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

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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


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.'
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 ($A = A_{0P}$).

1.1. \[ E_1 + A_{i-1P} \xrightleftharpoons[k_{3i-1}]{k_{3i-2}} A_{i-1P} E_1 \xrightarrow[k_{3i}]{k_{3i}} E_1 + A_{iP}, \quad i = 1, \ldots, n \]

1.1. \[ E_2 + A_{iP} \xrightleftharpoons[l_{3i-1}]{l_{3i-2}} A_{iP} E_2 \xrightarrow[l_{3i}]{l_{3i}} E_2 + A_{i-1P}, \quad i = 1, \ldots, n \]
With

\[ x_1 = E_1, \quad x_2 = A = A_0P, \quad x_3 = E_2, \]
\[ x_{1+3i} = A_{(i-1)}PE_1, \quad x_{2+3i} = A_iP, \quad x_{3+3i} = A_iPE_2 \]  \hspace{1cm} (1.2)

The network

\[
\begin{align*}
x_1 + x_{3i-1} & \xrightarrow{k_{3i-2}} x_{3i+1} & \xrightarrow{k_{3i}} x_1 + x_{3i+2}, \\
x_2 + x_{3i+2} & \xrightarrow{l_{3i-2}} x_{3i+3} & \xrightarrow{l_{3i}} x_2 + x_{3i-1}
\end{align*}
\]  \hspace{1cm} (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

\[ \kappa := \text{col} \left( \kappa(1), \ldots, \kappa(n) \right) \in \mathbb{R}_{>0}^{6n}. \]  \hspace{1cm} (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 $\mathcal{Y} = (y_1, \ldots, y_{6n}) \in \mathbb{R}^{(3+3n) \times 6n}$.

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

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

(1.5)

and the

Dynamical system with mass action kinetics

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

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

\[
e^{\mu} = \text{col}(e^{\mu_i}), \quad \ln(\mu) = \text{col}(\ln(\mu_i)), \quad a^{\ell^T} := \prod_{i=1}^{m} a_i^{\ell_i} = e^{\ell^T \ln(a)},
\]

$$g^L = \text{col}(g^{L\text{row }i}) \text{ for } g \in \mathbb{R}^{m}, L \in \mathbb{Z}^{n \times m}.$$
For $n = 3$

The stoichiometric matrix $S$ and the rate exponent matrix $Y^T$:
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

$$
Z = \begin{bmatrix}
1 & 0 & 0 \\
-1 & 1 & -1 \\
0 & 0 & 1
\end{bmatrix}
\begin{bmatrix}
1 & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & 1
\end{bmatrix}
\cdots
\begin{bmatrix}
1 & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & 1
\end{bmatrix}
\in \mathbb{R}^{3 \times (3+3n)}.
$$

(1.7)

**Definition of Multistationarity**

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

$$
SR(\kappa, a) = 0, 
$$

(1.8a)

$$
SR(\kappa, b) = 0, 
$$

(1.8b)

$$
Z a = Z b. 
$$

(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 $S$. 

(144x267)
2.1 Characterization of Multistationarity

2. General Reduction Results

Consider the Mass Action Network

\[ \dot{x} = S R(\kappa, x) = S \text{diag}(\kappa) x^{Y^T} \] (2.1)

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

\[ E := \begin{bmatrix} E_0 & \cdots & E_0 \end{bmatrix} \in \mathbb{R}^{6n \times 3n}_{>0} \text{ with } E_0 := \begin{bmatrix} 1 & 0 & 1 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \\ 0 & 1 & 1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \] (2.2)

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

The steady state relations for the cone \( C \) are of the form

\[ \text{diag}(\kappa) a^{Y^T} = E \lambda, \quad \text{diag}(\kappa) b^{Y^T} = E \nu \quad \text{for} \quad \lambda, \nu \in \mathbb{R}^{3n}_{\geq 0}. \]
2.1 Characterization (3D Reduction)

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

\[ \kappa = \kappa(a, \lambda) := \text{diag} \left( a - Y^T \right) E \lambda. \]  

(2.3)

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

\[ b = \text{diag}(e^\mu) a = \text{diag} \left( \frac{1}{\kappa(a, \lambda)} \right) E \nu \quad (\mu \in \mathbb{R}^{3n+3}) \]

with

\[ Y^T \mu = \ln \left[ \frac{E \nu}{E \lambda} \right]. \]  

(2.4)

Two Facts

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

Consequence

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

(2.5)

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

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

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

(2.6a)

for

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

(2.6b)

In the expression $b = \text{diag}(g^L) a$, the $g_1$-exponents are given by the 1st column of $L$ and hence $\pm 1$ (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 \( a \in \mathbb{R}^{3n+3}_\geq 0 \):

3D Multistationarity Condition / Coset Condition

\[
\Theta(g, a) := Z \left( \text{diag}(g^L) - Id \right) a = 0, \\
\Theta = (\Theta_1, \Theta_2, \Theta_3)^T, \quad g = (g_1, g_2, g_3)^T \in \mathbb{R}^3_\geq 0.
\] (2.7)

3D Reduction

For \( g \neq 1 \) satisfying the rational \( 3 \times 3 \)-system (2.7), the steady states

\[
a \quad \text{and} \quad b := \text{diag}(g^L) a
\]

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

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

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

with linear

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

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

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

Like $\xi u - v = \xi v - v + \xi (u - v) = \xi u - u + (u - v)$.

The resulting $\Theta_2 \uparrow 0$ can be written in polynomial form as

$$Q(\xi) := J_0(\xi) F_3(\xi) - J_1(\xi) [F_1(\xi)]^2 - J_2(\xi) F_1(\xi) F_3(\xi) \uparrow 0.$$
2.2 cont.

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

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

or equivalently as

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

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

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

(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 $\xi_0$ 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!
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

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

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

$$b = \text{diag}(g^L) a \neq a$$

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. 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

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

where the \( n \) parameters

\[
a_{3j+1} \quad \text{for} \quad j = 1, 2, ..., n
\]

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 \( a \in \mathbb{R}^{3+3} \) and fix the rate constant vector

\[
\kappa = \kappa(a) = \text{diag } \left( a^{1/3} \right) E \lambda \quad \text{with} \quad \lambda = 1
\]

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

\[
a^* = (1, 1, 1|a_4, 1, 1|a_7, 1, 0.1|a_{10}, 0.32, 60)^T \in \mathbb{R}_{>0}^{12}
\]

(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

\[
a_4 := a_4^* = 5.9026(84)\ldots, \quad a_7 := a_7^* = 2.1344(85)\ldots, \quad a_{10} := a_{10}^* = 248.9413(34)\ldots.
\]

(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 \((\delta, \xi)\)-plane.
3.1 Triple phosphorylation \((n = 3)\)

### 3.1 Bifurcation diagram

![Bifurcation Diagram](image)

**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 \((\delta_-, \xi_-) = (-0.04488..., 0.66691(4)...)\) and \((\delta_+, \xi_+) = (0.03352..., 0.41262(522)...)\). For \(\delta = 0\) one encounters the prescribed triple zero \(\xi = 1\), the zero \(\xi = \frac{1}{2}\) and an additional zero near \(0.36222(562)\).

| Solid lines correspond to \(\xi\)'s yielding exponentially stable steady states, dashed lines to \(\xi\)'s yielding unstable steady states. |
3.1 Numerical values

For $\delta = -0.03$, 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.

<table>
<thead>
<tr>
<th>Phos. #</th>
<th>$b^{(1)}$</th>
<th>$b^{(2)}$</th>
<th>$b^{(3)}$</th>
<th>$b^{(4)} \equiv a$</th>
<th>$b^{(5)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.4730</td>
<td>1.2198</td>
<td>1.0793</td>
<td>1</td>
<td>0.9618</td>
</tr>
<tr>
<td></td>
<td>4.7498</td>
<td>2.4000</td>
<td>1.4726</td>
<td>1</td>
<td>0.7700</td>
</tr>
<tr>
<td></td>
<td>4.2424</td>
<td>2.1440</td>
<td>1.3722</td>
<td>1</td>
<td>0.8246</td>
</tr>
<tr>
<td>1</td>
<td>41.3012</td>
<td>17.2813</td>
<td>9.3826</td>
<td>5.9026</td>
<td>4.3718</td>
</tr>
<tr>
<td></td>
<td>1.6493</td>
<td>1.3655</td>
<td>1.1583</td>
<td>1</td>
<td>0.8980</td>
</tr>
<tr>
<td></td>
<td>6.9970</td>
<td>2.9277</td>
<td>1.5895</td>
<td>1</td>
<td>0.7406</td>
</tr>
<tr>
<td>2</td>
<td>5.1859</td>
<td>3.5554</td>
<td>2.6688</td>
<td>2.1344</td>
<td>1.8438</td>
</tr>
<tr>
<td></td>
<td>0.5726</td>
<td>0.7768</td>
<td>0.9112</td>
<td>1</td>
<td>1.0474</td>
</tr>
<tr>
<td></td>
<td>0.2429</td>
<td>0.1665</td>
<td>0.1250</td>
<td>0.1</td>
<td>0.0863</td>
</tr>
<tr>
<td>3</td>
<td>209.9882</td>
<td>235.8919</td>
<td>244.8175</td>
<td>248.9113</td>
<td>250.7710</td>
</tr>
<tr>
<td></td>
<td>0.0636</td>
<td>0.1414</td>
<td>0.2293</td>
<td>0.32</td>
<td>0.3909</td>
</tr>
<tr>
<td></td>
<td>50.6175</td>
<td>56.8616</td>
<td>59.0132</td>
<td>60</td>
<td>60.4482</td>
</tr>
</tbody>
</table>

$\xi$ | 0.3472 | 0.5689 | 0.7866 | 1 | 1.1662 |

**Table:** The five admissible steady states $b^{(j)}$ of (1.6) for $\delta = -0.03$ 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 $a \in \mathbb{R}_{>0}^{15}$ as

\[
\begin{align*}
  a_1 &= 1, & a_2 &= 1, & a_3 &= 1, & a_4 &= 1.983448, & a_5 &= 1, & a_6 &= 1, \\
  a_7 &= 469.6162955, & a_8 &= 1, & a_9 &= 400, & a_{10} &= 73.8036, & a_{11} &= .32, & a_{12} &= 60, \\
  a_{13} &= .5807998, & a_{14} &= 7, & a_{15} &= 1.8.
\end{align*}
\]

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 \(\xi\)’s yielding exponentially stable steady states, dashed lines to \(\xi\)’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

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

\[
\text{sign} \left( \ln \left( \frac{b^{(j+1)}}{b^{(j)}} \right) \right) = (-, -, -|-, -, -|-, +, -|+, +, +)^T = s_2
\]

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 $SR(\kappa, a) = SR(\kappa, b) = 0$, $Z(b - a) = 0$.

**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:

$$\Gamma^T(0) = (\Gamma E_1, \Gamma A, \Gamma E_2) = \left(\frac{b_1}{a_1}, \frac{b_2}{a_2}, \frac{b_3}{a_3}\right)$$

and

$$\xi = \frac{\Gamma E_1}{\Gamma E_2} = \frac{b_1}{a_1}/b_2/a_3,$$

with

$$\Gamma^T(1) = (\Gamma A\Gamma E_1, \xi\Gamma A, \xi\Gamma A\Gamma E_2) = \left(\frac{b_4}{a_4}, \frac{b_5}{a_5}, \frac{b_6}{a_6}\right)$$

and

$$\Gamma^T(i) = (\Gamma A(i-1)P E_1, \Gamma A i P, \Gamma A i P E_2) = \xi^{i-1} \left(\frac{b_4}{a_4}, \frac{b_5}{a_5}, \frac{b_6}{a_6}\right) = \left(\frac{b_{1+3i}}{a_{1+3i}}, \frac{b_{2+3i}}{a_{2+3i}}, \frac{b_{3+3i}}{a_{3+3i}}\right)$$

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

$$\xi = \frac{\Gamma E_1}{\Gamma E_2} = \frac{\Gamma A P}{\Gamma A} = \frac{\Gamma A(i+1)P}{\Gamma A i P} = \frac{\Gamma A i P E_1}{\Gamma A(i-1)P E_1} = \frac{\Gamma A(i+1)P E_2}{\Gamma A i P E_2}. \quad (4.1)$$
Consider the experimental investigation of a specific multisite phosphorylation system (1.1) whereby the rate constants $\kappa$ 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 $A, A_P, \ldots, A_{nP}$ two different sets of steady state values have been measured, i.e., there exists data for $a_2, a_5, \ldots, a_{2+3n}$ and $b_2, b_5, \ldots, b_{2+3n}$.

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 $A, \ldots, A_{nP}$ suffices to exclude multistationarity in case the points $(\alpha_i, \beta_i)$ are not collinear.


