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Lyapunov functions for Michaelis Menten approximation of processive phosphorylation futile cycles

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Abstract— **In this paper, we analyze a class of processive phosphorylation futile cycles in which a given protein substrate is modified by more than two enzymes. This analysis is based on the assumption that the initial concentration of the protein substrate is much higher than the concentrations of the enzymes. This assumption allows us to reduce the dynamics of the system to its Michaelis Menten approximation using the steady state approach. We then prove that for the given system of equations, there exists a unique equilibrium concentration vector in every positive stoichiometric compatibility class. We further prove that this unique equilibrium is globally asymptotically stable using two different Lyapunov functions. The first Lyapunov function is a piecewise linear in rates (PWLR) Lyapunov function. The construction of the second Lyapunov function is based on the property of strict convexity of the exponential function.**

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I. INTRODUCTION

In this paper, we consider a chemical reaction network of the following type.

$$\begin{aligned} P_0 + E_1 &\rightleftharpoons C_{11} \rightleftharpoons C_{12} \rightleftharpoons \cdots\cdots \rightleftharpoons C_{1m_1} \longrightarrow P_1 + E_1 \\ P_1 + E_2 &\rightleftharpoons C_{21} \rightleftharpoons C_{22} \rightleftharpoons \cdots\cdots \rightleftharpoons C_{2m_2} \longrightarrow P_2 + E_2 \\ &\vdots \qquad\qquad \vdots \qquad\qquad \vdots \\ P_{n-1} + E_n &\rightleftharpoons C_{n1} \rightleftharpoons C_{n2} \rightleftharpoons \cdots\cdots \rightleftharpoons C_{nm_n} \longrightarrow P_0 + E_n \end{aligned} \tag{1}$$

With regards to the above network, P_{i-1} denotes a substrate protein and E_i denotes an enzyme modifying P_{i-1} to P_i in the i^{th} reaction chain of the network. C_{i1}, C_{i2}, ..., C_{im_i} are intermediate complexes that are involved in the modification of the protein substrate P_{i-1} to P_i in the i^{th} reaction chain. Since in every reaction chain, the enzyme catalyzing the modification of the corresponding protein substrate is neither produced nor consumed, and the protein substrate consumed in one reaction chain is produced in equal amounts in another reaction chain, the reaction network (1) is a futile cycle.

With $n = 2$, i.e., with only two reaction chains, the network (1) denotes the model of a multisite phosphorylation futile cycle as studied in [1], in which the first reaction chain models the phosphorylation of P_0 to P_1 under a *processive* mechanism and the second reaction chain models the dephosphorylation of P_1 back to P_0 also under a processive mechanism. 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 and their mechanisms, the reader is referred to [2]. The mechanism (1) presented in this paper is a generalization of the processive phosphorylation model studied in [1], in which the phosphorylation and dephosphorylation of a protein substrate is carried out by more than two enzymes.

In addition to processive mechanism of phosphorylation and dephosphorylation, there exists another popular mechanism, which is called *distributive* mechanism whose dynamics have been studied extensively in [3]–[8]. The dynamics of processive futile cycles have also been studied extensively in [1], [9], [10].

In [9], under the assumption that each reaction is governed by the law of *mass action kinetics*, the futile cycle (1) was analyzed for equlibria and stability and it was proved that corresponding to a given set of nonnegative initial concentrations, there exists a unique positive equilibrium concentration vector to which the dynamics of the system globally asymptotically converges. In this paper, we consider a Michaelis Menten approximation of the futile cycle (1) and show similar results for the approximate model. 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. In order to derive the Michaelis Menten approximation of the network (1), we make use of the King Altman approach [11]–[13] and the steady state approach [14]. We show using intermediate value property of continuous functions that the Michaelis Menten approximation of (1) admits a unique equilibrium corresponding to a given total concentration of the substrate and its phosphorylated forms.

The main contribution of the paper is the construction of two different Lyapunov functions to prove stability of the unique positive equilibrium concentration vector corresponding to a given total substrate concentration, of the Michaelis Menten approximation of the network (1). The first Lyapunov function is a *PieceWise Linear in Rates* (PWLR) Lyapunov function very similar to the one used to prove global asymptotic stability of the network (1) in [9]. The construction of piecewise linear Lyapunov functions in order to prove stability of systems, specially chemical reaction networks, is not uncommon in the literature.

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Recently, Lyapunov functions that are piecewise linear in reaction rates have been used to prove stability of chemical reaction networks in [15], [16]. A piecewise linear Lyapunov function in the time-derivative of the states was also used to prove stability of nonlinear compartmental systems in [17], [18]. The second Lyapunov function used in this paper is an alternative Lyapunov function that can also be used to prove global stability in our case. The construction of this Lyapunov function is based on the strict convexity of the exponential function.

The paper is organized as follows. In Section II, we derive the Michaelis Menten approximation model of the chemical reaction network (1) using the steady state approach. In this section, we also prove nonnegativity and boundedness of the solution trajectories of the approximate model. In Section III, we prove that for the approximate model, there exists a unique positive equilibrium concentration vector corresponding to a given total substrate concentration. We then prove the global asymptotic stability of this equilibrium concentration vector using two different Lyapunov functions in Section IV. In Section V, we present the conclusions of the paper.

II. MICHAELIS MENTEN APPROXIMATION USING THE STEADY STATE APPROACH

In order to derive the Michaelis Menten approximation of the network (1), we begin with the assumption that each reaction in the network (1) is governed by the *law of mass action kinetics* which 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. Consider the reaction

$$X_1 + X_2 \underset{k_r}{\overset{k_f}{\rightleftharpoons}} X_3 \tag{2}$$

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 (2) 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 $\dot{x} := \frac{dx}{dt}$.

Now consider the i^{th} reaction chain of the network (1) where $i \in \{1, \ldots, n\}$. Assuming that each reaction in this reaction chain is governed by mass action kinetics, and defining $P_n := P_0$, take the (positive) reaction constants of the different reactions in the chain as shown below.

$$P_{i-1} + E_i \underset{k_{-i1}}{\overset{k_{i1}}{\rightleftharpoons}} C_{i1} \underset{k_{-i2}}{\overset{k_{i2}}{\rightleftharpoons}} \cdots\cdots \underset{k_{-im_i}}{\overset{k_{im_i}}{\rightleftharpoons}} C_{im_i} \overset{k_{i(m_i+1)}}{\longrightarrow} P_i + E_i$$

Let p_i, e_i and c_{ij} denote the concentrations of P_i, E_i and C_{ij} respectively. For $i = 1, \ldots, n$, define

$$r_i := k_{i(m_i+1)} c_{im_i}; \qquad q_i := k_{i1} p_{i-1} e_i - k_{-i1} c_{i1}. \tag{3}$$

Then, we have the rate equations

$$\dot{p}_i = r_i - q_{i+1} \tag{4}$$

for $i = 1, \ldots, n-1$ and

$$\dot{p}_0 = r_n - q_1 \tag{5}$$

We now derive the Michaelis-Menten approximation for the network (1). Note that this can be done using *singular perturbation theory* as was done for the case of a distributive 2-site phosphorylation system known as a *dual futile cycle* in [6], [19]. In this paper, instead we make use of a combination of the *King-Altman approach* [11], [13] and the *steady-state approach* [14]. First of all, in order to derive the Michaelis-Menten approximation of any enzyme-kinetic reaction mechanism, it is assumed that the concentration of the substrates are much higher than those of the enzymes and their complexes. Under these conditions, the reactions between the enzymes and their complexes (E_i, C_{ij}) occur at a much faster time-scale than those involving the substrates and products (P_i).

The first step in King Altman approach is to consider the reactions occuring in the faster time-scale, i.e., those reactions that involve the enzymes and their complexes. Since these reactions occur rapidly compared with those that involve the substrates and products, the concentrations of substrates and products are assumed constants while deriving the rate equations of these reactions. Consider now the faster time-scale reactions in the i^{th} reaction chain of (1) where $i \in \{1, 2, \ldots, n\}$.

$$E_i \underset{k_{-i1}}{\overset{k_{i1}p_{i-1}}{\rightleftharpoons}} C_{i1} \underset{k_{-i2}}{\overset{k_{i2}}{\rightleftharpoons}} \cdots\cdots \underset{k_{-im_i}}{\overset{k_{im_i}}{\rightleftharpoons}} C_{im_i} \overset{k_{i(m_i+1)}}{\longrightarrow} E_i$$

When a steady state of the above network is reached, we have

$$k_{i1} p_{i-1} e_i - k_{-i1} c_{i1} = k_{i2} c_{i1} - k_{-i2} c_{i2} = \cdots\cdots =$$

$$k_{im_i} c_{i(m_i-1)} - k_{-im_i} c_{im_i} = k_{i(m_i+1)} c_{im_i} \tag{6}$$

For $j = 1, \ldots, m_i$, we prove by induction that there exists a $\delta_{ij} \in \mathbb{R}_+$, such that

$$c_{ij} = \delta_{ij} c_{im_i}. \tag{7}$$

Note that $\delta_{im_i} = 1 > 0$. Assume that $c_{i(j+1)} = \delta_{i(j+1)} c_{im_i}$ with $\delta_{i(j+1)} > 0$ and $j \leq m_i - 1$. Then from equations (6), it follows that

$$k_{i(j+1)} c_{ij} - k_{-i(j+1)} c_{i(j+1)} = k_{i(m_i+1)} c_{im_i}.$$

This implies that

$$c_{ij} = \frac{1}{k_{i(j+1)}} (k_{i(m_i+1)} + k_{-i(j+1)} \delta_{i(j+1)}) c_{im_i} = \delta_{ij} c_{im_i}$$

where

$$\delta_{ij} := \frac{1}{k_{i(j+1)}}(k_{i(m_i+1)} + k_{-i(j+1)}\delta_{i(j+1)}).$$

It follows that $\delta_{ij} > 0$ if $\delta_{i(j+1)} > 0$. Since $\delta_{im_i} > 0$, by induction, we obtain $\delta_{ij} > 0$ for $i = 1, 2, \ldots, m_i - 1$.

From equations (6), it also follows that $r_i = q_i$, i.e.,

$$k_{i1}p_{i-1}e_i - k_{-i1}c_{i1} = k_{i(m_i+1)}c_{im_i}.$$

This implies that

$$p_{i-1}e_i = \frac{1}{k_{i1}}(k_{i(m_i+1)} + k_{-i1}\delta_{i1})c_{im_i} = \delta_{i0}c_{im_i} \tag{8}$$

where

$$\delta_{i0} := \frac{1}{k_{i1}}(k_{i(m_i+1)} + k_{-i1}\delta_{i1}) > 0.$$

We have from equation (8),

$$c_{im_i} = \frac{p_{i-1}}{\delta_{i0}}e_i \tag{9}$$

Observe that with respect to the i^{th} reaction chain, we have

$$\dot{e}_i + \sum_{j=1}^{m_i} \dot{c}_{ij} = 0$$

due to which we have the conservation relation

$$e_i + \sum_{j=1}^{m_i} c_{ij} = e_{it} \tag{10}$$

where e_{it} is a constant that depends on i. Now substituting equations (7) and (9) in the conservation relation (10), we get

$$e_i = \frac{e_t}{1 + a_i p_{i-1}}, \tag{11}$$

where

$$a_i := \frac{\sum_{j=1}^{m_i} \delta_{ij}}{\delta_{i0}}. \tag{12}$$

Substituting equations (9), (11) and (12) in (3), we get

$$r_i = \frac{v_i a_i p_{i-1}}{1 + a_i p_{i-1}} \tag{13}$$

where

$$v_i := \frac{k_{i(m_i+1)}e_{it}}{\sum_{j=1}^{m_i} \delta_{ij}}$$

Since $r_i = q_i$ for $i = 1, \ldots, n$, equations (4) and (5) may be rewritten in the following way to obtain the Michaelis Menten approximation model for the network (1)

$$\dot{p}_i = r_i - r_{i+1}$$

for $i = 1, \ldots, n-1$ and

$$\dot{p}_0 = r_n - r_1.$$

This set of equations may be written in matrix form as follows using the expressions for $r_i (i = 1, \ldots, n)$ in equation (13).

$$\frac{d}{dt}\begin{bmatrix} p_0 \\ p_1 \\ \vdots \\ p_{n-1} \end{bmatrix} = \begin{bmatrix} -1 & 0 & \cdots & 1 \\ 1 & -1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & -1 \end{bmatrix} \begin{bmatrix} \frac{v_1 a_1 p_0}{1+a_1 p_0} \\ \frac{v_2 a_2 p_1}{1+a_2 p_1} \\ \vdots \\ \frac{v_n a_n p_{n-1}}{1+a_n p_{n-1}} \end{bmatrix} \tag{14}$$

Define

$$p := \begin{bmatrix} p_0 \\ p_1 \\ \vdots \\ p_{n-1} \end{bmatrix};\ N := \begin{bmatrix} -1 & 0 & \cdots & 1 \\ 1 & -1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & -1 \end{bmatrix};\ r := \begin{bmatrix} r_1 \\ r_2 \\ \vdots \\ r_n \end{bmatrix} \tag{15}$$

Then equation (14) may be rewritten as

$$\dot{p} = Nr. \tag{16}$$

This equation corresponds to the scheme of reactions shown below

$$P_0 \xrightarrow{1} P_1 \xrightarrow{2} P_2 \cdots\cdots\cdots P_{n-1} \xrightarrow{n} P_0 \tag{17}$$

With regards to the above scheme, r_i denotes the rate of the reaction i for $i = 1, \ldots, n$. Note that for $i = 1, \ldots, n$, a_i is the reciprocal of the Michaelis constant associated with the substrate P_{i-1} of reaction i. Note also that v_i is the maximum possible rate of reaction i (at infinite substrate concentration p_{i-1}) in scheme (17).

Let $\mathbb{R}^n_+$ denote the set of n-dimensional vectors with positive real elements. Let p_{in} denote the vector of initial substrate concentrations, i.e., $p_{\text{in}} = p(0)$. Assume that each component of p_{in} is nonnegative. Then it is easy to see from equation (16) that

$$p - p_{\text{in}} \in \text{im}(N)$$

The space of concentrations

$$\mathcal{S}_{p_{\text{in}}} := \{p \in \mathbb{R}^n_+ \quad | \quad p - p_{\text{in}} \in \text{im}(N)\}$$

is the *positive stoichiometric compatibility class* corresponding to p_{in} as defined in [20]–[22]. $\mathcal{S}_{p_{\text{in}}}$ is the space of substrate concentration vector p with positive components that can be reached if the initial concentration vector is equal to p_{in}.

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

$$\sum_{i=0}^{n-1} \dot{p}_i = 0$$

due to which we have the only conservation relation corresponding to (16) given by

$$\sum_{i=0}^{n-1} p_i = p_t \tag{18}$$

where p_t is a constant, i.e., the total substrate concentration is conserved. Notice that the vector p stays in $\mathcal{S}_{p_{\text{in}}}$ as long as $p \in \mathbb{R}^n_+$ and $\mathbb{1}_n^\top p = p_t$.

We now prove *nonnegativity* of system (14), i.e., we prove that the nonnegative orthant is invariant with respect to the system of equations (14). This will enable us to prove the boundedness of the system trajectories in the corollary that follows the Lemma below.

Lemma 1: Consider the dynamical system described by equations (14) with $a_i, v_i > 0$ for $i = 1, \ldots, n$. If the initial

values of $p_0, p_1, \ldots, p_{n-1}$ are nonnegative, then they remain nonnegative at all future times.

Proof: If we assume that at any particular instant of time, any one of $p_0, p_1, \ldots, p_{n-1}$ has value zero, and the rest are nonnegative, then from the dynamical equations (14), it follows that the rate of change of the variable with value zero, is either positive or zero. This proves that the nonnegative orthant is invariant with respect to the differential equations (14), since any point on the boundary of the nonnegative orthant will either be pushed into the positive orthant or will stay in the boundary. In other words, if the initial values of $p_0, p_1, \ldots, p_{n-1}$ are nonnegative, they remain nonnegative at all future times. ■

Corollary 2: Consider the system of equations (14) with $a_i, v_i > 0$ for $i = 1, \ldots, n$. Define p_t as in (18). If $p_i(0) \geq 0$ for $i = 1, \ldots, n-1$, then the solution trajectories of (14) are bounded with $0 \leq p_i(t) \leq p_t$ for $i \in \{0, 1, \ldots, n-1\}$ and $t \geq 0$.

Proof: The statement follows from Lemma 1 and the conservation relation (18). ■

III. UNIQUENESS OF EQUILIBRIUM

In this section, we prove that system (14) admits a unique positive equilibrium for a given positive total substrate concentration p_t.

Theorem 3: With regards to the system (14), let p_t be defined by equation (18). Assume that $a_i, v_i > 0$ for $i = 1, \ldots, n$. Then corresponding to a given positive value of p_t, the system (14) has a unique positive equilibrium concentration vector.

Proof: For $i = 0, \ldots, n-1$, let p_i^* denote the concentration of p_i at an equilibrium. For $i = 1, \ldots, n$ define r_i as in equation (13). Let s denote the index for which v_s is minimum among the elements of $\{v_1, v_2, \ldots, v_n\}$. From equation (14), it is easy to see that an equilibrium point occurs when $r_i = r_{i+1}$ for $i = 1, \ldots, n-1$. We will use this fact to parametrize p_i^* in terms of p_{s-1}^* for $i = 0, 1, \ldots, n-1$. Notice that $r_i = r_s$ at an equilibrium point implies that

$$\frac{v_i a_i p_{i-1}^*}{1 + a_i p_{i-1}^*} = \frac{v_s a_s p_{s-1}^*}{1 + a_s p_{s-1}^*}$$

which in turn implies that

$$p_{i-1}^* = \frac{b_i p_{s-1}^*}{1 + \alpha_i p_{s-1}^*} \tag{19}$$

where $b_i := \frac{v_s a_s}{v_i a_i}$ and $\alpha_i := a_s(1 - \frac{v_s}{v_i}) \geq 0$. Using conservation relation (18), we get

$$\sum_{i=1}^{n} p_{i-1}^* = p_t \tag{20}$$

which implies that

$$\sum_{i=1}^{n} \frac{b_i p_{s-1}^*}{1 + \alpha_i p_{s-1}^*} - p_t = 0 \tag{21}$$

We prove that there exists a unique $p_{s-1}^* \in (0, p_t)$ that satisfies the above equation. Define

$$f(x) := \sum_{i=1}^{n} \frac{b_i x}{1 + \alpha_i x} - p_t$$

Since $b_s = 1$ and $\alpha_s = 0$,

$$f(p_t) = b_s p_t + \sum_{i=1, i \neq s}^{n} \frac{b_i p_t}{1 + \alpha_i p_t} - p_t = \sum_{i=1, i \neq s}^{n} \frac{b_i p_t}{1 + \alpha_i p_t} > 0$$

Observe that $f(0) = -p_t < 0$. Since $f(x)$ is a continuous function for $x \geq 0$, by intermediate value property, it follows that f has at least one root in the open interval $(0, p_t)$. Now observe that

$$f'(x) = \sum_{i=1}^{n} \frac{b_i}{(1 + \alpha_i x)^2} > 0 \quad \forall \quad x.$$

Since f is monotonously increasing, f has precisely one root in the open interval $(0, p_t)$. This implies that the value of p_{s-1}^* that satisfies equation (21) is unique and positive. From equation (19) it follows that for a given positive value of p_t, p_{i-1}^* is unique and positive for $i = 1, \ldots, n$. From the conservation relation (20), it follows that $p_i^* \in (0, p_t)$ for $i = 0, \ldots, n-1$. This concludes the proof. ■

From Theorem 3, it follows that there is a unique equilibrium in every positive stoichiometric compatibility class $\mathcal{S}_{p_{\text{in}}}$ corresponding to a nonnegative, nonzero initial substrate concentration vector p_{in}.

IV. GLOBAL ASYMPTOTIC STABILITY

In this section we prove the unique equilibrium concentration vector of the system (14) corresponding to a given positive total substrate concentration p_t is globally asymptotically stable. As mentioned in the introduction, we prove this using two different Lyapunov functions.

Theorem 4: Consider the system of equations (14) with $a_i, v_i > 0$ for $i = 1, \ldots, n$. Define p_t as in (18) and assume that $p_t > 0$. If $p_i(0) \geq 0$ for $i = 1, \ldots, n-1$, then the solution trajectories of (14) converge to the unique positive equilibrium corresponding to the value of p_t.

Remark 5: In the following we give two proofs of the above theorem based on two different Lyapunov functions. The first proof is based on the construction of a *PieceWise Linear in Rates* (PWLR) Lyapunov function coupled with the use of *LaSalle's invariance principle* [23], [24, Section 4.2], [25, pp. 188-189]. The construction of the Lyapunov function in the second proof is based on the strict convexity of the exponential function.

Proof: For $i = 1, \ldots, n$, define r_i as in equation (13). Define p, N and r as in equation (15). Let p^* denote the unique equilibrium concentration vector corresponding to p_t. Thus

$$p^* = \begin{bmatrix} p_0^* \\ p_1^* \\ \vdots \\ p_{n-1}^* \end{bmatrix}$$

Proof 1: Define the PWLR Lyapunov function

$$V(r) = \|r\|_\infty = \max_{1\le j\le n} r_j$$

It is easy to see that V is a piecewise linear function of the components of r. Observe that $V(r)$ is continuous in time, since each component of r is continuous in time. Let

$$V(r) = r_i = \frac{v_i a_i p_{i-1}}{1 + a_i p_{i-1}}. \tag{22}$$

Define $r_0 := r_n$. Differentiating (22) with respect to time, we get

$$\frac{d}{dt}V(r) = \frac{\partial r_i}{\partial p_{i-1}} \cdot \frac{dp_{i-1}}{dt} = \frac{v_i a_i (r_{i-1} - r_i)}{(1 + a_i p_{i-1})^2} \le 0$$

since $r_i = \|r\|_\infty$. Let $\mathfrak{E}$ denote the set of all vectors r for which $\frac{d}{dt}V(r) = 0$. We prove that any $r \in \mathfrak{E}$ that is positively invariant satisfies $r \in \text{span}(\mathbb{1}_n)$ where as before, $\mathbb{1}_n$ denotes a vector of dimension n that has every entry equal to 1. Observe that $[\frac{d}{dt}V(r) = 0] \Longrightarrow [r_{i-1} = r_i]$. For invariance, we need to have

$$\frac{dr_{i-1}}{dt} = \frac{dr_i}{dt} = 0.$$

This implies that $r_{i-2} = r_{i-1} = r_i$ if $i \neq 1$ and $r_{n-1} = r_n = r_1$ if $i = 1$. Thus by induction, it follows that for invariance, we need to have

$$r_1 = r_2 = \cdots = r_n$$

which corresponds to the equilibrium concentration vector p^*. It follows that the only vector $r \in \mathfrak{E}$ that is positively invariant corresponds to the unique equilibrium concentration vector p^*. Since $\frac{d}{dt}V(r) \le 0$ and r is a continuous function of the concentration vector p, by LaSalle's invariance principle, it follows that all solution trajectories of (14) converge to p^*.

Proof 2: We have

$$r_1 = r_2 = \cdots = r_n =: \rho(\text{say})$$

when $p = p^*$, i.e., at the positive equilibrium corresponding to p_t. Define

$$G := \sum_{i=1}^{n} p_{i-1} \ln r_i - p_t \ln \rho - \sum_{i=1}^{n} \frac{1}{a_i} \ln\left(\frac{1 + a_i p_{i-1}}{1 + a_i p^*_{i-1}}\right) \tag{23}$$

We now prove that G is a Lyapunov function for the system (14), by proving the following

1) $G \ge 0$ with equality holding only if $p = p^*$;
2) $\dfrac{dG}{dt} \le 0$ with equality holding only if $p = p^*$.

Observe that the expression for G can be rewritten as follows

$$G = \sum_{i=1}^{n} p_{i-1} \ln\left(\frac{r_i}{\rho}\right) - \sum_{i=1}^{n} \frac{1}{a_i} \ln\left(\frac{\frac{1}{a_i} + p_{i-1}}{\frac{1}{a_i} + p^*_{i-1}}\right)$$

$$= \sum_{i=1}^{n} \left[p_{i-1} \ln\left(\frac{p_{i-1}}{p^*_{i-1}}\right) - \left(p_{i-1} + \frac{1}{a_i}\right) \ln\left(\frac{\frac{1}{a_i} + p_{i-1}}{\frac{1}{a_i} + p^*_{i-1}}\right)\right]$$

We now prove that

$$p_{i-1} \ln\left(\frac{p_{i-1}}{p^*_{i-1}}\right) \ge \left(p_{i-1} + \frac{1}{a_i}\right) \ln\left(\frac{\frac{1}{a_i} + p_{i-1}}{\frac{1}{a_i} + p^*_{i-1}}\right) \tag{24}$$

with equality holding only if $p_{i-1} = p^*_{i-1}$. Define

$$y := (x + \ell) \ln\left(\frac{x + \ell}{x + \ell^*}\right)$$

with ℓ and ℓ^* being constants. Then it can be verified that

$$\frac{dy}{dx} = 1 - z + \ln z$$

where

$$z := \frac{x + \ell}{x + \ell^*}$$

From the strict concavity of the logarithmic function, we have

$$\frac{dy}{dx} = 1 - z + \ln z \le 0$$

with equality holding only if $z = 1$, i.e., if $\ell = \ell^*$. This implies that

$$\ell \ln\left(\frac{\ell}{\ell^*}\right) \ge (x + \ell) \ln\left(\frac{x + \ell}{x + \ell^*}\right)$$

for $x > 0$. Substituting $\ell = p_{i-1}$; $\ell^* = p^*_{i-1}$ and $x = \frac{1}{a_i}$ proves inequality (24) with equality holding only if $p_{i-1} = p^*_{i-1}$. This in turn implies that $G \ge 0$ with equality holding only if $p = p^*$.

We now prove that $\dfrac{dG}{dt} \le 0$ with equality holding only if $p = p^*$. Define

$$G^* := p_t \ln \rho - \sum_{i=1}^{n} \frac{1}{a_i} \ln(1 + a_i p^*_{i-1})$$

From equations (23) and (13), it follows that

$$\begin{aligned} G = & - \sum_{i=1}^{n} \left(p_{i-1} + \frac{1}{a_i}\right) \ln(1 + a_i p_{i-1}) \\ & + \sum_{i=1}^{n} p_{i-1} \ln(v_i a_i p_{i-1}) - G^* \end{aligned}$$

For some $k \in \{0, \ldots, n-1\}$,

$$\begin{aligned} \frac{\partial G}{\partial p_k} = & - \left(p_k + \frac{1}{a_{k+1}}\right) \frac{a_{k+1}}{1 + a_{k+1} p_k} - \ln(1 + a_{k+1} p_k) \\ & + \ln(v_{k+1} a_{k+1} p_k) + p_k \left(\frac{1}{p_k}\right) \\ = & \ln\left(\frac{v_{k+1} a_{k+1} p_k}{1 + a_k p_k}\right) \end{aligned}$$

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

$$\frac{\partial G}{\partial p_k} = \ln r_{k+1}.$$

Define $\Gamma := \begin{bmatrix} \ln r_1 & \ln r_2 & \cdots & \ln r_n \end{bmatrix}$. Now

$$\frac{dG}{dt} = \sum_{i=0}^{n-1} \frac{\partial G}{\partial p_i} \cdot \frac{dp_i}{dt} = \Gamma \frac{dp}{dt} = \Gamma N r \tag{25}$$

For $i = 1, \ldots, n$, define $\gamma_i := \ln r_i$. From equation (25), we get

$$\begin{aligned} \frac{dG}{dt} &= (\gamma_2 - \gamma_1)e^{\gamma_1} + (\gamma_3 - \gamma_2)e^{\gamma_2} + \cdots \\ &+ (\gamma_n - \gamma_{n-1})e^{\gamma_{n-1}} + (\gamma_1 - \gamma_n)e^{\gamma_n} \end{aligned}$$

From the strict convexity of the exponential function, we have

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

for any $\gamma_i, \gamma_j \in \mathbb{R}$, and the equality occurs only when $\gamma_i = \gamma_j$. Hence

$$\frac{dG}{dt} \leq e^{\gamma_2} - e^{\gamma_1} + e^{\gamma_3} - e^{\gamma_2} + \cdots + e^{\gamma_n} - e^{\gamma_{n-1}} + e^{\gamma_1} - e^{\gamma_n} = 0$$

From the above, it follows that $\frac{dG}{dt} = 0$, only when $\gamma_1 = \gamma_2 = \cdots = \gamma_n$. i.e., when $r_1 = r_2 = \cdots = r_n$, which corresponds with the unique positive equilibrium $p = p^*$ associated with the total substrate concentration p_t in our case.

This implies that G is a Lyapunov function for the system (14). Therefore, all solution trajectories of (14) converge to the unique positive equilibrium corresponding to p_t. ■

V. CONCLUSION

In this paper, we have derived the Michaelis Menten approximation of a model of processive phosphorylation futile cycle in which the phosphorylation and the subsequent dephosphorylation of the substrate protein is carried out by more than two enzymes. We have proved that the approximate model admits a unique equilibrium in every positive stoichiometric compatibility class using intermediate value property of continuous functions. We have constructed two different Lyapunov functions for the model in order to prove the global asymptotic stability of the unique equilibrium in every positive stoichiometric compatibility class. The first one is a PWLR Lyapunov function which is very similar to the one in [9] that has been used to prove global stability of the original mass action kinetics model (1). The construction of the second Lyapunov function is based on the strict convexity of the exponential function and is very similar to the Lyapunov function used to prove stability of complex balanced mass action kinetics chemical reaction networks in [26]. It should be noted that the uniqueness and global stability of the equilibrium of the approximate model considered in this paper can also be proved using principles from monotone systems theory described in [27], [28].

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