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Stability analysis of the Michaelis–Menten approximation of a mixed mechanism of a phosphorylation system

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ABSTRACT

In this paper, we consider a mixed mechanism of a n-site phosphorylation system in which the mechanism of phosphorylation is distributive and that of dephosphorylation is processive. It is assumed that the concentrations of the substrates are much higher than those of the enzymes and their intermediate complexes. This assumption enables us to reduce the system using the steady-state approach to a Michaelis–Menten approximation of the system. It is proved that the resulting system of nonlinear ordinary differential equations admits a unique positive equilibrium in every positive stoichiometric compatibility class using the theory of quadratic equations. We then consider two special cases. In the first case, we assume that the Michaelis constants associated with the different substrates in the phosphorylation reactions are equal and construct a Lyapunov function to prove asymptotic stability of the system. In the second case, we assume that there are just two sites of phosphorylation and dephoshorylation and prove that the resulting system is asymptotically stable using Poincaré Bendixson theorem.

1. Introduction

Multisite phosphorylation systems are intracellular futile cycles in which one enzyme catalyzes the attachment of phosphate groups onto a protein at multiple sites and another enzyme detaches the phosphate groups from the protein. Such futile cycles play a vital role in biological processes like cellular signalling and cell cycle control and consequently their dynamical properties are of great interest. For a detailed exposition on phosphorylation systems, the reader is referred to [1].

There are primarily two mechanisms of multisite phosphorylation, namely, *distributive* and *processive* mechanisms. We illustrate these two mechanisms for a 2-site phosphorylation system. Given below is the schematic for distributive mechanism of a 2-site phosphorylation system with S_0 denoting the protein substrate, S_1 and S_2, the phosphorylated proteins, E and F the enzymes catalyzing the phosphorylation and dephosphorylation respectively, and C_1, C_2, D_1 and D_2 denoting the enzyme complex intermediates.

$$\begin{aligned} S_0 + E &\rightleftharpoons C_1 \to S_1 + E \rightleftharpoons C_2 \to S_2 + E \\ S_2 + F &\rightleftharpoons D_1 \to S_1 + F \rightleftharpoons D_2 \to S_0 + F \end{aligned}$$

Likewise the schematic for processive mechanism of a 2-site phosphorylation system is given below.

$$\begin{aligned} S_0 + E &\rightleftharpoons C_1 \to C_2 \to S_2 + E \\ S_2 + F &\rightleftharpoons D_1 \to D_2 \to S_0 + F \end{aligned}$$

Recently there has been some research on the dynamical properties of the two mechanisms of phosphorylation systems. It has been shown [2,3] that a distributive multisite phoshorylation mechanism can exhibit the property of multistationarity if there are at least two sites of phosphorylation, meaning that there can be more than one equilibrium corresponding to any given set of initial conditions of the system. Distributive mechanisms are also found to exhibit *bistability* [4–6]. For processive mechanisms, it has been shown [7] that there is a unique equilibrium corresponding to a given set of initial conditions and this equilibrium is globally asymptotically stable. This result has recently been generalized [8,9] for the case of processive mechanisms where the protein substrate is modified by more than two enzymes.

In this paper, we consider a mixed mechanism of a *n*-site phosphorylation system where the mechanism of phosphorylation is distributive and that of dephosphorylation is processive. The dynamics of such mechanisms have been studied recently in [10]. The authors of [10] have shown that such a mechanism admits a unique equilibrium corresponding to any given set of initial conditions. Furthermore, they have shown that such a mechanism exhibits sustained oscillations under certain conditions.

In this paper, we consider a Michaelis Menten approximation of a

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mixed mechanism of a n-site phosphorylation system and show using analytical methods that such a mechanism admits a unique equilibrium corresponding to a given set of initial conditions. In order to derive the Michaelis Menten approximation of a mixed mechanism of n-site phosphorylation system, we make use of the steady state approach [11]. The Michaelis–Menten approximation of the mechanism is derived under the assumption that the concentrations of the substrate and phosphorylated proteins are much higher than those of the enzymes and their intermediate complexes and therefore the results obtained in this paper are valid under those assumptions.

We then consider two special cases of the mechanism and prove asymptotic stability in both the cases. In the first case, we assume that the Michaelis constants associated with the different substrates in the phosphorylation reactions in the mechanism are equal and then construct a Lyapunov function in order to prove the asymptotic stability of such a system. In the second case, we assume that there are just two sites of phosphorylation and dephosphorylation and prove that the resulting system is asymptotically stable using Poincaré Bendixson theorem.

2. Preliminaries

In this section, we provide some preliminaries that are required to understand the results presented in this paper. We begin with the law of mass action kinetics for chemical reactions.

2.1. Law of mass action kinetics

The law of mass action kinetics is the most common rate governing law of chemical reactions. According to this law, the rate of a reaction is proportional to the concentrations of the different species on the substrate side of the reaction. We now describe this law with the help of an example of a reversible reaction. Consider the reaction

$$X_1 + X_2 \underset{k_r}{\overset{k_f}{\rightleftharpoons}} X_3 \tag{1}$$

In the reaction above, k_f and k_r are positive constants known as the forward and the reverse rate constants. Let x_i denote the concentration of X_i for $i = 1, 2, 3$. The mass action reaction rate of the forward reaction is $k_f x_1 x_2$, and the rate of the reverse reaction is $k_r x_3$. Therefore the overall reaction rate in the forward direction of the reversible reaction (1) is $r = k_f x_1 x_2 - k_r x_3$. In this case, the rates of change of concentrations of the different species of the reaction are given by

$$\dot{x}_1 = \dot{x}_2 = -\dot{x}_3 = -r$$

where $\dot{x} := \frac{dx}{dt}$.

2.2. Michaelis–Menten approximation and the steady state approach

Consider the following simple reaction mechanism in which an enzyme E catalyzes the formation of a product P from a substrate S by involving an intermediate complex C.

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \overset{k_2}{\rightarrow} P + E \tag{2}$$

Let e, s, c, p respectively denote the concentrations of E, S, C, P at any instant of time. Applying the law of mass action kinetics, we have

$$\begin{aligned} \dot{c} &= -\dot{e} = k_1 es - (k_{-1} + k_2)c \\ \dot{s} &= -k_1 es + k_{-1} c \\ \dot{p} &= k_2 c \end{aligned}$$

Since $\frac{d}{dt}(e + c) = 0$, we have

$$e + c = e_t \tag{3}$$

where e_t is a constant. The *Michaelis–Menten approximation* for the dynamics of the above reaction mechanism is obtained by assuming that after an initial short transient, the system reaches a steady state at which the rate of formation of the intermediate complex C is equal to the rate at which it is consumed. Therefore at steady state, we have

$$\dot{c} = k_1 es - (k_{-1} + k_2)c = 0 \tag{4}$$

This assumption is called the *steady state assumption*. Substituting for e from (3) in Eq. (4), we get

$$c = \frac{e_t s}{s + K_m}$$

where $K_m := \frac{k_{-1} + k_2}{k_1}$ is called the *Michaelis constant* associated with the substrate S and

$$\dot{p} = k_2 c = \frac{V_m s}{s + K_m} \tag{5}$$

where $V_m := k_2 e_t$ is the maximum possible value of $\dot{p}$ (at infinite s). Eq. (5) is called the Michaelis–Menten approximation for the dynamics of the reaction mechanism (2) and the approach described above for deriving the equation is called the *steady state approach*.

Let e_0, c_0 and s_0 denote the initial concentrations of E, S and C in the reaction mechanism (2). It can be shown using *singular perturbation analysis* (see e.g. [12]) that the Michaelis–Menten approximation (5) is valid when the initial concentration of the substrate is much higher than those of the enzyme E and the intermediate complex C, i.e., when

$$\frac{e_0}{s_0} \ll 1 \quad \text{and} \quad \frac{c_0}{s_0} \ll 1. \tag{6}$$

However, Lee Segel [13] proved that this is not a necessary condition for the validity of Eq. (5). In [13], Lee Segel proved that if $c_0 = 0$, then (5) is valid whenever

$$\frac{e_0}{K_m + s_0} \ll 1. \tag{7}$$

The above condition for the validity of Eq. (5) was derived using the *reactant stationary approximation* (RSA), where it is assumed that during the initial short transient before the steady state, there is a negligible depletion of the substrate concentration s. For the case when $c_0 > 0$, using RSA, the authors of [14] proved that Eq. (5) is valid whenever

$$\left| \frac{-e_0 + \frac{k_{-1} c_0}{k_1 s_0}}{K_m + s_0} \right| \ll 1. \tag{8}$$

Notice that while (6) implies (7) and (8), the converse is not true. For the mixed mechanism phosphorylation network considered in this paper, we will not use RSA to derive the exact condition for the validity of the Michaelis Menten approximation of the system. We will instead assume that the concentrations of the substrates are much higher than those of the enzymes and their complexes. It can be easily proved using the singular perturbation method that the Michaelis Menten approximation is always valid under this condition.

2.3. Some mathematical preliminaries

In proving the results presented in this paper, we make use of the following well known results.

Lemma 1 (*Jensen's inequality*). *Given a real strictly convex function ϕ, x_i in the domain of ϕ and $\sigma_i > 0$ for $i = 1, \ldots, n$, the following inequality holds.*

$$\frac{\sum_{i=1}^n \sigma_i \phi(x_i)}{\sum_{i=1}^n \sigma_i} \geq \phi\left(\frac{\sum_{i=1}^n \sigma_i x_i}{\sum_{i=1}^n \sigma_i} \right)$$

Equality holds in the above only if $x_1 = x_2 = \ldots = x_n$.

Theorem 2 (*Bendixson's criterion*). *If on a simply connected region $D \subset \mathbb{R}^2$, the expression $\frac{\partial f}{\partial x} + \frac{\partial g}{\partial y}$ is not identically equal to zero and does not change sign, then the system*

$$\dot{x} = f(x, y)$$
$$\dot{y} = g(x, y)$$

has no periodic orbits lying entirely in D.

Theorem 3 (*Poincaré Bendixson Theorem*). *Given a system of ordinary differential equations* $\frac{d\mathbf{x}}{dt} = \mathbf{F}(\mathbf{x})$, *where* $\mathbf{x}$ *is two dimensional, let* $\mathbf{x}(t)$ *represent a solution trajectory of the system which is bounded. Then either* $\mathbf{x}(t)$ *converges as* $t \longrightarrow \infty$ *to an equilibrium point of the system, or it converges to a single periodic cycle.*

The next result is a property of convex functions which is frequently used in chemical reaction network theory (see for e.g., [15, p. 334], [16, p. 2410]).

Lemma 4. *Given a real strictly convex function* ϕ, *define* $\Phi(x) := \phi'(x) = \frac{d}{dx}\phi(x)$. *Then for two numbers* γ_i *and* γ_j *in the domain of* ϕ,

$$(\gamma_i - \gamma_j)\Phi(\gamma_j) \leq \phi(\gamma_i) - \phi(\gamma_j) \tag{9}$$

with equality holding only if $\gamma_i = \gamma_j$.

Proof. If $\gamma_i = \gamma_j$, then (9) holds with an equality sign. Assume that $\gamma_i \neq \gamma_j$. We consider two cases.

Case 1: $\gamma_i > \gamma_j$. By mean value theorem and strict convexity of ϕ, there exists a number $c \in (\gamma_j, \gamma_i)$ such that

$$\Phi(c) = \frac{\phi(\gamma_i) - \phi(\gamma_j)}{\gamma_i - \gamma_j} > \Phi(\gamma_j)$$

Hence

$$(\gamma_i - \gamma_j)\Phi(\gamma_j) < \phi(\gamma_i) - \phi(\gamma_j).$$

Case 2: $\gamma_i < \gamma_j$. By mean value theorem and strict convexity of ϕ, there exists a number $c \in (\gamma_i, \gamma_j)$ such that

$$\Phi(c) = \frac{\phi(\gamma_j) - \phi(\gamma_i)}{\gamma_j - \gamma_i} < \Phi(\gamma_j)$$

Hence

$$(\gamma_i - \gamma_j)\Phi(\gamma_j) < \phi(\gamma_i) - \phi(\gamma_j).$$

□

3. Mixed mechanism of a n-site phosphorylation system

We now consider a mixed mechanism of a n-site phosphorylation system as described in the introduction. We start with the assumption that each reaction in the mechanism is governed by the law of mass action kinetics. Consider a mixed mechanism of a n-site phosphorylation system with rate constants as given below.

$$\begin{array}{ccccccccccccc} S_0 + E & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & C_1 & \xrightarrow{m_1} & S_1 + E & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & C_2 & \xrightarrow{m_2} & S_2 + E & \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} & \cdots & C_n & \xrightarrow{m_n} & S_n + E \\ S_n + F & \underset{l_{-1}}{\overset{l_1}{\rightleftharpoons}} & D_1 & & \xrightarrow{l_2} & & D_2 & & \xrightarrow{l_3} & & \cdots & D_n & \xrightarrow{l_{n+1}} & S_0 + F \end{array} \tag{10}$$

We assume that all the rate constants in the above scheme are strictly positive. For $i = 1, \ldots, n$, let c_i, d_i and s_i denote the concentrations of C_i, D_i and S_i respectively and let e and f denote the concentrations of the enzymes E and F respectively. Let s_0 denote the concentration of S_0. Define for $i = 1, \ldots, n$,

$$p_i := k_i e s_{i-1} - k_{-i} c_i; \qquad r_i := m_i c_i \tag{11}$$

Then using the law of mass action kinetics, we have

$$\dot{s}_0 = l_{n+1} d_n - p_1 \tag{12}$$

$$\dot{s}_n = r_n - (l_1 s_n f - l_{-1} d_1) \tag{13}$$

$$\dot{d}_1 = l_1 s_n f - (l_{-1} + l_2) d_1. \tag{14}$$

For $i = 1, \ldots, n-1$, we have

$$\dot{s}_i = r_i - p_{i+1}; \tag{15}$$

for $i = 1, \ldots, n$, we have

$$\dot{c}_i = p_i - r_i \tag{16}$$

and for $i = 2, \ldots, n$, we have

$$\dot{d}_i = l_i d_{i-1} - l_{i+1} d_i. \tag{17}$$

The last two rate equations for the reaction network (10) are

$$\dot{e} = \sum_{i=1}^{n} (-p_i + r_i) = -\sum_{i=1}^{n} \dot{c}_i$$

and

$$\dot{f} = -l_1 s_n f + l_{-1} d_1 + l_{n+1} d_n = -\sum_{i=1}^{n} \dot{d}_i$$

from which we have the conservation relations

$$e + \sum_{i=1}^{n} c_i = e_t \tag{18}$$

$$f + \sum_{i=1}^{n} d_i = f_t \tag{19}$$

where e_t and f_t are constants.

We now assume that the concentrations of the substrate and the phosphorylated proteins are much higher than those of the enzymes and their intermediate complexes and derive a Michaelis–Menten approximation for the network (10). Note that this can be done using *singular perturbation theory* as was done for the case of a pure distributive 2-site phosphorylation system known as a *dual futile cycle* in [5,17]. In this paper, instead we make use of the *steady-state approach* [11] as described in Section 2.2. In this approach, it is assumed that after an initial transient, the network reaches a steady state at which the overall rates of change of concentrations of all intermediate complexes C_i and D_i equate to zero.

Define

$$a_i := \frac{k_i}{k_{-i} + m_i}$$

When the steady state is reached, from Eq. (16), we have

$$\dot{c}_i = p_i - r_i = 0$$

and therefore,

$$c_i = a_i s_{i-1} e$$

for $i = 1, \ldots, n$. Using the conservation relation (18), we have

$$e\left(1 + \sum_{j=1}^{n} a_j s_{j-1}\right) = e_t$$

Therefore

$$e = \frac{e_t}{1 + \sum_{j=1}^{n} a_j s_{j-1}}; \qquad c_i = \frac{e_t a_i s_{i-1}}{1 + \sum_{j=1}^{n} a_j s_{j-1}} \qquad i = 1, \ldots, n.$$

The above equations are expressions for the steady concentrations of the enzyme E and enzyme complexes C_i attained as a result of rapid equilibriation. It follows from (11) that

$$r_i = \frac{v_i a_i s_{i-1}}{1 + \sum_{j=1}^{n} a_j s_{j-1}} \tag{20}$$

where $v_i := m_i e_t$.

Since $\dot{d}_i = 0$ for $i = 1, \ldots, n$ at steady state, from Eqs. (14) and (17), it follows that

$$l_2 d_1 = l_3 d_2 = \cdots = l_{n+1} d_n = l_1 s_n f - l_{-1} d_1 =: r_0 \text{(say)}. \tag{21}$$

This implies that

$$d_i = \frac{r_0}{l_{i+1}} \tag{22}$$

for $i = 1, \ldots, n$. From the last of the equations in (21), we get

$$l_1 s_n f = l_{-1} d_1 + r_0 = r_0 \left(1 + \frac{l_{-1}}{l_2}\right).$$

This implies that

$$r_0 = \frac{l_1 l_2 s_n f}{l_{-1} + l_2}. \tag{23}$$

Substituting (22) in the conservation relation (19), we get

$$f_t = f + r_0 \left(\frac{1}{l_2} + \frac{1}{l_3} + \cdots + \frac{1}{l_{n+1}}\right) \tag{24}$$

Defining

$$a_0 := \frac{l_1 l_2}{l_{-1} + l_2} \sum_{i=1}^{n} \frac{1}{l_{i+1}} \tag{25}$$

and substituting Eq. (23) in (24), we get

$$f_t = f(1 + a_0 s_n). \tag{26}$$

It follows from Eqs. (23), (25) and (26) that

$$r_0 = \frac{v_0 a_0 s_n}{1 + a_0 s_n}, \tag{27}$$

where

$$v_0 := \frac{f_t}{\sum_{i=1}^{n} \frac{1}{l_{i+1}}}.$$

In order to obtain the Michaelis–Menten approximation of (10), we need to substitute the steady state expressions that we have obtained for $r_i (i = 0, \ldots, n)$ in Eqs. (12), (13) and (15).

Since $r_i = p_i$ for $i = 1, \ldots, n$ due to rapid equilibriation, it follows from Eqs. (12), (13), (15) and (21) that

$$\begin{aligned} \dot{s}_0 &= r_0 - r_1 \\ \dot{s}_1 &= r_1 - r_2 \\ &\vdots \\ \dot{s}_{n-1} &= r_{n-1} - r_n \\ \dot{s}_n &= r_n - r_0 \end{aligned}$$

with r_i defined by Eqs. (27) and (20) for $i = 0, 1, \ldots, n$. This set of equations may be written in matrix form as follows using the expressions for $r_i (i = 0, 1, \ldots, n)$ in Eqs. (27) and (20).

$$\frac{d}{dt}\begin{bmatrix} s_0 \\ s_1 \\ \vdots \\ s_{n-1} \\ s_n \end{bmatrix} = \begin{bmatrix} 1 & -1 & \cdots & 0 & 0 \\ 0 & 1 & \cdots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & 1 & -1 \\ -1 & 0 & \cdots & 0 & 1 \end{bmatrix} \begin{bmatrix} \frac{v_0 a_0 s_n}{1 + a_0 s_n} \\ \frac{v_1 a_1 s_0}{1 + \sum_{j=1}^{n} a_j s_{j-1}} \\ \frac{v_2 a_2 s_1}{1 + \sum_{j=1}^{n} a_j s_{j-1}} \\ \vdots \\ \frac{v_n a_n s_{n-1}}{1 + \sum_{j=1}^{n} a_j s_{j-1}} \end{bmatrix} \tag{28}$$

Define

$$s := \begin{bmatrix} s_0 \\ s_1 \\ \vdots \\ s_{n-1} \\ s_n \end{bmatrix}; \qquad N := \begin{bmatrix} 1 & -1 & \cdots & 0 & 0 \\ 0 & 1 & \cdots & 0 & 0 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & 1 & -1 \\ -1 & 0 & \cdots & 0 & 1 \end{bmatrix}; \qquad \rho := \begin{bmatrix} r_0 \\ r_1 \\ \vdots \\ r_{n-1} \\ r_n \end{bmatrix} \tag{29}$$

Then Eq. (28) may be rewritten as

$$\frac{d}{dt} s = N\rho. \tag{30}$$

This equation corresponds to the scheme of reactions shown below

$$S_0 \xrightarrow{1} S_1 \xrightarrow{2} S_2 \cdots\cdots\cdots S_{n-1} \xrightarrow{n} S_n \xrightarrow{0} S_0 \tag{31}$$

With regards to the above scheme r_i denotes the rate of the reaction i for $i = 0, \ldots, n$ and N denotes the *stoichiometric matrix* of the reaction scheme. Note that a_0 is the reciprocal of the Michaelis constant associated with the substrate S_n in the dephosphorylation reaction $S_n \longrightarrow S_0$ and for $i = 1, \ldots, n$, a_i is the reciprocal of the Michaelis constant associated with S_{i-1} in the phosphorylation reactions (reactions 1 to n). Note also that v_i is the maximum possible rate of reaction i (at infinite substrate concentrations) in scheme (31).

Let $\mathbb{R}_+^n$ denote the set of n-dimensional vectors with positive real elements. Let s_{in} denote the vector of initial substrate concentrations, i.e., $s_{\text{in}} = s(0)$. Assume that each component of s_{in} is nonnegative. Then it is easy to see from Eq. (30) that

$$s - s_{\text{in}} \in \text{Im}(N)$$

The space of concentrations

$$\mathscr{S}_{s_{\text{in}}} := \{s \in \mathbb{R}_+^{n+1} \mid s - s_{\text{in}} \in \text{Im}(N)\}$$

is the *positive stoichiometric compatibility class* corresponding to s_{in} as defined in [15,18,19]. $\mathscr{S}_{s_{\text{in}}}$ is the space of substrate concentration vector s with positive components that can be reached if the initial concentration vector is equal to s_{in}.

It is easy to see that $\ker(N^{\top}) = \text{Im}(\mathbb{1}_{n+1})$, where $\mathbb{1}_{n+1}$ denotes a vector of dimension $n + 1$, each of whose entries is equal to 1. We therefore have $\mathbb{1}_{n+1}^{\top}\left(\frac{ds}{dt}\right) = 0$, i.e.,

$$\frac{d}{dt} \sum_{i=0}^{n} s_i = 0$$

due to which we have the only conservation relation corresponding to (30) given by

$$\sum_{i=0}^{n} s_i = s_t \tag{32}$$

where s_t is a constant, i.e., the total substrate concentration is conserved. Notice that the vector s stays in $\mathscr{S}_{s_{\text{in}}}$ as long as $s \in \mathbb{R}_+^{n+1}$ and $\mathbb{1}_{n+1}^{\top} s = s_t$.

It can be proved that the nonnegative orthant is forward invariant with respect to the system of equations (28), i.e., if the initial values of $s_0, s_1, \ldots, s_n$ are nonnegative, then they remain nonnegative at all future times. This property commonly referred to as *nonnegativity* is well known for chemical reaction networks including the one considered in this paper. The reader is referred to [20, pp. 613–615] for a simple proof of nonnegativity for chemical reaction networks in which (a) the reaction rate vector ρ has nonnegative elements for nonnegative species concentrations, (b) each element of ρ is a continuously differentiable function of the species concentrations and (c) the reaction rate of any given reaction is equal to zero whenever a species in the substrate of the reaction has zero concentration. A similar proof of nonnegativity may be found in [21, p. 1042], where it is assumed that in addition to conditions (a) and (c), each element of ρ is locally Lipschitz in the species concentrations. In the following lemma, we provide a formal statement of nonnegativity of the system of equations (28).

Lemma 5. *Consider the dynamical system described by equations (28) with $a_i, v_i > 0$ for $i = 0, \ldots, n$. If the initial values of $s_0, s_1, \ldots, s_n$ are nonnegative, then they remain nonnegative at all future times.*

Using the above lemma, it can be proved that the trajectories of the system are bounded.

Corollary 6. *Consider the system of equations (28) with $a_i, v_i > 0$ for $i = 0, \ldots, n$. Define s as in (29) and s_t as in (32). If the components of $s(0)$*

are all nonnegative, then the solution trajectories of (28) are bounded with $0 \le s_i(t) \le s_t$ *for* $i \in \{0, 1, \ldots, n\}$ *and* $t \ge 0$.

Proof. The statement follows from Lemma 5 and the conservation relation (32). □

4. Uniqueness of equilibrium

In this section, we prove that system (28) admits a unique positive equilibrium for a given total substrate concentration.

Theorem 7. *With regards to the system (28), let* s_t *be defined by equation (32). Assume that* a_i, $v_i > 0$ *for* $i = 0, \ldots, n$. *Then corresponding to a given positive value of* s_t, *the system (28) has a unique positive equilibrium.*

Proof. With regards to Eq. (30), an equilibrium occurs when ρ lies in the right kernel of N, i.e., when

$$r_0 = r_1 = \cdots = r_n.$$

Let $\bar{s} = [\bar{s}_0 \;\; \bar{s}_1 \;\; \ldots \;\; \bar{s}_n]^\top$ denote an equilibrium point. From Eqs. (20), we get

$$\bar{s}_{i-1} = \beta_i \bar{s}_0 \tag{33}$$

where

$$\beta_i := \frac{v_1 a_1}{v_i a_i}$$

for $i = 1, \ldots, n$. Since at equilibrium, $r_0 = r_1$, we get

$$\frac{v_1 a_1 \bar{s}_0}{1 + \sum_{j=1}^{n} a_j \bar{s}_{j-1}} = \frac{v_0 a_0 \bar{s}_n}{1 + a_0 \bar{s}_n} \tag{34}$$

Using the conservation relation (32), we get

$$\bar{s}_n = s_t - \sum_{j=1}^{n} \bar{s}_{j-1} = s_t - \sum_{j=1}^{n} \beta_j \bar{s}_0$$

Define $\gamma := \sum_{j=1}^{n} \beta_j$. Then

$$\bar{s}_n = s_t - \gamma \bar{s}_0. \tag{35}$$

Define $\delta := v_1 a_1 \sum_{j=1}^{n} \frac{1}{v_j}$ and $\alpha := \frac{v_0 a_0}{v_1 a_1}$. Substituting Eqs. (33) and (35) in Eq. (34), we get

$$\frac{\bar{s}_0}{1 + \delta \bar{s}_0} = \frac{\alpha (s_t - \gamma \bar{s}_0)}{1 + a_0 (s_t - \gamma \bar{s}_0)}$$

This leads to a quadratic equation in $\bar{s}_0$ given by

$$(\delta\alpha - a_0)\gamma \bar{s}_0^2 + [(1 + \gamma\alpha) - (\delta\alpha - a_0)s_t]\bar{s}_0 - s_t\alpha = 0. \tag{36}$$

Let us now construct a quadratic equation in $\bar{s}_n$ similar to (36). From Eq. (35), we have

$$\bar{s}_0 = \frac{s_t - \bar{s}_n}{\gamma} \tag{37}$$

Substituting Eqs. (37) and (33) in Eq. (34), we get

$$\frac{(s_t - \bar{s}_n)}{\gamma + \delta(s_t - \bar{s}_n)} = \frac{\alpha \bar{s}_n}{1 + a_0 \bar{s}_n}$$

This leads to the following quadratic equation in $\bar{s}_n$.

$$(\delta\alpha - a_0)\bar{s}_n^2 - [(1 + \gamma\alpha) + (\delta\alpha - a_0)s_t]\bar{s}_n + s_t = 0 \tag{38}$$

We now consider 3 cases and in each one of the three cases, we prove the existence of a unique positive equilibrium corresponding to a given positive value of s_t.

Case 1: $\delta\alpha - a_0 > 0$.

In this case, since α, a_0, γ, δ, s_t are all positive, it follows that the discriminant Δ_1 of the quadratic Eq. (36) is positive, and hence there are two real roots, of which one is positive and the other is negative. Let $s_{01} < 0$ and $s_{02} > 0$ denote these roots.

The discriminant Δ_2 of Eq. (38) is given by

$$\begin{aligned}\Delta_2 &= [(1 + \gamma\alpha) + (\delta\alpha - a_0)s_t]^2 - 4s_t(\delta\alpha - a_0) \\ &= [(1 + \gamma\alpha) - (\delta\alpha - a_0)s_t]^2 + 4\gamma\alpha(\delta\alpha - a_0)s_t > 0\end{aligned}$$

Therefore Eq. (38) has two real roots, which are both positive. Let s_{n1} and s_{n2} denote these roots with $s_{n2} > s_{n1}$. From Eq. (35), it is easy to see that the roots s_{01} and s_{n2} correspond to one equilibrium and the roots s_{02} and s_{n1} correspond to the other equilibrium, which is a positive equilibrium. Thus we have

$$\gamma s_{02} + s_{n1} = s_t$$

and $\bar{s} = [s_{02} \;\; \beta_2 s_{02} \;\; \beta_3 s_{02} \;\; \cdots \;\; \beta_n s_{02} \;\; s_{n1}]^\top$ is a unique positive equilibrium of (28).

Case 2: $\delta\alpha - a_0 = 0$.

In this case Eqs. (36) and (38) can be simplified as follows:

$$\bar{s}_0[1 + \gamma\alpha] - s_t\alpha = 0.$$

$$-(1 + \gamma\alpha)\bar{s}_n + s_t = 0$$

It is easy to see that the roots of the above equations, $\bar{s}_0$ and $\bar{s}_n$ are both positive, which implies that $\bar{s}_i > 0$ for $i = 1, \ldots, n-1$. This implies that $\bar{s}$ is unique and has positive components.

Case 3: $\delta\alpha - a_0 < 0$.

In this case, the discriminant Δ_1 of Eq. (36) is given by

$$\begin{aligned}\Delta_1 &= [(1 + \gamma\alpha) + (a_0 - \delta\alpha)s_t]^2 - 4(a_0 - \delta\alpha)\gamma\alpha s_t \\ &= [(1 + \gamma\alpha) - (a_0 - \delta\alpha)s_t]^2 + 4(a_0 - \delta\alpha)s_t > 0\end{aligned}$$

Therefore Eq. (36) has two real roots, which are both positive. Let s_{01} and s_{02} denote these roots with $s_{02} > s_{01}$. Now consider Eq. (38). It is easy to see that the discriminant Δ_2 of this equation is positive. Consequently the roots of this equation are real, with one of them being positive and the other negative. Let $s_{n1} < 0$ and $s_{n2} > 0$ denote the two roots of (38). From Eq. (35), it can be inferred that the roots s_{01} and s_{n2} correspond to one equilibrium, which is positive and the roots s_{02} and s_{n1} correspond to the other equilibrium. Thus we have

$$\gamma s_{01} + s_{n2} = s_t$$

and $\bar{s} = [s_{01} \;\; \beta_2 s_{01} \;\; \beta_3 s_{01} \;\; \cdots \;\; \beta_n s_{01} \;\; s_{n2}]^\top$ is a unique positive equilibrium of (28). □

From Theorem 7, it follows that there is a unique equilibrium in every positive stoichiometric compatibility class $\mathscr{S}_{s_{\text{in}}}$ corresponding to a nonnegative, nonzero initial substrate concentration vector s_{in}.

5. Asymptotic stability of two special cases

We now consider two special cases as mentioned in the introduction section and prove asymptotic stability of the unique positive equilibrium of the system (28). In the first case, we assume that the Michaelis constant associated with the different substrates involved in the phosphorylation reactions are equal. In the second case, we assume that there are just two sites of phosphorylation and dephosphorylation.

5.1. The case of equal Michaelis constants

With reference to the system (28), we assume that $a_1 = a_2 = \cdots = a_n$. With this assumption, we construct a Lyapunov function in order to prove asymptotic stability of the corresponding unique equilibrium.

Theorem 8. *Consider the system of equations (28) with* $v_i > 0$ *for* $i = 0, \ldots, n$ *and* $a_1 = a_2 = \cdots = a_n > 0$. *Define* s *as in (29) and* s_t *as in (32). If the components of* $s(0)$ *are all nonnegative and* $s(0) \neq 0$, *then the solution trajectories of (28) converge to the unique positive equilibrium corresponding to the value of* s_t.

Proof. Define $a = a_1$. Then for $i = 1, \ldots, n$, the rate r_i of reaction i in scheme (31) is given by

$$r_i = \frac{v_i a s_{i-1}}{1 + a\sum_{j=0}^{n-1} s_j}. \tag{39}$$

Let $\bar{s} = [\bar{s}_0 \ \ \bar{s}_1 \ \cdots \ \bar{s}_n]^\top$ denote the unique positive equilibrium substrate concentration vector corresponding to the total substrate concentration s_t. With reference to Eq. (30), ρ lies in the right kernel of N at equilibrium.

Thus

$$r_0 = r_1 = \cdots = r_n = :r \,(\text{say})$$

when $s = \bar{s}$, i.e., at the positive equilibrium corresponding to s_t. Define

$$G := \sum_{i=1}^{n} s_{i-1}\ln r_i + s_n \ln r_0 - s_t \ln r - \frac{1}{a}\ln\left(\frac{1 + a\sum_{i=0}^{n-1} s_i}{1 + a\sum_{i=0}^{n-1}\bar{s}_i}\right) - \frac{1}{a_0}\ln\left(\frac{1 + a_0 s_n}{1 + a_0 \bar{s}_n}\right) \tag{40}$$

where it is assumed that $s_{i-1}\ln r_i = 0$ if $s_{i-1} = 0$ for some $i \in \{1, \ldots, n\}$ and $s_n \ln r_0 = 0$ if $s_n = 0$. Note that this assumption is reasonable since for $i \in \{0, \ldots, n-1\}$

$$\lim_{s_i \to 0^+} s_i \ln r_{i+1} = \lim_{s_i \to 0^+} s_i \ln\left(\frac{v_{i+1} a s_i}{1 + a\sum_{j=0}^{n-1} s_j}\right) = 0$$

We now prove that G is a Lyapunov function for the system (28), by proving the following

1. $G \geq 0$ with equality holding only if $s = \bar{s}$;
2. $\frac{dG}{dt} \leq 0$ with equality holding only if $s = \bar{s}$.

We first prove that $G \geq 0$ with equality holding only if $s = \bar{s}$. From Eq. (40), we have

$$\begin{aligned} G &= \sum_{i=1}^{n} s_{i-1}\ln\left(\frac{r_i}{r}\right) + s_n \ln\left(\frac{r_0}{r}\right) - \frac{1}{a}\ln\left(\frac{1 + a\sum_{i=0}^{n-1} s_i}{1 + a\sum_{i=0}^{n-1}\bar{s}_i}\right) - \frac{1}{a_0}\ln\left(\frac{1 + a_0 s_n}{1 + a_0 \bar{s}_n}\right) \\ &= \sum_{i=0}^{n} s_i \ln\left(\frac{s_i}{\bar{s}_i}\right) - \left(\sum_{i=0}^{n-1} s_i + \frac{1}{a}\right)\ln\left(\frac{\sum_{j=0}^{n-1} s_j + \frac{1}{a}}{\sum_{j=0}^{n-1}\bar{s}_j + \frac{1}{a}}\right) - \left(s_n + \frac{1}{a_0}\right)\ln\left(\frac{s_n + \frac{1}{a_0}}{\bar{s}_n + \frac{1}{a_0}}\right) \end{aligned} \tag{41}$$

We now prove the following inequalities:

$$\sum_{i=0}^{n-1} s_i \ln\left(\frac{s_i}{\bar{s}_i}\right) \geq \left(\sum_{i=0}^{n-1} s_i + \frac{1}{a}\right)\ln\left(\frac{\sum_{j=0}^{n-1} s_j + \frac{1}{a}}{\sum_{j=0}^{n-1}\bar{s}_j + \frac{1}{a}}\right) \tag{42}$$

with equality holding only if $s_i = \bar{s}_i$ for $i = 0, \ldots, n$ and

$$s_n \ln\left(\frac{s_n}{\bar{s}_n}\right) \geq \left(s_n + \frac{1}{a_0}\right)\ln\left(\frac{s_n + \frac{1}{a_0}}{\bar{s}_n + \frac{1}{a_0}}\right) \tag{43}$$

with equality holding only if $s_n = \bar{s}_n$. In order to prove inequalities (42) and (43), we make use of the following lemma which can be proved using *Jensen's inequality*.

Lemma 9. *Define*

$$H(s, \bar{s}) := \begin{cases} s\ln\left(\frac{s}{\bar{s}}\right) & \text{if} \quad s > 0 \\ 0 & \text{if} \quad s = 0 \end{cases}$$

Then

$$\sum_{i=1}^{n} H(s_i, \bar{s}_i) \geq H\left(\sum_{i=1}^{n} s_i + b, \sum_{i=1}^{n}\bar{s}_i + b\right) \tag{44}$$

where $b > 0$ and for $i = 1, \ldots, n$, $s_i \geq 0$ and $\bar{s}_i > 0$. The equality in (44) holds only if $s_i = \bar{s}_i$ for $i = 1, \ldots, n$.

Proof. We first prove that for any $s_1, s_2 \geq 0$ and $\bar{s}_1, \bar{s}_2 > 0$,

$$H(s_1, \bar{s}_1) + H(s_2, \bar{s}_2) \geq H(s_1 + s_2, \bar{s}_1 + \bar{s}_2) \tag{45}$$

with equality holding only if $\frac{s_1}{\bar{s}_1} = \frac{s_2}{\bar{s}_2}$.

If $s_1 = 0$ and $s_2 > 0$, then the left hand side of (45) is equal to

$$s_2 \ln\left(\frac{s_2}{\bar{s}_2}\right) > s_2 \ln\left(\frac{s_2}{\bar{s}_1 + \bar{s}_2}\right)$$

If $s_1 = s_2 = 0$, then (45) reduces to an equality and $\frac{s_1}{\bar{s}_1} = \frac{s_2}{\bar{s}_2}$ holds. Now assume that $s_1 > 0$, $s_2 > 0$. Define

$$\phi(x) := \ln\left(\frac{1}{x}\right)$$

and note that ϕ is a real strictly convex function. Applying Jensen's inequality (Lemma 1) on ϕ with $n = 2$, $\sigma_i := s_i$ and $x_i := \frac{\bar{s}_i}{s_i}$ gives

$$s_1 \ln\left(\frac{s_1}{\bar{s}_1}\right) + s_2 \ln\left(\frac{s_2}{\bar{s}_2}\right) \geq (s_1 + s_2)\ln\left(\frac{s_1 + s_2}{\bar{s}_1 + \bar{s}_2}\right)$$

with equality holding only if $\frac{s_1}{\bar{s}_1} = \frac{s_2}{\bar{s}_2}$.

It is easy to verify by induction that

$$\sum_{i=1}^{n} H(s_i, \bar{s}_i) \geq H\left(\sum_{i=1}^{n} s_i, \sum_{i=1}^{n}\bar{s}_i\right) \tag{46}$$

with equality holding only if

$$\frac{s_1}{\bar{s}_1} = \frac{s_2}{\bar{s}_2} = \ldots = \frac{s_n}{\bar{s}_n}$$

Now observe that $H(b, b) = 0$. Adding $H(b, b)$ to both sides of inequality (46), we get

$$\sum_{i=1}^{n} H(s_i, \bar{s}_i) \geq H\left(\sum_{i=1}^{n} s_i, \sum_{i=1}^{n}\bar{s}_i\right) + H(b, b) \geq H\left(\sum_{i=1}^{n} s_i + b, \sum_{i=1}^{n}\bar{s}_i + b\right)$$

with equality holding only if

$$\frac{s_1}{\bar{s}_1} = \frac{s_2}{\bar{s}_2} = \ldots = \frac{s_n}{\bar{s}_n} = \frac{b}{b} = 1,$$

i.e., if $s_i = \bar{s}_i$ for $i = 1, \ldots, n$. □

Observe that inequalities (42) and (43) directly follow from Lemma 9. From Eq. (41), it follows that $G \geq 0$ with equality holding only if $s = \bar{s}$, i.e. at equilibrium.

We now prove that $\frac{dG}{dt} \leq 0$ with equality holding only if $s = \bar{s}$. Define

$$\overline{G} := s_t \ln r - \frac{1}{a}\ln\left(1 + a\sum_{i=0}^{n-1}\bar{s}_i\right) - \frac{1}{a_0}\ln(1 + a_0\bar{s}_n)$$

From Eqs. (40) and (39), it follows that

$$G = \sum_{i=1}^{n} s_{i-1}\ln(v_i a s_{i-1}) - \left(\sum_{i=1}^{n} s_{i-1} + \frac{1}{a}\right)\ln\left(1 + a\sum_{j=0}^{n-1} s_j\right) + s_n \ln(v_0 a_0 s_n) - \left(s_n + \frac{1}{a_0}\right)\ln(1 + a_0 s_n) - \overline{G}$$

Define $\gamma_i := \ln r_i$ for $i = 0, 1, \ldots, n$. For some $k \in \{0, \ldots, n-1\}$,

$$\frac{\partial G}{\partial s_k} = \ln(v_{k+1}as_k) + s_k\left(\frac{1}{s_k}\right) - \ln\left(1 + a\sum_{i=0}^{n-1} s_i\right) - \left(\sum_{i=0}^{n-1} s_i + \frac{1}{a}\right)\frac{a}{1 + a\sum_{i=0}^{n-1} s_i} = \ln\left(\frac{v_{k+1}as_k}{1 + a\sum_{i=0}^{n-1} s_i}\right)$$

Thus for $k \in \{0, \ldots, n-1\}$,

$$\frac{\partial G}{\partial s_k} = \gamma_{k+1}.$$

Taking partial derivative of G with respect to s_n, we similarly have

$$\frac{\partial G}{\partial s_n} = \gamma_0$$

Define

$$\Gamma := \begin{bmatrix} \gamma_1 \\ \gamma_2 \\ \vdots \\ \gamma_n \\ \gamma_0 \end{bmatrix}.$$

Now

$$\frac{dG}{dt} = \sum_{i=0}^{n} \frac{\partial G}{\partial s_i}\cdot\frac{ds_i}{dt} = \Gamma^{\top}\frac{ds}{dt} = \Gamma^{\top}N\rho \tag{47}$$

From Eq. (47), we get

$$\frac{dG}{dt} = (\gamma_1 - \gamma_0)e^{\gamma_0} + (\gamma_2 - \gamma_1)e^{\gamma_1} + \cdots + (\gamma_n - \gamma_{n-1})e^{\gamma_{n-1}} + (\gamma_0 - \gamma_n)e^{\gamma_n}$$

Since the exponential function is strictly convex, we can apply Lemma 4 with $\phi(x) := e^x$ to obtain

$$(\gamma_i - \gamma_j)e^{\gamma_j} \leq e^{\gamma_i} - e^{\gamma_j}$$

for any $\gamma_i, \gamma_j \in \mathbb{R}$ with equality holding only if $\gamma_i = \gamma_j$. Hence

$$\frac{dG}{dt} \leq e^{\gamma_1} - e^{\gamma_0} + e^{\gamma_2} - e^{\gamma_1} + \cdots + e^{\gamma_n} - e^{\gamma_{n-1}} + e^{\gamma_0} - e^{\gamma_n} = 0$$

From the above, it follows that $\frac{dG}{dt} = 0$, only when $\gamma_0 = \gamma_1 = \cdots = \gamma_n$. i.e., when $r_0 = r_1 = \cdots = r_n$, which corresponds with the unique positive equilibrium $s = \bar{s}$ associated with the total substrate concentration s_t in our case. This implies that G is a Lyapunov function for the system (28). Therefore, all solution trajectories of (28) converge to the unique positive equilibrium corresponding to s_t. □

5.2. The case of two sites of phosphorylation and dephosphorylation

In this section, we consider the system (28) with $n = 2$, and prove asymptotic stability of the unique equilibrium corresponding to a given total substrate concentration. The proof proceeds by reduction of the 3-state Michaelis–Menten approximation of the system given by (28) with $n = 2$ into a system of nonlinear ordinary differential equations comprising of two states using the conservation law (32). This reduction enables us to make use of the Bendixson's criterion in order to prove the non existence of periodic orbits in the system. Finally we make use of the Poincaré Bendixson theorem to conclude that the unique equilibrium is a global attractor. This method has been used in order to prove a similar result for the case of the Michaelis Menten approximation of the *dual phosphorylation futile cycle* in [22], in which the mechanisms of both phosphorylation and dephosphorylation are distributive.

Theorem 10. *Consider the system of equations (28) with $n = 2$ and a_i, $v_i > 0$ for $i = 0, 1, 2$. Define s as in (29) and s_t as in (32). If the components of $s(0)$ are all nonnegative and $s(0) \neq 0$, then the solution trajectories of (28) converge to the unique positive equilibrium corresponding to the value of s_t.*

Proof. From the conservation relation (32), we have

$$s_2 = s_t - s_0 - s_1 \tag{48}$$

Substituting for s_2 in the first two equations of (28), we get

$$\begin{aligned} \dot{s_0} &= -\frac{v_1a_1s_0}{1 + a_1s_0 + a_2s_1} + \frac{v_0a_0(s_t - s_0 - s_1)}{1 + a_0(s_t - s_0 - s_1)} := f(s_0, s_1) \\ \dot{s_1} &= \frac{v_1a_1s_0}{1 + a_1s_0 + a_2s_1} - \frac{v_2a_2s_1}{1 + a_1s_0 + a_2s_1} := g(s_0, s_1) \end{aligned} \tag{49}$$

Replace the third equation in (28) with Eq. (48). Observe that the system (49) has only two states and therefore we can make use of the celebrated *Bendixson's criterion* (Theorem 2) in order to investigate the existence of periodic orbits. We have

$$\frac{\partial f}{\partial s_0} + \frac{\partial g}{\partial s_1} = -\frac{v_0a_0}{(1 + a_0(s_t - s_0 - s_1))^2} - \frac{[v_1a_1(1 + a_2(s_0 + s_1)) + v_2a_2(1 + a_1s_0)]}{(1 + a_1s_0 + a_2s_1)^2} \tag{50}$$

Since each component of $s(0)$ is nonnegative, from Lemma 5, it follows that they remain nonnegative at all future times, and therefore from Eq. (50), it follows that $\frac{\partial f}{\partial s_0} + \frac{\partial g}{\partial s_1} < 0$ at all times $t \geq 0$. From Bendixson's criterion, it follows that the system (49) does not admit any sustained oscillations because of the non existence of periodic orbits in the nonnegative quadrant. We now make use of the Poincare–Bendixson Theorem (Theorem 3) in order to prove that the unique equilibrium of the system is a global attractor.

From Corollary 6, it follows that every solution trajectory of the system (49) is bounded. From the assumptions made in the statement of the Theorem and from Theorem 7, it follows that the system (28) and hence (49) admit a unique positive equilibrium. Since (49) does not have any periodic orbit, it follows from Poincaré Bendixson Theorem that every solution trajectory of (49) and hence of (28) converges to the corresponding unique positive equilibrium. □

6. Conclusion

In this paper, we have proved that the Michaelis Menten approximation of a mixed mechanism of a phosphorylation system admits a unique equilibrium in every positive stoichiometric compatibility class using the theory of quadratic equations. Further, we have proved that if the Michaelis constants associated with the different substrates in the phosphorylation reactions are equal, then the equilibrium is asymptotically stable. We have proved this by making use of the convexity of the exponential function in a way similar to the proof of asymptotic stability of complex balanced networks as presented in [16]. In the case of the number of sites of phosphorylation and dephosphorylation being equal to two, we have proved that the resulting unique equilibrium is asymptotically stable irrespective of the values of the parameters of the system. Here, we have made use of Bendixson's criterion and Poincare Bendixson theorem, as has been already done for the case of the Michaelis Menten approximation of the dual futile cycle in [22].

The results obtained in this paper are valid under the assumption that the concentrations of the substrate and the phosphorylated proteins are much higher than those of the enzymes catalyzing the phosphorylation and dephosphorylation processes and their intermediate complexes. In the case where this assumption does not hold, it is possible that the system admits sustained oscillations as shown numerically in [10].

The results about uniqueness and asymptotic stability of a positive equilibrium obtained in the paper hold also if the mechanisms of phosphorylation and dephosphorylation are swapped, i.e., if the phosphorylation occurs in a processive way and the dephosphorylation in a distributive way. Currently, research is being carried out to prove asymptotic stability of the unique positive equilibrium of the Michaelis Menten approximation of a mixed mechanism of phosphorylation corresponding to a given total substrate concentration with no condition on the parameters of the system, since numerical simulations have shown to support this conjecture.

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