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An all-encompassing global convergence result for processive multisite phosphorylation systems

Mitchell Eithun[a,*], Anne Shiu[b]

[a] Department of Mathematical Sciences, Ripon College, Ripon, WI 54971, USA
[b] Department of Mathematics, Texas A&M University, College Station, TX 77843-3368, USA

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ABSTRACT

Phosphorylation, the enzyme-mediated addition of a phosphate group to a molecule, is a ubiquitous chemical mechanism in biology. Multisite phosphorylation, the addition of phosphate groups to multiple sites of a single molecule, may be distributive or processive. Distributive systems, which require an enzyme and substrate to bind several times in order to add multiple phosphate groups, can be bistable. Processive systems, in contrast, require only one binding to add all phosphate groups, and were recently shown to be globally stable. However, this global convergence result was proven only for a specific mechanism of processive phosphorylation/dephosphorylation (namely, all catalytic reactions are reversible). Accordingly, we generalize this result to allow for processive phosphorylation networks in which each reaction may be irreversible, and also to account for possible product inhibition. We accomplish this by first defining an all-encompassing processive network that encapsulates all of these schemes, and then appealing to recent results of Marcondes de Freitas et al. that assert global convergence by way of monotone systems theory and network/graph reductions (corresponding to removing intermediate complexes). Our results form a case study into the question of when global convergence is preserved when reactions and/or intermediate complexes are added to or removed from a network.

1. Introduction

We address the question of when global dynamics, such as global convergence to a unique equilibrium, are preserved when reactions and/or intermediate complexes are added to or removed from a biochemical network. Our work forms a case study into this question, by analyzing networks that describe the processive multisite phosphorylation/dephosphorylation of a molecule (a so-called "multiple futile cycle"). We now recall possible mechanisms underlying such a network.

1.1. Mechanisms of processive multisite phosphorylation

A biological process of great importance, *phosphorylation* is the enzyme-mediated addition of a phosphate group to a protein substrate. This process often modifies the function of the substrate. The reactions underlying this mechanism are: $S_0 + E \rightleftharpoons S_0E \to S_1 + E$, where S_i is the substrate with i phosphate groups attached and E is the enzyme.

Additionally, many substrates have more than one *site* at which phosphate groups can be attached. Such multisite phosphorylation may be *distributive* or *processive*, or somewhere in between [1,2]. In distributive phosphorylation, each binding of an enzyme to a substrate results in at most one addition of a phosphate group. In contrast, in processive phosphorylation, when an enzyme catalyzes the addition of a phosphate group, phosphate groups are added to all sites before the enzyme and substrate dissociate.

Most studies on the mathematics of multisite phosphorylation have focused on phosphorylation under a sequential and fully *distributive* mechanism [3–7]. These systems admit bistability [8,9], and the set of steady states is parametrized by monomials [10–12].

As for *processive* phosphorylation, Conradi and Shiu [13] considered the following processive n-site phosphorylation/dephosphorylation network:

* Corresponding author.
E-mail addresses: eithunm@ripon.edu (M. Eithun), annejls@math.tamu.edu (A. Shiu).

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$$S_0 + K \underset{k_2}{\overset{k_1}{\rightleftharpoons}} S_0K \underset{k_4}{\overset{k_3}{\rightleftharpoons}} S_1K \underset{k_6}{\overset{k_5}{\rightleftharpoons}} \dots \underset{k_{2n}}{\overset{k_{2n-1}}{\rightleftharpoons}} S_{n-1}K \xrightarrow{k_{2n+1}} S_n + K$$

$$S_n + F \underset{\ell_{2n}}{\overset{\ell_{2n+1}}{\rightleftharpoons}} S_nF \underset{\ell_{2n-2}}{\overset{\ell_{2n-1}}{\rightleftharpoons}} \dots \underset{\ell_4}{\overset{\ell_5}{\rightleftharpoons}} S_2F \underset{\ell_2}{\overset{\ell_3}{\rightleftharpoons}} S_1F \xrightarrow{\ell_1} S_0 + F \tag{1}$$

They proved that every resulting dynamical system (arising from mass-action kinetics), in contrast with distributive systems, does *not* admit bistability and, moreover, exhibits rigid dynamics. Specifically, each invariant set (specified by conservation laws) contains a unique steady state, which is a global attractor [13]. Conradi and Shiu proved this result via monotone systems theory, by generalizing a result of Angeli and Sontag [14]. Subsequently, using other means, Ali Al-Radhaw [15, Section 8.3], Rao [16], and Marcondes de Freitas et al. [17] established the same global convergence result.

However, in addition to (1), there are other mechanisms for processive phosphorylation, the following being the most common [18]:

$$S_0 + K \underset{k_2}{\overset{k_1}{\rightleftharpoons}} S_0K \xrightarrow{k_3} S_1K \xrightarrow{k_5} \dots \xrightarrow{k_{2n-1}} S_{n-1}K \xrightarrow{k_{2n+1}} S_n + K$$

$$S_n + F \underset{\ell_{2n}}{\overset{\ell_{2n+1}}{\rightleftharpoons}} S_nF \xrightarrow{\ell_{2n-1}} \dots \xrightarrow{\ell_5} S_2F \xrightarrow{\ell_3} S_1F \xrightarrow{\ell_1} S_0 + F \tag{2}$$

Here, in contrast with network (1), the catalytic reactions are not reversible.

Another possible mechanism incorporates *product inhibition*. Instead of detaching when the final phosphate group is attached or removed (e.g., $S_{n-1}K \to S_n + K$), the substrate and enzyme remain bound (e.g., $S_{n-1}K \to S_nK$), and then subsequently detach (e.g., $S_nK \to S_n + K$). Also, the final product (e.g., S_n) may rebind to the enzyme, thereby inhibiting its activity (e.g., $S_nK \leftarrow S_n + K$). Thus, a processive realization of this scheme is:

$$S_0 + K \rightleftharpoons S_0K \longrightarrow S_1K \longrightarrow \dots \longrightarrow S_{n-1}K \longrightarrow S_nK \rightleftharpoons S_n + K$$

$$S_n + F \rightleftharpoons S_nF \longrightarrow \dots \longrightarrow S_2F \longrightarrow S_1F \longrightarrow S_0F \rightleftharpoons S_0 + F \tag{3}$$

There are distributive systems with such product inhibition [9, Scheme 2].

Can the global stability result for (1) be generalized to incorporate the other mechanisms (2–3)? Indeed, we accomplish this in this work:

Theorem 1.1. *For any mass-action kinetics*[1] *system arising from network (1), (2), or (3) and any choice of rate constants, each invariant set $\mathcal{P}$ contains a unique positive steady state and it is the global attractor of $\mathcal{P}$.*

The proof of Theorem 1.1 appears in Section 4. For now, we describe briefly the ideas behind the proof.

1.2. Proving global stability via an all-encompassing network

To prove Theorem 1.1, we construct an *all-encompassing network* that subsumes all three networks (1)–(3), and then prove the global convergence result for this network. In this all-encompassing network, each reaction may be reversible or irreversible, there are m reaction components rather than 2, and the number of binding sites in each component is allowed to differ.

In addition to incorporating networks (1)–(3) as special cases, our all-encompassing network also specializes to 1-site phosphorylation networks (futile cycles) and certain cyclic networks introduced by Rao [16]. Hence, our global convergence result for the all-encompassing network generalizes prior global convergence results, including those of Angeli and Sontag [14] and Donnell and Banaji [19] (for the 1-site network), Conradi and Shiu [13] and Marcondes de Freitas et al. [17] (network (1)), and Rao [16].

To prove our global convergence result, we use monotone systems theory and network/graph reductions. Specifically, we use a graph-theoretic criterion for global convergence from monotone systems theory. This criterion, due to Angeli et al. [20], asserts that a given network is globally convergent if two graphs built from the network, the so-called *R*- and *SR*-graphs, satisfy certain properties. To apply this result efficiently, in light of the fact that our network has many intermediate complexes such as S_0K and S_nF, we additionally use recent results that allow us to remove many of these intermediate complexes before applying the global-convergence criterion. These results, due to Marcondes de Freitas et al. [17,20], state that if the convergence criterion holds after removing intermediate complexes, then the criterion also holds for the original network.

1.3. Outline

The outline of our work is as follows. Section 2 defines reaction networks and their associated dynamical systems. Section 3 introduces the all-encompassing network, Section 4 states the main global convergence result, and Section 5 provides the proof. In Section 6, we mention other approaches to proving global stability, and, in Section 7, we comment on how the systems analyzed in this work compare to other related phosphorylation systems. A discussion appears in Section 8. Finally, Appendix A explains how we check a technical detail, namely, bounded-persistence.

[1] In fact, other kinetics besides mass-action also work (see Remark 5.1).