade this means a concentration 10^4 -higher than the protein concentration in the first cycle which is not common in signalling pathways [15, 37, 38]. Due to these assumptions the mechanistic and the Goldbeter-Koshland-like representation will differ in the dynamic and the steady state behavior. In a recent investigation of a MAPK-cascade model with negative feedback, see Figure 6, the authors of [36] have shown that in a more detailed representation the oscillations predicted in [28] do not emerge. The reason is the overestimated ultrasensitive behavior of the subsequent steps due to the neglect of the complex concentrations. At least for the chosen set of parameters, both models differ even though they use the same parameters. In other words, parameter estimation of a given model strongly depends on the model structure. If we compare the Michaelis constant derived from known rate constants of a mechanistic model and the Michaelis constant derived from a Goldbeter-Koshland model they may differ for the same set of experimental data. Due to the underlying assumptions in both models the parameters are not comparable.

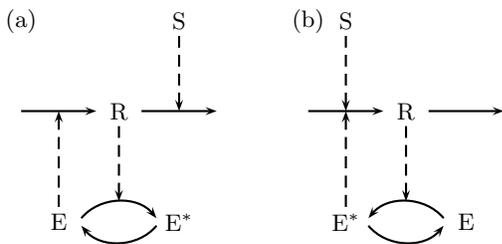


FIG. 7: Diagrammatic representation of a homoeostatic system (a) and a mutually activated system (b) according to [45].

Furthermore, the assumption of a quasi-steady state for the intermediate complexes is crucial. If substrate concentration and the modifying enzyme concentration are similar, a small rate coefficient k_2 is the consequence. Furthermore an enzyme saturation is, either not reached or only for a short time.

VIII. QUASI-STATIONARY STATES IN FEEDBACK MECHANISMS

The behavior of signalling networks is regulated and controlled by positive and/or negative feedback. It is often assumed that the reactions involved in the feedback loops are much faster than the regulated branch of the network (e.g. in the cell cycle [6, 7]). In this case, the component feeding back can be treated as a steady state variable. The regulated component is assumed as approximately constant, or quasi-stationary, during the period needed to establish the steady state of the feedback loop. The changes in the dynamics of a system due to the applied quasi-stationary approximation shall be discussed in this section. We consider simple models consisting of a linear reaction scheme, which is regulated in an autocatalytic manner through an enzyme having an active and an inactive form.

For the first example, we use a minimal model for a homoeostatic system, shown in Figure 7. The signalling component R is synthesized in a catalytic reaction and degraded in a bimolecular reaction with an external constant stimulus S

$$\frac{dR}{dt} = k_0 E - k_2 SR. \quad (21)$$

The enzyme dynamics follow the modification scheme (12). In Figure 8 we compare the dynamical treatment

$$\frac{dE}{dt} = \frac{k_3(1-E)}{K_{M3} + 1 - E} - \frac{k_4 ER}{K_{M4} + E} \quad (22)$$

with the quasi-stationary model published in [45], where the enzyme E is assumed in its steady state

$$E(R) = G(k_3, k_4 R, K_{M3}, K_{M4}). \quad (23)$$

Due to the negative feedback, the system can display damped oscillations. The dynamic description of the

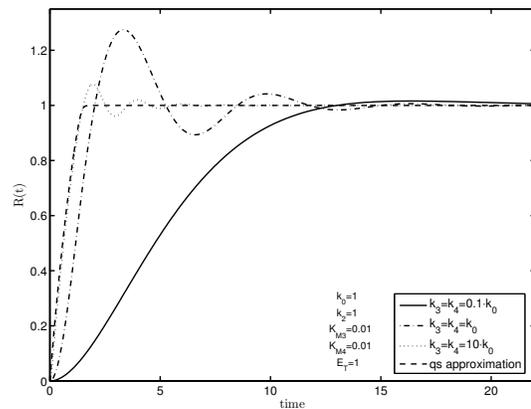


FIG. 8: Temporal evolution of the homoeostatic model [45] for the quasi-stationary approximation of enzyme activation (23) and the detailed model (22). Due to a negative feedback loop, the detailed model shows damped oscillations, which are dependent on the ratio of the rate coefficients. Only, if the enzyme activation cycle reaches its equilibrium rapidly, both models agree. For a very slow relaxation, the curve shows aperiodic behavior and takes much longer to equilibrate as is predicted by the quasi-stationary model.

feedback loop leads to a qualitatively different dynamic behavior than the model using the quasi-stationary approximation, which cannot show oscillations. Note that the steady state is unaffected because the individual steady states of the subsystems remain unaffected. In a more complex signalling network this can lead to a qualitatively new behavior of the system as a whole. For example, damped oscillations may occur or thresholds (bifurcation points) may be passed. The faster the enzyme activation in comparison to the synthesis of the signalling component R, the smaller the deviations between a detailed and the quasi-stationary model.

In addition to a change in the dynamics of a signal, the quasi-stationary state assumption can affect other characteristic properties of biochemical networks. As an example, we consider the model of a one-way switch (Figure 7(b)) discussed in [45]. The behavior of this system is linked to biological processes for which a fundamental, possibly irreversible decisions between two states are made. This is, for instance, the case in cell differentiation and in developmental processes [46–48]. The synthesis of the signalling component R

$$\frac{dR}{dt} = k_0 E^* + k_1 S - k_2 R \quad (24)$$

is increased by the modified enzyme E*. Enzyme modification is controlled by the signalling component R itself. Again, we compare the dynamic model

$$\frac{dE^*}{dt} = \frac{k_3(1-E^*)R}{K_{M3} + 1 - E^*} - \frac{k_4 E^*}{K_{M4} + E^*} \quad (25)$$

with the quasi-stationary approximation

$$E^*(R) = G(k_3 R, k_4, K_{M3}, K_{M4}). \quad (26)$$

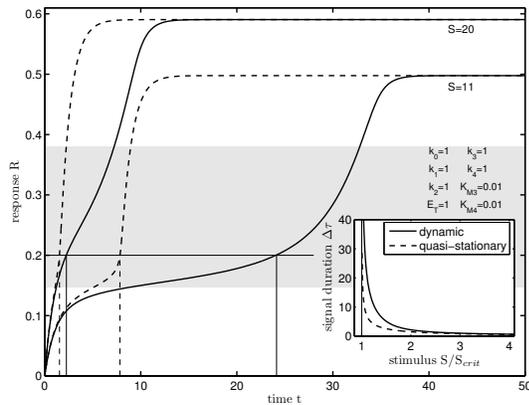


FIG. 9: Relaxation of the one-way switch (24) to steady state for two different, constant supercritical stimuli in quasi-stationary approximation (dashed) and in dynamic treatment (solid). The horizontal line marks the concentration which has to be exceeded for the system to reach to the upper stable branch after the external stimulus is switched off. In the grey region no stable steady state solution exists. The inset shows the critical signal duration time $\Delta\tau$ as function of the stimulus for both approximations.

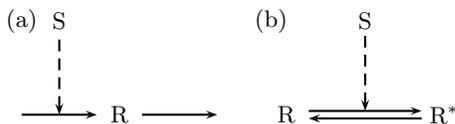


FIG. 10: Diagrammatic representation of a linear (a) and a hyperbolic system (b) [45].

Whereas both models show a similar dynamic behavior (see Figure 9), some characteristic times differ. An important time for a bistable system is the signal duration $\Delta\tau$ needed to reach the upper stable branch, if the supercritical external stimulus is switched off after $\Delta\tau$. This characteristic time is compared in the inset of Figure 9. In the quasi-stationary approximation the steady state is reached much faster than in a dynamical model. Especially for small supercritical signals the signal duration deviates from each other.

Note, in Figure 8 we vary the rate coefficients determining the subsystems; a more general measure for this purpose is the relaxation time τ . This characteristic time is a measure for the time the biochemical reaction needs to relax from a perturbed state to its steady state. Especially for complex signalling networks this is not only dependent on the rate coefficients k_μ , but also on the concentrations of other participating species. For instance, the relaxation time of a simple linear system (Figure 10(a)) is

$$\tau_{\text{lin}} = k_2^{-1}, \quad (27)$$

that is, the inverse of the degradation rate constant k_2 . If we consider a hyperbolic system (Figure 10(b)), the

relaxation time is

$$\tau_{\text{hyb}} = (k_2 + k_1 S)^{-1}, \quad (28)$$

where S is the external stimulus. A change in the concentration thus varies the ratios of the relaxation times. Unfortunately, the estimation of characteristic times is exceedingly difficult if no analytical expressions are known [49].

A more detailed description of feedback loops can change the dynamic behavior of the system and its characteristic parameters in comparison to a quasi-stationary approximation, whereas the steady state is unchanged. The approximation of a quasi-stationary state may apply if one subsystem settles into a steady state much faster than the other. One must however be aware that the ratio of the relaxation times depends on the current conditions of the considered system. For the dynamic modelling of signalling networks this approximation has to be validated for each model.

IX. DISCUSSION

The framework of chemical kinetics is a frequently used approach to dynamic modelling of complex signalling networks. The resulting systems of coupled ordinary nonlinear differential equations can usually only be solved numerically. To reduce the complexity of an analysis, one uses exact relations, like conservation relations, and different approximations like steady-state assumptions or one neglects components. Such a reduction simplifies the mathematical dimension of biochemical networks and makes the model amenable to the estimation of parameter values from experimental data. The downside is that an approximation can misguide the analysis of the real dynamical behavior of the pathway.

As shown in Section V, the time until reaching a steady state can bring about further differences between the predicted time course and the one observed in experiments. A similar conclusion can be drawn if a quasi-stationary state approximation is used, e.g. for a feedback loop. The altered behavior of this single subunit of the model can change the behavior of the system as a whole. It can happen that this loop induces oscillations into the network or the unstable region is shifted to other external stimuli.

Furthermore a comparison of model parameters is difficult due to the uncertainty in determining rate coefficients from Michaelis constants. Due to the possible changes in the dynamic behavior, the set of parameters of a detailed model must be derived from experimental data.

As shown, steady-state approximations can lead to a reduction of the dynamical system but since the complete system takes a finite time to establish the assumed state, the complete system and its approximation will differ during a transient period. This delay modifies the dynamics of a pathway dramatically. In particular, for

the treatment of feedback loops one has to take care using these approximations. Small changes in the dynamics of the loop can change the overall behavior substantially. Common for the steady-state approximations are, that they do not influence the steady state value. In contrast, the neglect of the finite concentration of intermediates, influences the steady-state values and leads to a weakening or disappearance of ultrasensitive characteristics. In signalling cascades consisting of a combination of activation/deactivation cycles, even small changes in the ultrasensitivity can change the overall behavior. Furthermore, pathways are often regulated by multiple feedback loops. Hence, the combination of steady-state assumptions and neglect of components may result in very different dynamics.

As shown in Section VI, each activation cycle can show ultrasensitive behavior depending on the associated Michaelis constants and the ratio of the ‘substrate’ protein and the kinase. A weakening or disappearance of ultrasensitivity, due to finite complex concentrations in the cycles, can change the behavior of the whole cascade qualitatively. For the same set of parameters (Michaelis constants and limiting rates) the amplification, possible oscillations or multistability can change dramatically. Notice, one ultrasensitive step in the cascade is enough

to create such nonlinear behavior.

Whereas steady state properties are more robust against approximations, the dynamic behavior is sensitive to the approximations used. Hence, if transient, dynamic behavior is most relevant, (e.g. in cell signalling) some frequently used approximations have to be justified.

Finally, what we have shown is that dynamic pathway modelling requires careful considerations with regard to the role of mathematical models in explaining observations, hypothesizing phenomena and supporting the design of experiments: Systems biology is the *art* of making appropriate assumptions.

Acknowledgments

T.M. and O.W. acknowledge the support of the German Federal Ministry of Education and Research (BMBF) grant 01GR0475 as part of the National Genome Research Network (NGFN). Parts of this work were supported by an EU FP6 grant LSHG-CT-2004-512060 within the project Computational Systems Biology of Cell Signalling(COSBICS). The responsibility for the content lies with the authors.

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