N-site phosphorylation systems with 2N-1 steady states

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Introduction 1.1: The Origin

1. Introduction

1.1 The Origin: JMB-Paper by L. Wang & E.D. Sontag

On the number of steady states in a multiple futile cycle

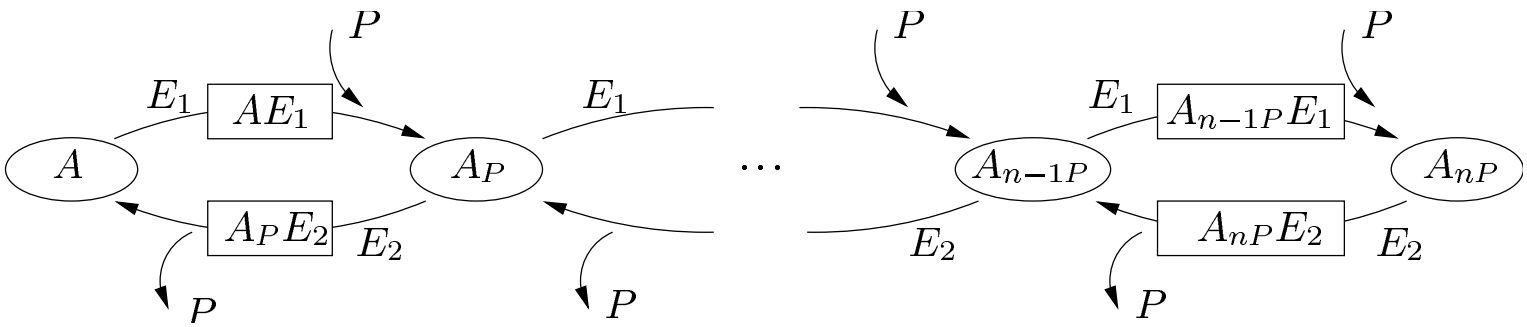
Journal of Math. Biology 57:29–52, 2008

For N-site phosphorylation systems, there are no more than 2N-1 steady states.

'We do not expect the number of steady states to exceed $N+1$ if N is even and N if N is odd.'

'So a natural conjecture would be that the number of steady states never exceeds $N+1$ under any condition.'

1.2 The Network



The network for the sequential distributive phosphorylation and dephosphorylation of protein A at n-sites by a kinase E_1 a phosphatase E_2.
The phosphorylated forms of A are denoted by the subscript nP denoting the number of phosphorylated sites ($A = A_{0\cdot P}$).

$$
\begin{aligned}
E_1 + A_{i-1P} &\underset{k_{3i-1}}{\overset{k_{3i-2}}{\rightleftharpoons}} A_{i-1P}\,E_1 \xrightarrow{k_{3i}} E_1 + A_{iP}, \quad i = 1, \ldots, n \\
E_2 + A_{iP} &\underset{l_{3i-1}}{\overset{l_{3i-2}}{\rightleftharpoons}} A_{iP}\,E_2 \xrightarrow{l_{3i}} E_2 + A_{i-1P}, \quad i = 1, \ldots, n\ .
\end{aligned}
\tag{1.1}
$$

1.2 cont.

With

$$\begin{aligned} x_1 &= E_1\,, \qquad x_2 = A = A_{0P}\,, \qquad x_3 = E_2\,, \\ x_{1+3i} &= A_{(i-1)P}E_1\,, \qquad x_{2+3i} = A_{iP}\,, \qquad x_{3+3i} = A_{iP}E_2 \end{aligned} \tag{1.2}$$

The network

$$\begin{aligned} x_1 + x_{3i-1} &\underset{k_{3i-1}}{\overset{k_{3i-2}}{\rightleftharpoons}} x_{3i+1} \xrightarrow{k_{3i}} x_1 + x_{3i+2}, \\ x_2 + x_{3i+2} &\underset{l_{3i-1}}{\overset{l_{3i-2}}{\rightleftharpoons}} x_{3i+3} \xrightarrow{l_{3i}} x_2 + x_{3i-1} \end{aligned} \tag{1.3}$$

for $i = 1, \ldots, n$ and for $\kappa_{(i)} := (k_{3i-2}, k_{3i-1}, k_{3i}, l_{3i-2}, l_{3i-1}, l_{3i})^T$. Define

$$\kappa := \mathrm{col}\left(\kappa_{(1)}, \ldots, \kappa_{(n)}\right) \in \mathbb{R}^{6n}_{>0}\,. \tag{1.4}$$

.

1.3 The Mass Action ODE-System

From (1.1), one can derive for every n the

– stoichiometric matrix $S \in \mathbb{R}^{(3+3n)\times 6n}$ and the
– rate exponent matrix $\mathcal{Y} = (y_1, ..., y_{6n}) \in \mathbb{R}^{(3+3n)\times 6n}$.

These define two monomial functions $\Phi : \mathbb{R}^{3+3n} \to \mathbb{R}^{6n}$ and $R(\kappa, \cdot) : \mathbb{R}^{3+3n} \to \mathbb{R}^{6n}$ via

$$\Phi(x) := x^{\mathcal{Y}^T} \equiv \mathrm{col}\,(x^{y_1}, \ldots, x^{y_{6n}}) \quad \text{and} \quad R(\kappa, x) := \mathrm{diag}\,(\kappa)\,\Phi(x)\,. \tag{1.5}$$

and the

Dynamical system with mass action kinetics

$$\dot{x} = S\,R(\kappa, x) = S\,\mathrm{diag}\,(\kappa)\,x^{\mathcal{Y}^T}\,. \tag{1.6}$$

The $6n$-dimensional vector $R(\kappa, x)$ is called the reaction rate vector.

$e^{\mu} = \mathrm{col}\,(e^{\mu_i})$, $\ln(\mu) = \mathrm{col}\,(\ln(\mu_i))$, $a^{\ell^T} := \prod_{i=1}^{m} a_i^{\ell_i} = e^{\ell^T \ln(a)}$,

$g^L = \mathrm{col}\,(g^{L_{row\,i}})$ for $g \in \mathbb{R}^m_{>0}$, $L \in \mathbb{Z}^{n\times m}$.

For $n = 3$

The stoichiometric matrix S and the rate exponent matrix $\mathcal{Y}^T$:

$$\left[\begin{array}{ccc|ccc|ccc|ccc|ccc|ccc}
-1 & 1 & 1 & 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 \\
-1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 & -1 & 1 & 1 & 0 & 0 & 0 & -1 & 1 & 1 \\
\hline
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 1 & -1 & 1 & 0 & -1 & 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\hline
0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 & -1 & 1 & 0 & 0 & 0 & 1 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\
\hline
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 1 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & -1
\end{array}\right]$$

$$\mathcal{Y}^T = \left[\begin{array}{ccc|ccc|ccc|ccc|ccc|ccc}
1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\hline
0 & 0 & 1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\hline
1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\hline
0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\hline
1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\hline
0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0
\end{array}\right].$$

1.4 Multistationarity, e.g. for Switching

A matrix Z of conservation laws, providing a basis for the left kernel of S, is given by

$$Z = \left[\begin{array}{ccc|ccc|c|ccc} 1 & 0 & 0 & 1 & 0 & 0 & & 1 & 0 & 0 \\ -1 & 1 & -1 & 0 & 1 & 0 & \cdots & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 & 1 & & 0 & 0 & 1 \end{array}\right] \in \mathbb{R}^{3\times(3+3n)}. \tag{1.7}$$

Definition of Multistationarity

The system $\dot{x} = S\,R(\kappa, x)$ from (1.6) is said to exhibit multistationarity if and only if there exist a positive vector $\kappa \in \mathbb{R}^{6n}_{>0}$ and at least two distinct positive vectors a and b in $\mathbb{R}^{3+3n}_{>0}$ with

$$S\,R(\kappa, a) = 0\,, \tag{1.8a}$$
$$S\,R(\kappa, b) = 0\,, \tag{1.8b}$$
$$Z\,a = Z\,b. \tag{1.8c}$$

(1.8a) and (1.8b) describe the steady state property of a and b whereas (1.8c) asks for these steady states to belong to the same coset of the stoichiometric matrix S.

2.1 Characterization (Generator matrix E)

2. General Reduction Results

Consider the

Mass Action Network

$$\dot{x} = S\,R(\kappa, x) = S\,diag(\kappa)\,x^{\mathcal{Y}^{\mathrm{T}}} \tag{2.1}$$

- with positive pointed polyhedral cone $\mathcal{C} = ker(S) \bigcap \mathbb{R}^{3n+3}_{>0}$ and its generator matrix $E \in \mathbb{R}^{6n\times 3n}_{>0}$ given below,
- and left kernel basis matrix Z (conservation laws, $ZS = 0$).

$$E := \begin{bmatrix} E_0 & & \\ & \ddots & \\ & & E_0 \end{bmatrix} \in \mathbb{R}^{6n\times 3n}_{>0} \quad \text{with} \quad E_0 := \begin{bmatrix} 1 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \tag{2.2}$$

where that the columns of E also form a basis of $\ker(S)$.

The steady state relations for the cone $\mathcal{C}$ are of the form

$$diag(\kappa)\,a^{\mathcal{Y}^{\mathrm{T}}} = E\lambda\,, \quad diag(\kappa)\,b^{\mathcal{Y}^{\mathrm{T}}} = E\nu \quad \text{for} \quad \lambda, \nu \in \mathbb{R}^{3n}_{\geq 0}\,.$$

2.1 Characterization (3D Reduction)

For a given positive steady state a one has the rate constant vector(s)

$$\kappa \;=\; \kappa(a,\lambda) := \operatorname{diag}\left(a^{-\mathcal{Y}^T}\right) E\,\lambda\,. \tag{2.3}$$

A further positive steady state b for this $\kappa \;=\; \kappa(a,\lambda)$ can be written as

$$b \;=\; \operatorname{diag}(e^{\mu})\,a \;=\; \operatorname{diag}\left(\frac{1}{\kappa(a,\lambda)}\right) E\,\nu \qquad (\mu \in \mathbb{R}^{3n+3})$$

with

$$\mathcal{Y}^T\,\mu \;=\; \ln\left[\frac{E\nu}{E\lambda}\right]. \tag{2.4}$$

Two Facts

- The right hand side of (2.4) is in a 2-dimensional subspace (by Fredholm).
- The right kernel of $\mathcal{Y}^T$ is 1-dimensional.

Consequence

$$\mu = L\,\ln(g) \quad \text{for} \quad g = (g_1, g_2, g_3)^T \in \mathbb{R}^3_{>0} \tag{2.5}$$

for the matrix $L \in \mathbb{Z}^{(3+3n)\times 3}$ given below and $b = \operatorname{diag}(e^{\mu})\,a = \operatorname{diag}(g^L)\,a$.

2.1 Characterization (Matrix L)

We choose the matrix L in an (obviously) clever way as

$$L \equiv (L_1, L_2, L_3) := \begin{bmatrix} L(0) \\ L(1) \\ \vdots \\ L(n) \end{bmatrix} \in \mathbb{Z}^{(3+3n)\times 3} \tag{2.6a}$$

for

$$L(0) := \begin{bmatrix} 1 & n-1 & -1 \\ -1 & -n & 0 \\ 1 & n-2 & -1 \end{bmatrix}, \; L(i) := \begin{bmatrix} 0 & i-2 & -1 \\ -1 & i-n & 0 \\ 0 & i-2 & -1 \end{bmatrix} \tag{2.6b}$$

In the expression $b = \mathrm{diag}(g^L)\, a$,

the g_1-exponents are given by the 1st column of L and hence ± 1 (or 0),

the g_3-exponents are given by the 3rd column of L and hence -1 (or 0),

the g_2-exponents are given by the 2nd column of L .

One has: $\ker\left(\mathcal{Y}^T\right) = [L_1]$.

2.1 Characterization (Coset Condition)

With the parameter $a \in \mathbb{R}^{3n+3}_{>0}$:

3D Multistationarity Condition/Coset Condition

$$\Theta(g,a) := Z\left(\operatorname{diag}(g^L) - Id\right) a \;=\; 0\,, \tag{2.7}$$

$$\Theta = (\Theta_1, \Theta_2, \Theta_3)^T\,, \quad g = (g_1, g_2, g_3)^T \in \mathbb{R}^3_{>0}\,.$$

3D Reduction

For $g \neq \underline{1}$ satisfying the rational 3×3-system (2.7), the steady states

$$a \quad \text{and} \quad b := \operatorname{diag}(g^L)\, a$$

are distinct positive steady states for the network $\dot{x} = S\, R(\kappa(a,\lambda), x)$ within <u>one</u> coset of the stoichiometric matrix S.

2.2 Scalar Determinig Equation

Exploiting the properties of our choice of L and taking $g_2 \equiv \xi$: The system

$$\Theta_1 = 0\,, \quad \Theta_3 = 0$$

is linear wrt. g_1 and g_3. Suppressing the a-dependence:

$$g_1 = g_1(\xi) := \quad \xi^{1-n} F_1(\xi)/\Delta(\xi) \overset{!}{>} 0, \tag{2.8a}$$

$$g_3 = g_3(\xi) := \quad \xi^{-1} F_3(\xi)/\Delta(\xi) \overset{!}{>} 0 \tag{2.8b}$$

with linear

$$\Delta(\xi) := \frac{a_1 \xi}{\omega_1} - \frac{a_3}{\omega_3} = \frac{a_1}{\omega_1}(\xi - \xi^*) \tag{2.8c}$$

and with polynomials F_1 and F_3 in ξ of degree $n-1$ and n where F_3 is affine in F_1:

$$F_3(\xi) \;=\; F_{31}(\xi) + \frac{a_1 \xi}{\omega_1} F_1(\xi) \;=\; F_{33}(\xi) + \frac{a_3}{\omega_3} F_1(\xi)\,, \quad \omega := Z\,a\,. \tag{2.8d}$$

Like $\xi \boldsymbol{u} - \boldsymbol{v} \;=\; \xi \boldsymbol{v} - \boldsymbol{v} + \xi(\boldsymbol{u} - \boldsymbol{v}) \;=\; \xi \boldsymbol{u} - \boldsymbol{u} + (\boldsymbol{u} - \boldsymbol{v})$.

The resulting $\Theta_2 \overset{!}{=} 0$ can be written in polynomial form as

$$Q(\xi) := \; J_0(\xi)\, F_3(\xi) \;-\; J_1(\xi) \left[F_1(\xi)\right]^2 \;-\; J_2(\xi)\, F_1(\xi)\, F_3(\xi) \overset{!}{=} \; 0\,.$$

2.2 cont.

The resulting $\Theta_2 \stackrel{!}{=} 0$ can be written in polynomial form as

$$Q(\xi) \equiv Q_1(\xi) := Q_{10}(\xi)\Delta^2(\xi) - Q_{11}(\xi)\,\Delta(\xi)\,F_1(\xi) + Q_{12}(\xi)\,F_1^2(\xi) \stackrel{!}{=} 0\,.$$

or equivalently as

$$Q(\xi) \equiv Q_3(\xi) := Q_{30}(\xi)\Delta^2(\xi) - Q_{31}(\xi)\,\Delta(\xi)\,F_1(\xi) + Q_{32}(\xi)\,F_1^2(\xi) \stackrel{!}{=} 0\,.$$

We now take linear combinations with nonnegative h_1 and h_3, $h := (h_1, h_3) \neq (0,0)$, and define

$$P_h(\xi) := \omega_2 h_1 Q_1(\xi) + \omega_2 h_3 Q_3(\xi) = A_h(\xi)\Delta^2(\xi) + B_h(\xi)\Delta(\xi)F_1(\xi) - C_h(\xi)F_1^2(\xi) \tag{2.9}$$

with certain polynomials $A_h(\xi)$, $B_h(\xi)$ and $C_h(\xi)$. Note: P_h is of degree $2n+1$.

A zero ξ_0 of P_h will be called admissible if it satisfies

$$\xi_0 > 0\,, \quad g_1(\xi_0) > 0 \quad (\text{i.e.,} \Delta(\xi_0)F_1(\xi_0) > 0)$$

and hence automatically $g_3(\xi_0) > 0$. Note: nonlinear a-dependence!

2.2 cont.

In the 'symmetric' case $h = (\omega_1, \omega_3)$ one finds $A_h(\xi) > 0$ and $C_h(\xi) > 0$ for $\xi > 0$ and thus

Scalar determining equation for $\xi > 0$, $\xi \neq \xi^*$

The determining equation for admissible solutions $g \in \mathbb{R}^3_{>0}$ of the coset condition (2.7) is given by

$$\theta(\xi, a) := 2C_h(\xi, a)F_1(\xi, a) - \Delta(\xi, a)\left[B_h(\xi, a) + \left(B_h^2(\xi, a) + 4A_h(\xi, a)C_h(\xi, a)\right)^{1/2}\right] = 0\ . \tag{2.10}$$

Any positive zero $\xi = \xi(a)$ of $\theta(\xi, a)$, different from $\xi^*(a)$, defines a positive steady state

$$b = \operatorname{diag}\left(g^L\right) a \neq a$$

of the network (1.6) for $g = (g_1(\xi(a), a), \xi(a), g_3(\xi(a), a))^T$ from (2.8).

In the 'unsymmetric' cases $h = (0, \omega_3)$ or $h = (\omega_1, 0)$ one has to check whether $A_h(\xi) > 0$ and $C_h(\xi) > 0$ hold for $\xi > 0$ in order to establish (2.10).

Remark: There are at most $2n - 1$ admissible zeros for P_h.

3.1 Computational Aspects for $n = 3$

3. Computational Aspects – Counterexamples

In the 'unsymmetric' case $h = (0, \omega_3)$ we denote $A_{(0,\omega_3)}$ by A_0 etc.
A_0 and C_0 turn out to be positive for $\xi > 0$ so that (2.10) applies.
Recalling (2.8c),

$$\Delta(\xi) = \frac{a_1 \xi}{\omega_1} - \frac{a_3}{\omega_3},$$

and suppressing the a-dependencies one has (2.10) in the form

$$\begin{aligned} 2C_0(\xi)F_1(\xi) + \quad & a_3\omega_1 \left[B_0(\xi) + \left(B_0^2(\xi) + 4A_0(\xi)C_0(\xi)\right)^{1/2}\right] \\ = \quad & a_1\omega_3\xi\left[B_0(\xi) + \left(B_0^2(\xi) + 4A_0(\xi)C_0(\xi)\right)^{1/2}\right] \end{aligned}$$

where the n parameters

$$a_{3j+1} \quad \text{for} \quad j = 1, 2, \ldots, n$$

appear just on the left-hand side and in a linear way. So they might be tuned to fulfill some prescribed constraints.

3.1 cont.($n = 3$)

For the triple phosphorylation ($n = 3$):

- We choose a positive $a \in \mathbb{R}^{3\cdot 3+3}_{>0}$ and fix the rate constant vector

$$\kappa = \kappa(a) = \operatorname{diag}\left(a^{-\mathcal{Y}^T}\right) E\,\lambda \quad \text{with} \quad \lambda = \underline{1}$$

  so that a is a positive steady state of the network (1.6).
- Obviously, one has $\theta_0(1, a) = 0$.
- In particular, we choose a of the form

$$a^* = \left(1, 1, 1 | a_4, 1, 1 | a_7, 1, 0.1 | a_{10}, 0.32, 60\right)^T \in \mathbb{R}^{12}_{>0} \tag{3.1}$$

  and compute *analytically* the remaining $n = 3$ parameters a_4, a_7 and a_{10} so that $\theta_0(\xi, a^*)$ has the triple zero $\xi = 1$ and a further zero $\xi = \frac{1}{2}$. The resulting numerical values (up to 4 decimals) are given by

$$a_4 := a_4^* = 5.9026(84)\ldots\,,\ a_7 := a_7^* = 2.1344(85)\ldots\,,\ a_{10} := a_{10}^* = 248.9413(34)\ldots\,. \tag{3.2}$$

  The rate constant vector $\kappa = \kappa(a^*)$ is positive.
- Finally, we vary the 10th component:

$$a = a^* + \delta\, e_{10}\,, \quad -.05 < \delta < .05\,,$$

  in (3.1), leading to the bifurcation diagram in Figure 1 in the (δ, ξ)-plane.

3.1 Bifurcation diagram

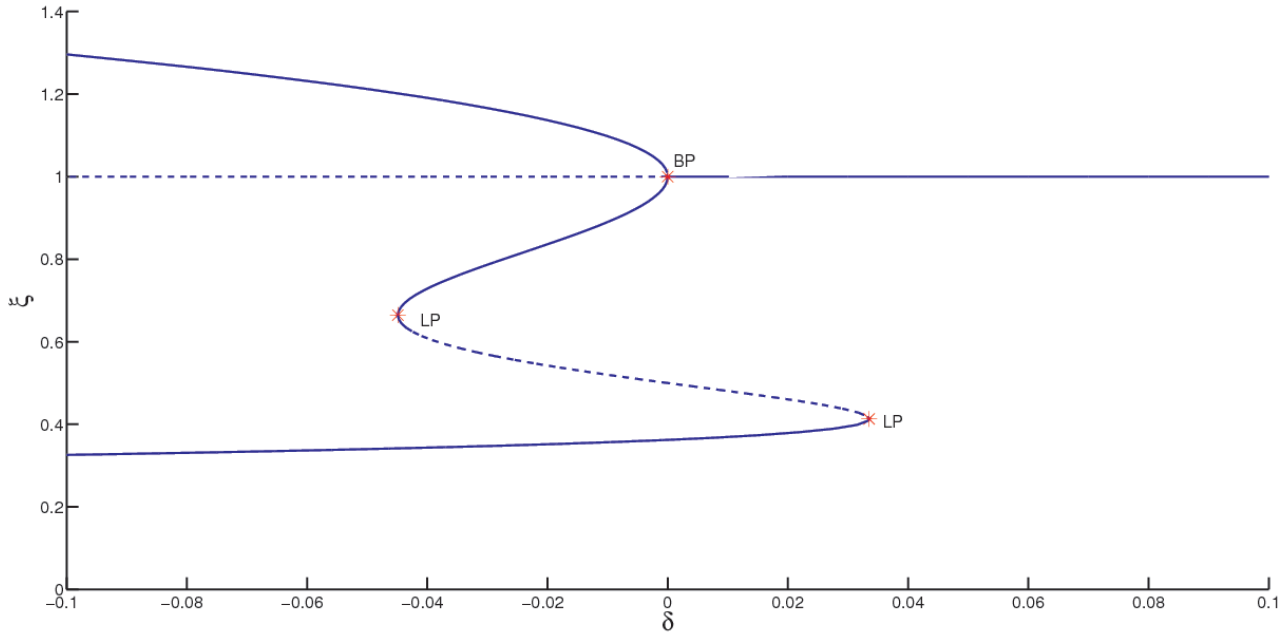


Figure: 4.1 Numerical continuation of $\theta_0(\xi, a) = 0$ from (2.10) with the data (3.1)&(3.2).

Pitchfork bifurcation at $(\delta_0, \xi_0) = (0, 1)$ (BP) and two saddle node bifurcations (LP) at $(\delta_-, \xi_-) = (-.04488..., .66691(4)...)$ and $(\delta_+, \xi_+) = (.03352..., .41262(522)...)$.
For $\delta = 0$ one encounters the prescribed triple zero $\xi = 1$, the zero $\xi = \frac{1}{2}$ and an additional zero near .36222(562)....
Solid lines correspond to ξ's yielding exponentially stable steady states, dashed lines to ξ's yielding unstable steady states.

3.1 Numerical values

For $\delta = -.03$, the numerical values for the five admissible zeros $\xi^{(j)}$ of (2.10) and the five admissible steady states $b^{(j)}$ of (1.6) can be found below.

Phos. #	$b^{(1)}$	$b^{(2)}$	$b^{(3)}$	$b^{(4)} \equiv a$	$b^{(5)}$
0	1.4730	1.2198	1.0793	1	0.9618
	4.7498	2.4000	1.4726	1	0.7700
	4.2424	2.1440	1.3722	1	0.8246
1	41.3012	17.2813	9.3826	5.9026	4.3718
	1.6493	1.3655	1.1583	1	0.8980
	6.9970	2.9277	1.5895	1	0.7406
2	5.1859	3.5554	2.6688	2.1344	1.8438
	0.5726	0.7768	0.9112	1	1.0474
	0.2429	0.1665	0.1250	.1	0.0863
3	209.9882	235.8919	244.8175	248.9113	250.7710
	0.0636	0.1414	0.2293	.32	0.3909
	50.6175	56.8616	59.0132	60	60.4482
ξ	0.3472	0.5689	0.7866	1	1.1662

Table: The five admissible steady states $b^{(j)}$ of (1.6) for $\delta = -.03$ and the corresponding zeros $\xi^{(j)}$ of (2.10) up to 4 decimals: the numerical values of the rate constant vectors $\kappa = \kappa(a)$ and $\kappa(a^*)$ coincide up to the first 4 decimals, but $\kappa_{14}(a) = \kappa_{15}(a) = 0.00401749\ldots$ and $\kappa_{14}(a^*) = \kappa_{15}(a^*) = 0.00401701\ldots$ differ.

3.2 Case $n = 4$

- For $n \geq 3$, the above argument can be applied to an n-site phosphorylation to create networks with $n+1$ steady states for (1.6) by tuning the n parameters a_{3j+1} so that

$$n+1 \text{ steady states may be prescribed.}$$

- For odd n, one is then generically expecting $n+2$ such steady states.

Using this rationale for even $n = 4$, we have constructed a phosphorylation network with a determining equation (2.10) with 5 prescribed zeros at 0.5, 1, 1.03, 1.05 and 1.07 by choosing $a \in \mathbb{R}^{15}_{>0}$ as

$$a_1 = 1\,, \quad a_2 = 1\,, \quad a_3 = 1\,, \quad a_4 = 1.983448\,, \quad a_5 = 1\,, \quad a_6 = 1\,,$$
$$a_7 = 469.6162955\,, \quad a_8 = 1\,, \quad a_9 = 400\,, \quad a_{10} = 73.8036\,, \quad a_{11} = .32\,, \quad a_{12} = 60\,,$$
$$a_{13} = .5807998\,, \quad a_{14} = 7\,, \quad a_{15} = 1.8\,.$$

As it turns out, this determining equation has two additional positive zeros, one near .59 and one near 51.07. By judicious guessing – see next figure.

3.2 cont.($n = 4$)

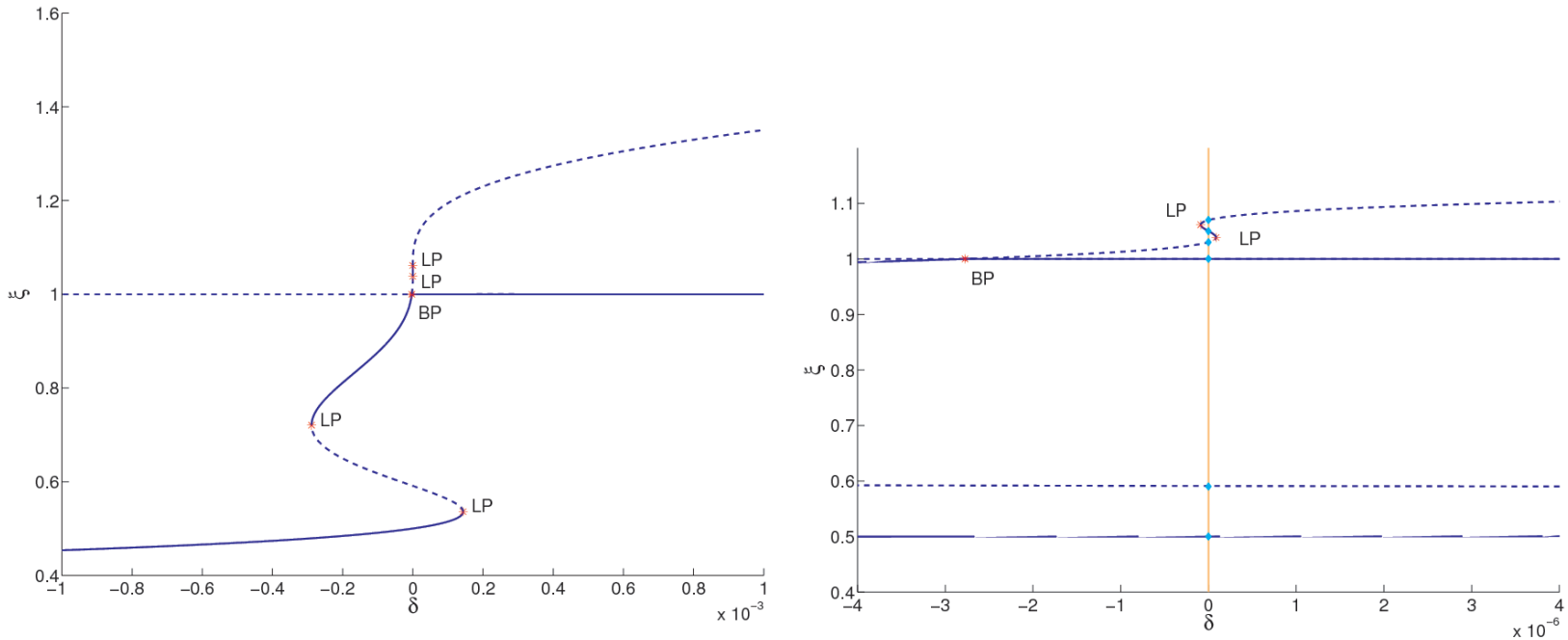


Figure: 4.2 Numerical continuation of $\theta_0(\xi, a) = 0$ from (2.10) with the above data – zoom on the right

There are 6 zeros 0.5, 0.5910929..., 1, 1.03, 1.05 and 1.07 and there is a 7th zero near $\xi = 51.07286$.
Solid lines correspond to ξ's yielding exponentially stable steady states, dashed lines to ξ's yielding unstable steady states. The label LP denotes saddle-node bifurcation points, the label BP transcritical bifurcation points.

4.1 Relations to sign patterns/orthants

4. Geometry of Multistationarity

Relations to sign patterns/orthants

- For the steady states of the 3-site phosphorylation system we observe that the sign vector for $\ln\left(b^{(j+1)}/b^{(j)}\right)$ is given by

$$sign\left(\ln\left(b^{(j+1)}/b^{(j)}\right)\right) = (-,-,-|-,-,-|-,+,-|+,+,+)^T =: s_2$$

  for $j = 1, 2, 3, 4$ so that these steady states are ordered with respect to s_2.

- The steady states of our 4-site phosphorylation system are not ordered in such a way.

4.2 Geometric constraints on multistationarity

Let $\kappa \in \mathbb{R}^{6n}_{>0}$ be given and assume network (1.1) admits two distinct positive vectors a and b with $S\,R(\kappa, a) = S\,R(\kappa, b) = 0,\ Z\,(b - a) = 0$.

Geometry and Reconstruction

Then the steady state concentrations a_1 and b_1 of the kinase together with the steady state concentrations a_3 and b_3 of the phosphatase and the steady state concentrations a_2 and b_2 of the unphosphorylated protein allow the reconstruction of the ratios

$$(g^L)_i = \frac{b_i}{a_i}\,, \quad i = 4, \ldots, 3 + 3n,$$

in the following way:

$$\Gamma^T_{(0)} = (\Gamma_{E_1}, \Gamma_A, \Gamma_{E_2}) = \Big(\frac{b_1}{a_1}, \frac{b_2}{a_2}, \frac{b_3}{a_3}\Big) \quad \text{and} \quad \xi = \frac{\Gamma_{E_1}}{\Gamma_{E_2}} = \frac{b_1/a_1}{b_2/a_3}\,,$$

with $\Gamma^T_{(1)} = (\Gamma_A\Gamma_{E_1},\ \xi\Gamma_A,\ \xi\Gamma_A\Gamma_{E_2}) = \Big(\frac{b_4}{a_4}, \frac{b_5}{a_5}, \frac{b_6}{a_6}\Big)$ and

$$\Gamma^T_{(i)} = (\Gamma_{A_{(i-1)P}E_1}, \Gamma_{A_{iP}}, \Gamma_{A_{iP}E_2}) = \xi^{i-1}\Big(\frac{b_4}{a_4}, \frac{b_5}{a_5}, \frac{b_6}{a_6}\Big) = \Big(\frac{b_{1+3i}}{a_{1+3i}}, \frac{b_{2+3i}}{a_{2+3i}}, \frac{b_{3+3i}}{a_{3+3i}}\Big)$$

for $i = 1, \ldots, n$. In particular one has for $i = 1, \ldots, n-1$

$$\xi = \frac{\Gamma_{E_1}}{\Gamma_{E_2}} = \frac{\Gamma_{A_P}}{\Gamma_A} = \frac{\Gamma_{A_{(i+1)P}}}{\Gamma_{A_{iP}}} = \frac{\Gamma_{A_{iP}E_1}}{\Gamma_{A_{(i-1)P}E_1}} = \frac{\Gamma_{A_{(i+1)P}E_2}}{\Gamma_{A_{iP}E_2}}\,. \tag{4.1}$$

4.2 cont.

Consider the experimental investigation of a specific multisite phosphorylation system (1.1) whereby the rate constants κ and the total concentrations are fixed, but might not (all) be known. Suppose we know a priori that the system exhibits multistationarity.

Then steady state data of the concentration of kinase, phosphatase and protein in two different steady states a and b (for these total concentrations) are sufficient to reconstruct all fractions $\frac{b_i}{a_i}$ of the two steady states. That is:

Measurements and Reconstruction

It suffices to measure a_1, a_2, a_3 and b_1, b_2, b_3 to reconstruct all the ratios

$\frac{b_i}{a_i}$, $i = 1, \ldots, 3 + 3n$.

4.4 A graphical test to exclude multistationarity

Suppose for the phosphoforms A, A_P, ..., A_{nP} two different sets of steady state values have been measured, i.e.,
there exists data for a_2, a_5, ..., a_{2+3n} and b_2, b_5, ..., b_{2+3n}.

If these belong to two steady states within one and the same coset, then the points

$$\alpha_i := \frac{a_{3i+2}}{a_{3i-1}}\,, \quad \beta_i := \frac{b_{3i+2}}{b_{3i-1}}\,, \quad i = 1, \ldots, n\,,$$

are collinear. Hence:

Exclusion of multistationarity

Measurement of two steady state values for A, ..., A_{nP} suffices to exclude multistationarity in case the points (α_i, β_i) are not collinear.

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