1. Introduction

The process of phosphorylation and dephosphorylation is a crucial chemical reaction network, which plays an important role in cell signaling [3, 8, 9, 12]. We consider a protein $S$ with $n$ phosphorylation sites, whose phosphorylation and dephosphorylation are respectively regulated by the enzymes kinase $E$ and phosphatase $F$. Following the notation in [1], the reaction network that governs the $n$-site phosphorylation cycle is:

$$S_0 + E \xrightarrow{k_1/k_2} S_1 + E \cdots \xrightarrow{k_{n+1}/k_{n+2}} S_{n+1} + E$$

$$\cdots \xrightarrow{k_{n+5}/k_{n+6}} S_{n-1} + E \xrightarrow{k_{n-5}/k_{n-4}} S_n + F$$

$$\cdots \xrightarrow{k_{n-1}/k_n} S_1 + F \xrightarrow{k_{n-2}/k_{n-3}} S_i + F \cdots$$

$$\cdots \xrightarrow{k_{n-1}/k_n} S_1 + F \xrightarrow{k_{n-2}/k_{n-3}} S_0 + F$$

(1)

where $S_i$ denotes the protein $S$ after $i$ phosphorylation steps. We denote the concentration of the reactants in network (1) as $e = [E]$, $f = [F]$, $s_i = [S_i]$ for $i = 0, \ldots, n$ and $u_i = [FS_{i+1}]$, $y_i = [ES_i]$ for $i = 0, \ldots, n - 1$. Under the mass-action assumption, the ODE system governing the evolution of the concentration of the species in time is as follows:

$$\frac{de}{dt} = -\sum_{i=0}^{n-1} \kappa_{6i+1} s_i e + \sum_{i=0}^{n-1} \kappa_{6i+1} (\kappa_{6i+2} + \kappa_{6i+3}) y_i, \quad \frac{df}{dt} = -\sum_{i=0}^{n-1} \kappa_{6i+4} s_i f + \sum_{i=0}^{n-1} (\kappa_{6i+5} + \kappa_{6i+6}) u_i,$$

(2)

$$\frac{dy_i}{dt} = \kappa_{6i-3} y_{i-1} - \kappa_{6i-1} s_i e + \kappa_{6i+2} y_i + \kappa_{6i+6} u_i - \kappa_{6i-2} s_i f + \kappa_{6i-1} u_{i-1}, \quad \text{for} \quad i = 0, \ldots, n,$$

$$\frac{du_i}{dt} = \kappa_{6i+1} s_i e - (\kappa_{6i+2} + \kappa_{6i+3}) y_i, \quad \frac{dy_0}{dt} = \kappa_{6i+4} s_i f - (\kappa_{6i+5} + \kappa_{6i+6}) u_i \quad \text{for} \quad i = 0, \ldots, n - 1,$$

with the agreement that $\kappa_j = 0$ if $j > 6n$ or $j < 0$. Each solution trajectory is confined in the subspace given by the conservation laws

$$e + \sum_{i=0}^{n-1} y_i = E_{tot}, \quad f + \sum_{i=0}^{n-1} u_i = F_{tot}, \quad s_0 + \sum_{i=1}^n s_i + \sum_{i=1}^{n-1} y_i + \sum_{i=0}^{n-1} y_i = S_{tot}.$$

(3)

where $E_{tot}, F_{tot}$ and $S_{tot}$ correspond to the total amounts of phosphatase, kinase and the protein $S$ in the system. The intersection of such level set with the non-negative orthant is called a stoichiometric compatibility class. The steady states of the system are found by setting the left-hand side of (2) to zero. We refer the reader to [5] or [6] for more information on chemical reaction network theory.

Following the notation in [1], we consider the set of parameters $K_i = \frac{\kappa_{6i+1}}{\kappa_{6i+2} + \kappa_{6i+3}}$ and $L_i = \frac{\kappa_{6i+4}}{\kappa_{6i+5} + \kappa_{6i+6}}$ for $i = 0, \ldots, n - 1$, which correspond to the inverses of the Michaelis-Menten constants (see e.g. Chapter 10) of the (de)phosphorylation events, and define $T_i = \prod_{j=0}^{i-1} \frac{\kappa_{6j+1} K_j}{\kappa_{6j+2} L_j}$ for $i = 0, \ldots, n - 1$ with the convention $T_{-1} = 1$. Then, the solution to the system given by the last 3$n$ steady state equations in the variables $s_{i+1}, y_i, u_i$ for $i = 0, \ldots, n - 1$ is

$$s_{i+1} = T_i e^{i+1} f^{-(i+1)} s_0 \quad y_i = K_i T_{i-1} e^{i+1} f^{-i} s_0 \quad u_i = L_i T_i e^{i+1} f^{-i} s_0.$$

(4)

This gives rise to a parametrization of the set of positive steady states in the variables $e, f, s_0$,

$$\varphi_\kappa : \mathbb{R}^3_{>0} \rightarrow \mathbb{R}^{3n+3}_{>0},$$

(5)

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that is, the image of \( \varphi_\kappa \) is precisely the set of positive steady states. We consider the polynomial function given by the right-hand side of (2), and by replacing its first three entries with the left-hand side of (3) we construct the polynomial function

\[
\psi_\kappa : \mathbb{R}^{3n+3} \rightarrow \mathbb{R}^{3n+3},
\]

whose image is the set if positive steady states in the compatibility class given by \( E_{\text{tot}}, F_{\text{tot}} \) and \( S_{\text{tot}} \).

If, for a given vector of reaction rate constants \( \kappa \), there exist positive \( E_{\text{tot}}, F_{\text{tot}} \), and \( S_{\text{tot}} \) such that some stoichiometric compatibility class has at least two positive steady states, then we say that \( \kappa \) enables multistationarity. If this is not the case, then \( \kappa \) is said to preclude multistationarity.

In this work, we first point out that the monostationarity of the system can be verified by checking the nonnegativity of a particular real polynomial as given in Theorem 3.3. Furthermore, we give a sufficient condition for multistationarity for \( n \)-site phosphorylation in Theorem 4.4. We introduce a general construction for sufficient symbolic multistationarity conditions in Remark 4.3. Moreover, in Theorem 4.4 we present an effective method for checking monostationarity if \( \kappa \) is given explicitly.

2. A Polynomial for Multistationarity

We follow the approach in [7], and reduce the problem of detecting multistationarity to the study of the signs of a multivariate rational function. We apply Theorem 1 in [4], and obtain the following result:

**Proposition 2.1.** Let \( \kappa \in \mathbb{R}_{>0}^{6n} \), \( \psi_\kappa \) be the polynomial function defined in (8) and \( \varphi_\kappa \) the parametrization of the set of positive steady states from (9). Define the following rational function

\[
q_\kappa(e, f, s_0) := (-1)^{3n} \det J_{\psi_\kappa}(\varphi_\kappa(e, f, s_0)).
\]

Then it holds that:

(i) If \( q_\kappa(e, f, s_0) > 0 \) for all \( (e, f, s_0) \in \mathbb{R}_{>0}^3 \), then \( \kappa \) precludes multistationarity.

(ii) If \( q_\kappa(e^*, f^*, s_0^*) < 0 \) for some \( (e^*, f^*, s_0^*) \in \mathbb{R}_{>0}^3 \), then \( \kappa \) enables multistationarity. In this case the stoichiometric compatibility class containing \( \varphi_\kappa(e^*, f^*, s_0^*) \) contains at least two positive steady states.

Based on Proposition 2.1 we study the signs \( q_\kappa \) attains on the positive orthant to determine the set of reaction rate constants that enable multistationarity. To this end, we first reduce the computation of the determinant of a matrix of size \( 3n+3 \), to the computation of the determinant of a matrix of size 3. Let \( \Phi \) be the linear map given by the left-hand side of (3).

**Proposition 2.2.** For any \( \kappa \in \mathbb{R}_{>0}^{6n} \) and \( (e, f, s_0) \in \mathbb{R}_{>0}^3 \), the sign of \( q_\kappa(e, f, s_0) \) agrees with the sign of

\[
\det J_{\Phi \circ \varphi_\kappa}(e, f, s_0).
\]

In view of Proposition 2.1 and 2.2, multistationarity is determined by considering the sign of the determinant of the Jacobian of \( \Phi \circ \varphi_\kappa \). Hence, \( \Phi \circ \varphi_\kappa \) depends on \( \kappa \) through the assembled parameters \( K_i, L_i, T_i \), and the determinant of its Jacobian agrees with the determinant of the following matrix:

\[
J := \begin{pmatrix}
1 + \sum_{i=0}^{n-1} (i+1) a_i x_1^2 x_3 & -\sum_{i=0}^{n-1} i a_i x_2^2 x_3 & \sum_{i=0}^{n-1} a_i x_1 x_2^2 \\
\sum_{i=0}^{n-1} (i+1) b_i x_1 x_3 & 1 - \sum_{i=0}^{n-1} i b_i x_2^2 x_3 & \sum_{i=0}^{n-1} b_i x_1 x_2^2 \\
-1 + \sum_{i=0}^{n-1} (i+1) c_i x_1^2 x_3 x_2 & -\sum_{i=0}^{n-1} i c_i x_1 x_2^2 x_3 & \sum_{i=0}^{n-1} c_i x_2^2 x_3^2
\end{pmatrix},
\]

where \( a_i, b_i, \) and \( c_i \) corresponds to \( K_{T_i-1}, L_i, T_i \) for \( i = 0, \ldots, n-1 \) respectively, and \( x_1 = e, x_2 = f \), and \( x_3 = s_0 \). The determinant of (7) is a polynomial in \( x_1, x_2, x_3 \) with coefficients depending on \( a_i, b_i, c_i \).

From Proposition 2.1 and Proposition 2.2 we see that the region of multistationarity is described by the nonnegativity of the polynomial \( p_\kappa(x_1, x_2, x_3) := \det J \).

**Proposition 2.3.** Let \( \kappa \in \mathbb{R}_{>0}^{6n} \), consider the corresponding parameter vector \( \eta \) and the polynomial \( p_\eta(x_1, x_2, x_3) := \det J \) for \( \kappa \) given in (7). Then:

(i) If \( p_\eta(x_1, x_2, x_3) > 0 \) for all \( (x_1, x_2, x_3) \in \mathbb{R}_{>0}^3 \), then \( \kappa \) precludes multistationarity.

(ii) If \( p_\eta(x_1^*, x_2^*, x_3^*) < 0 \) for some \( (x_1^*, x_2^*, x_3^*) \in \mathbb{R}_{>0}^3 \), then \( \kappa \) enables multistationarity, and the stoichiometric compatibility class containing \( \varphi_\kappa(x_1^*, x_2^*, x_3^*) \) contains at least two positive steady states.
3. Newton Polytope and Positivity

To analyze the possible signs that \( p_\eta(x_1, x_2, x_3) \) attains over the positive orthant, we study its Newton polytope, which we denote by \( N(p_\eta) \). We note that \( p_\eta \) is linear in \( x_1 \), and hence can be expressed as

\[
p_\eta(x_1, x_2, x_3) = P_0(x_2, x_3) + x_1 P_1(x_2, x_3).
\]

Therefore, each exponent vector in \( N(P_0) \) either belong to \( N(p_\eta) \cap \{ x \in \mathbb{N}^3 : x_1 = 0 \} = \{0\} \times N(P_0) \) or \( N(p_\eta) \cap \{ x \in \mathbb{N}^3 : x_1 = 1 \} = \{1\} \times N(P_1) \).

**Theorem 3.1.** For any \( n \), the set of vertices of the Newton polytope \( N(p_\eta) \) of the polynomial \( p_\eta \) consists of the following 10 points:

\[
\{(0, 0, 0), (0, n, 0), (0, 0, 1), (0, 2n, 1), (0, 2, 2), (0, 3n - 2, 2), (1, 0, 0), (1, n - 1, 0), (1, 1, 1), (1, 2n - 2, 1)\}.
\]

**Sketch of Proof:** It is sufficient to determine the vertices of \( N(P_0) \) and \( N(P_1) \). \( P_0 \) is quadratic in \( x_3 \), and it can be expressed as \( P_0(x_2, x_3) = A_2(x_2)x_3^2 + A_{10}(x_2)x_3 + A_{00}(x_2) \) for

\[
A_2(x_2) = \left(1 + \sum_{i=0}^{n-1} c_i x_2^{i+1}\right) \left(\sum_{i=0}^{2n-2} \sum_{i+j=\ell} (i-j)a_{ij} x_2^\ell + A_{10}(x_2)x_3 + A_{00}(x_2) \right), \quad A_{00}(x_2) = 1 + \sum_{i=0}^{n-1} c_i x_2^{i+1}.
\]

The vertices of \( N(P_0) \) are located along the lines \( L_i := \{ x_3 = i \} \), for \( i = 0, 1, 2 \), in the \((x_2, x_3)\)-plane, and correspond to the highest and lowest exponents of the polynomials given in (8). Therefore, the set of vertices of \( N(P_0) \) is contained in \( S_1 = \{(0, 0), (n, 0), (0, 1), (2n, 1), (2, 2), (3n - 2, 2)\} \). To show that this is exactly the set of vertices of \( N(P_0) \), it is enough to verify that the vertices on \( L_4 \) do not lie in the convex hull of the other four points in \( S_1 \). Therefore, the Newton polytope \( N(P_0) \) is the hexagon with vertex set \( S_1 \). \( P_0 \) is linear in \( x_3 \), and it can be expressed as \( P_1(x_2, x_3) = A_{11}(x_2)x_3 + A_{01}(x_2) \) for

\[
A_{11}(x_2) = (1 + x_2) \left(\sum_{i=0}^{2n-2} \sum_{i+j=\ell} (j-i)a_{ij} b_{ij} x_2^\ell \right), \quad A_{01}(x_2) = \sum_{i=0}^{n-1} (a_i + b_i)x_2^i.
\]

Following a similar argument, we see that the vertices of \( N(P_1) \) are \( S_2 = \{(0, 0), (1, n - 1, 0), (1, 1), (2n - 2, 1)\} \). Thus, we conclude that the set of vertices of \( N(p_\eta) \) is

\[
\{(0, 0, 0), (0, n, 0), (0, 0, 1), (0, 2n, 1), (0, 2, 2), (0, 3n - 2, 2), (1, 0, 0), (1, n - 1, 0), (1, 1, 1), (1, 2n - 2, 1)\}.
\]

\( \square \)

**Remark 3.2.** In the full article, we present the \( H \)-description of the Newton polytope \( N(p_\eta) \), and we show that it has 18 edges and 10 facets.

Newton polytopes of a polynomial can be used to determine properties of a polynomial, in particular, its positivity. In what follows, we use a well known result stating that if a polynomial \( P \) with Newton polytope \( N(P) \) is restricted to the polynomial \( P_F \) with support contained in a face \( F \) of \( N(P) \), then any sign which is attained by \( P_F \) is also attained by \( P \), see [7] Proposition 2.3. In particular, if the coefficient of the monomial supported at one of the vertices is negative, then there exists some \( x \in \mathbb{R}_{\geq 0}^n \) such that the polynomial is negative at \( x \).

**Theorem 3.3.** Fix \( \eta \in \mathbb{R}_{\geq 0}^{2n} \).

- The polynomial \( p_\eta \) attains a negative value over \( \mathbb{R}_{\geq 0}^3 \) if and only if \( P_0 \) attains negative values over \( \mathbb{R}_{\geq 0}^2 \).
- \( \eta \) enables multistationarity if and only if \( p_\eta \) attains negative values over \( \mathbb{R}_{\geq 0}^3 \) (equivalently \( P_0 \) attains negative values over \( \mathbb{R}_{\geq 0}^2 \)).

**Sketch of Proof:** By inspecting the coefficients of \( A_4(x_2) \), we conclude that only \( A_2, A_{10}, \) and \( A_{11} \) have negative coefficients. Furthermore, for a fixed \( x_2 > 0 \), we observe that \( A_2(x_2) \) is positive if and only if \( A_{11}(x_2) \) is positive, since both \( A_2 \) and \( A_{11} \) are positive multiples of \( \left(\sum_{\ell=1}^{2n-2} \sum_{i+j=\ell}(j-i)a_{ij} b_{ij} x_2^\ell\right) \). \( \square \)
By Theorem 3.3, multistationarity is completely characterized by the sign of \( P_\kappa \) over \( \mathbb{R}_{\geq 0}^3 \). We rewrite the polynomial as \( P_\kappa \), to indicate dependence on the parameter vector:

\[
P_\kappa(x_2, x_3) = A_2(x_2)x_2^3 + A_{10}(x_2)x_3 + A_{00}(x_2).
\]

This is a polynomial in two variables \( x_2 \) and \( x_3 \). If \( A_2(x_2) < 0 \), then \( P_\kappa \) attains also negative values.

4. Region of Multistationarity and its Connectedness

We investigate the nonnegativity of the polynomial to describe subsets in the parameter region that enable or preclude monostationarity. In order to do so, we utilize circuit polynomials, which are originally defined in [10], to find symbolic nonnegativity conditions.

**Theorem 4.1.** If \( \eta \) is such that \( \kappa_3\kappa_{12} - \kappa_6\kappa_9 < 0 \) or \( \kappa_{6n-9}\kappa_{6n} - \kappa_{6n-3}\kappa_{6n-6} < 0 \), then \( \eta \) enables multistationarity.

**Sketch of Proof.** The term that decides the sign of \( A_2(x_2) \) is

\[
\sum_{i+j=\ell} (i-j)a_ib_j x_2^{\ell+1} = \sum_{i+j=\ell} (i-j)(a_ib_j - a_jb_i)x_2^{\ell+1}.
\]

For \( i, j < n \), we have \( a_ib_j - a_jb_i = T_{i-1}T_{j-1}K_5K_1^{\kappa_{i+2}/\kappa_{i+6}} - \kappa_{i+2}/\kappa_{i+6} \). Therefore, the signs of the coefficients of \( A_2(x_2) \) are decided by the signs of the minors of the following matrix:

\[
M_\kappa = \begin{bmatrix} \kappa_3 & \kappa_9 & \cdots & \kappa_{6n-3} \\ \kappa_6 & \kappa_{9} & \cdots & \kappa_{6n-6} \end{bmatrix}
\]

Note that the two expressions in Theorem 4.1 are the first and last maximal minors of matrix \( M_\kappa \). \( \square \)

**Proposition 4.2.** Let \( a_i+b_i - a_ib_i > 0 \) for all \( i \in \{0, \ldots, n-1\} \). Then, all 2 by 2 minors of \( M_\kappa \) are positive, and \( A_2(x_2) \) is nonnegative.

For a given choice of parameters \( \eta \) that satisfies the conditions of Proposition 4.2, the only exponents of \( P_\kappa(x_2, x_3) \) with negative coefficients are

\[
A_- := \{(2, 1), (3, 1), \ldots, (2n - 2, 1)\}.
\]

If \( n = 2 \), then \( A_- \) consists of a single element, and \( M_\kappa \) has a unique 2 by 2 minor given by \( \kappa_3\kappa_{12} - \kappa_6\kappa_9 \). If \( \kappa_3\kappa_{12} - \kappa_6\kappa_9 > 0 \), then the nonnegativity of \( P_\kappa(x_2, x_3) \) only depends on the coefficient of the single exponent in \( A_- \). In [7] Theorem 3.5], authors provide a symbolic sufficient condition for monostationarity using sums of nonnegative circuit (SONC) polynomials. We further point out that \( P_\kappa(x_2, x_3) \) is nonnegative if and only if it is a SONC polynomial due to [13] Theorem 3.11] when \( n = 2 \). For \( n \geq 3 \), one can extend the the previous approach of decomposing the polynomial \( P_\kappa(x_2, x_3) \) into circuit polynomials, and then write a nonnegativity condition for each circuit polynomial.

**Remark 4.3.** For \( n \geq 2 \), each exponent in \( A_- \) is contained in both of the simplices given by \( \Delta_1 = \{(0, 0), (2, 2), (2n, 1)\} \) and \( \Delta_2 = \{(0, 1), (n, 0), (3n - 2, 2)\} \). One can form \( 2n - 3 \) circuit polynomials from each one of \( \Delta_1 \) and \( \Delta_2 \) by dividing the coefficients of each exponent in \( \text{Vert}(N(P_\kappa)) \) equally into \( 2n - 3 \) circuits. This yields two conditions for each exponent in \( A_- \), which arise from the nonnegativity condition for circuit polynomials (see [10] Theorem 3.8) of the polynomials that we consider for each element of \( A_- \).

The approach given in Remark 4.3 can be improved by considering the other elements of the support that are known to have positive coefficients. But it is not evident how to choose circuits optimally involving these extra exponents for general \( n \). However, if the vector of reaction rate parameters \( \eta \) is fixed from the beginning, then one can give a lower bound for \( P_\kappa(x_2, x_3) \) by solving a relative entropy program. Relative entropy programs are a class of convex problems, which can be solved effectively to a desired accuracy via interior point methods, see e.g. [2].

**Theorem 4.4.** For any \( \kappa \), given a fixed \( \kappa \) such that the conditions of Proposition 4.2 hold, one can find a non-decreasing sequence of lower bounds for \( P_\kappa \) by solving a relative entropy program at each step.

Furthermore, in the full article, we investigate the connectivity of the multistationarity region given by \( X := \{\eta = (\kappa_3, \kappa_6, \ldots, \kappa_{6n}, K_0, \ldots, K_{n-1}, L_0, \ldots, L_{n-1}) \in \mathbb{R}^{4n}_{\geq 0} : \eta \text{ enables multistationarity}\} \).

**Theorem 4.5.** The region \( X \) of multistationarity and the region \( \mathbb{R}^{4n}_{\geq 0}\backslash X \) of monostationarity is path connected for all \( n \).
References


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