

# Sharp equilibrium and stable-state capacities of multisite phosphorylation

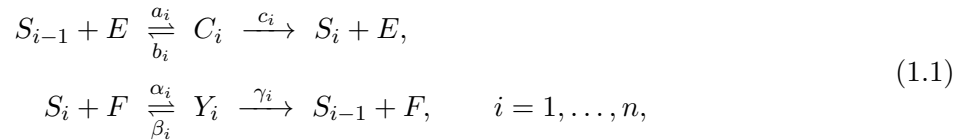
## Abstract

For the sequential distributive  $n$ -site phosphorylation cycle with one kinase and one phosphatase, the maximal number of positive equilibria is  $2n - 1$  and the maximal number of locally asymptotically stable equilibria is  $n$ , for every  $n \geq 1$ . The stable-state ceiling includes non-hyperbolic attractors. Both bounds are attained simultaneously at common conserved totals by rational positive rate constants and rational hyperbolic equilibria:  $n$  sinks alternate with  $n - 1$  saddles, each with one unstable direction. This configuration persists on a nonempty open subset of the full parameter space. An explicit polynomial recurrence and interlacing construct the equilibria. Stability follows by separating equilibrium geometry from kinetic time scales, retaining enzyme-bound substrate in the slow variables, and controlling a path matrix with positive rank-one feedback. Further results give a diagonal Lyapunov certificate, a quantitative eigenvalue splitting law, exact finite-size witnesses and a three-site robustness and recovery certificate. An accompanying registered Lean theorem establishes equilibrium attainment; the broader capacity results are proved here by conventional arguments.

## 1 Introduction

### 1.1 Background

Reversible phosphorylation at several sites is one of the most common mechanisms by which cells regulate protein function [4, 34]. In the simplest mechanistic description a single kinase  $E$  adds phosphate groups to a substrate one site at a time in a fixed order, a single phosphatase  $F$  removes them in the reverse order, and each enzyme releases the substrate after every catalytic event. This is the *sequential distributive* mechanism, or multiple futile cycle. Under mass-action kinetics it is the reaction network



where  $S_i$  is the substrate with  $i$  phosphorylated sites,  $C_i$  and  $Y_i$  are the kinase–substrate and phosphatase–substrate complexes, and all  $6n$  rate constants are strictly positive. There are  $3n + 3$  species and three conserved totals,

$$E_T = E + \sum_{i=1}^n C_i, \quad F_T = F + \sum_{i=1}^n Y_i, \quad S_T = \sum_{i=0}^n S_i + \sum_{i=1}^n (C_i + Y_i). \tag{1.2}$$

The set of nonnegative concentrations with prescribed positive totals is a *compatibility class*; it is invariant under the dynamics and has dimension  $3n$ . Throughout this paper every statement about stability refers to the dynamics *within* a compatibility class, that is, with the three totals fixed.

Markevich, Hoek and Kholodenko observed that the two-site cycle can be bistable although it has no feedback other than competition for the shared enzymes [32]. This started a long line of work on the equilibria of (1.1); see [23, 7, 35, 18, 27, 8, 25, 6, 3, 15, 36] and the surveys [11, 13, 14]. The network has become a standard test case for algebraic and analytic methods in reaction network theory: it is large, biologically meaningful, and its steady-state variety has a monomial parametrization [33].

Three results frame the present work. Wang and Sontag [37] proved that for all rate constants and all totals there are at most  $2n - 1$  positive equilibria; they also exhibited  $n + 1$  ( $n$  even) or  $n$  ( $n$  odd) equilibria and conjectured that  $n + 1$  is never exceeded. Flockerzi, Holstein and Conradi [19] refuted that conjecture by constructing five equilibria for  $n = 3$  and seven for  $n = 4$ , so that the ceiling  $2n - 1$  is attained for  $n \leq 4$ ; their method requires the solution of a system of polynomial inequalities that grows with  $n$ . These constructions leave a gap between the universal ceiling and the known all-size lower bounds; related parameter-region results appear in [16]. Feliu, Rendall and Wiuf [17] proved “unlimited multistability”: for every  $n$  there are parameters with  $2\lfloor n/2 \rfloor + 1$  equilibria of which  $\lfloor n/2 \rfloor + 1$  are asymptotically stable. Their proof coalesces equilibria in a Michaelis–Menten limit and uses a centre-manifold and singular-perturbation argument, and they explain why that limit cannot produce  $2n - 1$  equilibria when  $n \geq 3$ . Explicit parameter regions with  $2\lfloor n/2 \rfloor + 1$  equilibria are described in [21], and numerical evidence for growth of the number of stable states with  $n$  goes back to [35]. Thus for  $n \geq 5$  there has been a gap between  $2n - 1$  and the largest number of equilibria known to occur, and for  $n \geq 3$  a gap between the largest number of stable equilibria known to occur for all  $n$ , namely  $\lfloor n/2 \rfloor + 1$ , and any proved ceiling. No upper bound on the number of stable equilibria other than the trivial  $2n - 1$  appears to have been stated.

## 1.2 Results

For a rate vector  $\kappa \in \mathbb{R}_{>0}^{6n}$  and totals  $T = (E_T, F_T, S_T) \in \mathbb{R}_{>0}^3$  let  $e(\kappa, T)$  be the number of positive equilibria of (1.1) in the class determined by  $T$ ; let  $h(\kappa, T)$  be the number of those at which the Jacobian of the vector field, restricted to the stoichiometric subspace, is Hurwitz (all eigenvalues in the open left half-plane); and let  $c(\kappa, T)$  be the number of those that are locally asymptotically stable relative to the class. Clearly  $h \leq c \leq e$ . We call

$$\mathcal{E}_n = \max_{\kappa, T} e(\kappa, T), \quad \mathcal{H}_n = \max_{\kappa, T} h(\kappa, T), \quad \mathcal{C}_n = \max_{\kappa, T} c(\kappa, T)$$

the *equilibrium capacity*, the *Hurwitz capacity* and the *stable-state capacity* of the  $n$ -site cycle. In each case the count is taken for one common rate vector and one common triple of totals; equilibria of different systems or different classes are never combined.

**Theorem 1.1** (Sharp capacities). *For every integer  $n \geq 1$ ,*

$$\mathcal{E}_n = 2n - 1, \quad \mathcal{H}_n = \mathcal{C}_n = n.$$

*More precisely:*

- (a) *For all positive rate constants and totals, the compatibility class contains at most  $n$  locally asymptotically stable equilibria. Equilibria whose linearization is singular or has imaginary eigenvalues are included in this count.*
- (b) *There are positive rational rate constants and totals for which the class contains exactly  $2n - 1$  equilibria, all positive, rational and hyperbolic;  $n$  of them are sinks and the other  $n - 1$  are saddles whose Jacobian has exactly one eigenvalue in the open right half-plane.*

- (c) *The configuration in (b) occurs on a nonempty open subset of  $\mathbb{R}_{>0}^{6n+3}$ , the space of all rate constants and totals.*

The inequality  $\mathcal{E}_n \leq 2n - 1$  is the theorem of Wang and Sontag, and  $\mathcal{E}_n = 2n - 1$  was known for  $n \leq 4$  [19]. For  $n = 1$  the unique equilibrium is globally attracting [1], and for  $n = 2$  two stable equilibria occur [25]; to our knowledge, a proof that  $n$  stable equilibria occur is new for every  $n \geq 3$ , and so is the ceiling (a) in the generality stated. Part (a) bounds the number of attracting equilibria. It does not bound the number of attractors of other kinds, such as periodic orbits, and the theorem does not assert that every trajectory converges to an equilibrium.

The equilibria in (b) are explicit. Order them by the ratio  $u = E/F$  of free kinase to free phosphatase and label them  $X_0, \dots, X_{2n-2}$ . The construction realizes any prescribed values of these ratios (Theorem 4.8), and in the stable realization the even-indexed equilibria are the sinks. The latter are obtained by splitting a single equilibrium of multiplicity  $2n - 1$ , and the splitting is governed by an exact law.

**Theorem 1.2** (Splitting law). *Let  $n \geq 2$  and let  $\xi_0 < \dots < \xi_{2n-2}$  be real numbers. In the family of systems constructed in Section 7, depending on a parameter  $\delta > 0$  and having equilibria  $X_0(\delta), \dots, X_{2n-2}(\delta)$  with  $\sqrt{1 + 8E/F} = 3 + \delta\xi_j$  at  $X_j(\delta)$ , the restricted Jacobian at  $X_j(\delta)$  has  $3n - 1$  eigenvalues whose real parts are negative and bounded away from zero, and one real eigenvalue*

$$\lambda_j(\delta) = -a\delta^{2n-2} \prod_{k \neq j} (\xi_j - \xi_k) (1 + O(\delta)), \quad \delta \rightarrow 0, \quad (1.3)$$

with a constant  $a > 0$  that does not depend on  $j$  and is given by (7.2). In particular  $\lambda_j < 0$  for even  $j$  and  $\lambda_j > 0$  for odd  $j$ .

Within this family the separation of the equilibria in any smooth observable is of order  $\delta$ , while the slowest relaxation time is of order  $\delta^{-(2n-2)}$ . Section 8 records this and further consequences of the proof: a closed formula for the stability margin of the limiting transverse dynamics, which decays like  $n^2(2/9)^n$ ; an explicit diagonal Lyapunov matrix, which makes the construction insensitive to the choice of the first  $n - 1$  catalytic currents; and the elementary corollary that a single cycle can hold  $q$  stable equilibrium labels if and only if  $n \geq q$ . Section 9 contains exact rational witnesses of Theorem 1.1(b) for  $n \leq 7$  with small parameters, and a three-site benchmark in declared physical units with a rigorous local certificate for independent perturbations of all 21 parameters.

### 1.3 Idea of the proof

*The ceiling.* The compatibility class is a compact convex polytope, the vector field points inward or is tangent on its boundary, and there are no equilibria on the boundary. Hence the Brouwer degree of the negative vector field on the interior of the class is  $+1$ . When all equilibria are nondegenerate this gives  $n_+ - n_- = 1$ , where  $n_{\pm}$  are the numbers of equilibria of index  $\pm 1$ , and with  $n_+ + n_- \leq 2n - 1$  we get  $n_+ \leq n$ . A sink has index  $+1$ . To include degenerate attractors and degenerate parameter values we use two classical facts: an asymptotically stable equilibrium has index  $+1$  whether or not it is hyperbolic, and, because the mass-action vector field depends linearly on the rate constants through a matrix of full rank, equilibria are nondegenerate for a dense set of rate vectors.

*The equilibria.* At a positive equilibrium all concentrations are monomials in  $u = E/F$ ,  $s = S_0$  and  $f = F$ , and the totals are  $E_T = uf(1 + sB(u))$ ,  $F_T = f(1 + suD(u))$ ,  $S_T = sA(u) + usf(B(u) + D(u))$  for polynomials  $A, B, D$  whose  $3n$  coefficients are arbitrary positive numbers determined by the rate constants. The enzyme totals determine  $s$  and  $f$ , and the equilibria are the solutions of one

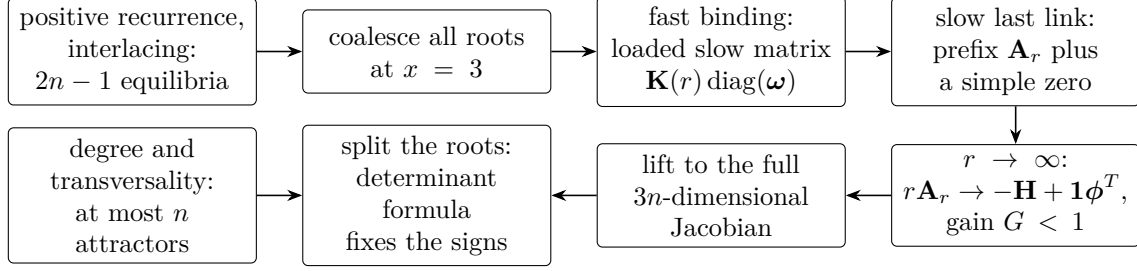


Figure 1: Structure of the proof. The ceiling (bottom left) is independent of the construction. The parameters are chosen in the order  $n$ ,  $r$ , last catalytic current, binding time scale, root separation.

scalar equation  $\Phi(u) = S_T$ . On the locus  $A = (1 + u)D$ ,  $S_T = E_T + F_T$ , the numerator of  $\Phi - S_T$  is a quadratic form whose discriminant is a square multiple of  $1 + 8u$ ; in the variable  $x = \sqrt{1 + 8u}$  it factors, and the equation becomes the linear interpolation condition  $(x - 1)(B - rD) = 2(r - u)D$ ,  $r = E_T/F_T$ . The even and odd parts of  $\prod_j (x - x_j)$  solve this problem for  $r = \infty$  and are generated by a recurrence with visibly positive coefficients; a Bézout-type quotient moves the solution to finite  $r$ ; and positivity of the quotient follows because the two polynomials have real, negative, interlacing roots.

*Stability.* The  $3n$  rate constants that do not enter the equilibrium equations can be chosen freely. We let all prescribed roots coalesce at  $x = 3$  and use this freedom in three steps (Figure 1). First, binding and unbinding are made fast. The slow variables are then the inventories  $\Lambda_i = S_i + C_{i+1} + Y_i$  of substrate with  $i$  phosphate groups, *including* the substrate bound to either enzyme; discarding the bound substrate would change the slow dynamics. The linearized slow dynamics is  $\mathbf{K} \text{diag}(\omega)$ , where  $\omega$  is the vector of catalytic currents and  $\mathbf{K} = (-\mathbf{R} + \mathbf{1}\mathbf{h}^T)\mathbf{M}^{-1}\mathbf{R}^T$  is formed from the path incidence matrix  $\mathbf{R}$ , a positive definite “mass matrix”  $\mathbf{M}$ , and a feedback vector  $\mathbf{h}$  that expresses enzyme sequestration. Second, the current through the last site is made small. The spectrum is then that of the leading  $(n - 1) \times (n - 1)$  block of  $\mathbf{K}$ , plus a simple zero that is forced by coalescence. Third, as the kinase excess  $r$  tends to infinity,  $r$  times this block converges to  $\mathbf{B}_* = -\mathbf{H} + \mathbf{1}\phi^T$ , where  $\mathbf{H}$  is the Dirichlet-type matrix of a weighted path and  $\phi$  is entrywise positive. This is a positive-feedback perturbation of a stable matrix, and its stability is decided by the scalar gain  $G = \phi^T \mathbf{H}^{-1} \mathbf{1}$ . The moments of the coalesced polynomials give

$$1 - G = \frac{v(50n - 45)}{9(14 - 8v)} > 0, \quad v = (3n - 2) \left(\frac{2}{9}\right)^{n-1}.$$

Finally the roots are split again. Every equilibrium inherits  $3n - 1$  stable eigenvalues, and the sign of the remaining real eigenvalue is read off from a determinant formula. The order of the choices is  $n$ , then  $r$ , then the last current, then the binding time scale, then the root separation; no estimate uniform in  $n$  is needed.

The general strategy of coalescing equilibria and then controlling the transverse spectrum by a singular perturbation is that of [17]; see also [25]. What is new here is an equilibrium geometry that reaches the maximal count, a reduction that keeps the enzyme-bound substrate, and a transverse stability proof that works for every  $n$ .

## 1.4 Scope

Theorem 1.1 is a statement about the deterministic, well-mixed mass-action model (1.1) with irreversible catalytic steps. It counts stable equilibria. It is not a statement about Shannon capacity,

stochastic retention times, switching between states, or inheritance through cell division, and the open set in (c) comes without an estimate of its size. Section 11 returns to these points.

## 1.5 Formal verification and reproducibility

The equilibrium-attainment theorem is registered in Palomar. A later Lean development addresses the broader capacity and quantitative claims, but its complete independent verification encountered a memory limit. Section 10 distinguishes these records from the conventional proofs and exact computations used in this manuscript. The computational programs and saved outputs are supplied in a separate companion archive.

## 1.6 Organization

Section 2 parametrizes the equilibria, proves a determinant formula for the restricted Jacobian, and excludes boundary equilibria. Section 3 proves the ceiling. Section 4 constructs  $2n-1$  nondegenerate equilibria. Section 5 introduces the kinetic freedom and the loaded slow system. Section 6 proves stability of the transverse dynamics for large  $r$ . Section 7 lifts the spectrum to the full system and proves Theorems 1.1 and 1.2. Sections 8–11 contain consequences, exact examples, the formal verification, and a discussion.

# 2 The mechanism, its equilibria, and a determinant formula

Let  $V = V_\kappa$  be the mass-action vector field of (1.1) on  $\mathbb{R}^{3n+3}$ , with coordinates

$$(S_0, \dots, S_n, E, F, C_1, \dots, C_n, Y_1, \dots, Y_n),$$

and let  $W: \mathbb{R}^{3n+3} \rightarrow \mathbb{R}^3$  be the linear map (1.2). The totals are conserved, so  $V$  takes values in  $\ker W$ . For positive  $T = (E_T, F_T, S_T)$  the compatibility class is  $\mathcal{P}_T = \{X \geq 0 : WX = T\}$ .

## 2.1 Pools, the stoichiometric subspace, and the boundary

For  $1 \leq i \leq n$  let

$$Z_i = \sum_{k=i}^n S_k + \sum_{k=i+1}^n C_k + \sum_{k=i}^n Y_k \tag{2.1}$$

be the amount of substrate carrying at least  $i$  phosphate groups, where  $C_k$  is counted with  $k-1$  groups and  $Y_k$  with  $k$ . Binding and unbinding do not change  $Z_i$ , the kinase catalytic step at site  $i$  increases it, and the phosphatase catalytic step at site  $i$  decreases it; hence

$$\dot{Z}_i = c_i C_i - \gamma_i Y_i. \tag{2.2}$$

The functions  $(C_1, \dots, C_n, Y_1, \dots, Y_n, Z_1, \dots, Z_n)$  are linear coordinates on  $\ker W$ : from them and the totals one recovers  $S_n, \dots, S_1$  successively from (2.1), and then  $E, F, S_0$  from (1.2). In these coordinates a binding reaction changes exactly one complex coordinate and no  $Z_k$ , and the catalytic reactions at site  $i$  change  $Z_i$  and no other  $Z_k$ . Therefore the  $6n$  reaction vectors span  $\ker W$ , and  $\ker W$  is the stoichiometric subspace; its dimension is  $3n$ .

**Lemma 2.1.** *Let  $T \in \mathbb{R}_{>0}^3$ . The class  $\mathcal{P}_T$  is a compact convex polytope with nonempty relative interior, the vector field is inward or tangent on each of its faces, and every equilibrium in  $\mathcal{P}_T$  is strictly positive.*

*Proof.* Every concentration is bounded by one of the totals, so  $\mathcal{P}_T$  is compact; it is convex and closed by definition. An interior point is obtained by giving all complexes a small positive concentration and distributing the remaining substrate positively among the  $S_i$ . If a species has concentration zero, every reaction that consumes it has rate zero, so its derivative is nonnegative; this is the face condition. At an equilibrium,  $\dot{C}_i = \dot{Y}_i = 0$  gives  $C_i = p_i S_{i-1} E$  and  $Y_i = q_i S_i F$  with  $p_i = a_i/(b_i + c_i)$  and  $q_i = \alpha_i/(\beta_i + \gamma_i)$ . If  $E = 0$ , then all  $C_i$  vanish and  $E_T = 0$ ; so  $E > 0$ , and likewise  $F > 0$ . By (2.2),  $c_i p_i E S_{i-1} = \gamma_i q_i F S_i$ , so the  $S_i$  are all positive or all zero, and the latter would give  $S_T = 0$ . Then the complexes are positive as well.  $\square$

## 2.2 Parametrization of the positive equilibria

For given rate constants put

$$p_i = \frac{a_i}{b_i + c_i}, \quad q_i = \frac{\alpha_i}{\beta_i + \gamma_i}, \quad t_i = \prod_{k=1}^i \frac{c_k p_k}{\gamma_k q_k} \quad (t_0 = 1), \quad (2.3)$$

and define three polynomials with positive coefficients,

$$A(u) = \sum_{i=0}^n t_i u^i, \quad B(u) = \sum_{i=0}^{n-1} B_i u^i, \quad D(u) = \sum_{i=0}^{n-1} D_i u^i, \quad (2.4)$$

$$B_{i-1} = p_i t_{i-1}, \quad D_{i-1} = q_i t_i \quad (1 \leq i \leq n).$$

The letter  $D$  always denotes this polynomial; the phosphatase complexes are called  $Y_i$ .

**Proposition 2.2.** (i) *The positive equilibria of (1.1) are exactly the points*

$$\begin{aligned} S_i &= t_i u^i s \quad (0 \leq i \leq n), & E &= u f, & F &= f, \\ C_i &= B_{i-1} u^i s f, & Y_i &= D_{i-1} u^i s f \quad (1 \leq i \leq n), \end{aligned} \quad (2.5)$$

with  $(u, s, f) \in \mathbb{R}_{>0}^3$ . Their totals are

$$E_T = u f (1 + s B(u)), \quad F_T = f (1 + s u D(u)), \quad S_T = s A(u) + u s f (B(u) + D(u)). \quad (2.6)$$

(ii) *Conversely, let  $t_0 = 1$  and let  $t_1, \dots, t_n$ ,  $B_0, \dots, B_{n-1}$ ,  $D_0, \dots, D_{n-1}$  be arbitrary positive numbers. For any positive numbers  $b_i, \beta_i, \gamma_i$  ( $1 \leq i \leq n$ ), the rate constants*

$$c_i = \gamma_i \frac{D_{i-1}}{B_{i-1}}, \quad a_i = (b_i + c_i) \frac{B_{i-1}}{t_{i-1}}, \quad \alpha_i = (\beta_i + \gamma_i) \frac{D_{i-1}}{t_i} \quad (2.7)$$

*are positive and have the given numbers as their coefficients (2.4).*

*Proof.* At an equilibrium,  $C_i = p_i S_{i-1} E$ ,  $Y_i = q_i S_i F$  and, by (2.2),  $c_i C_i = \gamma_i Y_i$ , which gives  $S_i = (t_i/t_{i-1})(E/F)S_{i-1}$ . With  $u = E/F$ ,  $s = S_0$ ,  $f = F$  this is (2.5). Conversely, at a point of the form (2.5) the complex components of  $V$  vanish and  $c_i C_i = \gamma_i Y_i$  for every  $i$ . Then  $a_i S_{i-1} E - b_i C_i = c_i C_i$  and  $\alpha_i S_i F - \beta_i Y_i = \gamma_i Y_i$ , so  $\dot{S}_i = -c_{i+1} C_{i+1} + c_i C_i - \gamma_i Y_i + \gamma_{i+1} Y_{i+1} = 0$  (terms with index 0 or  $n+1$  are absent), and  $\dot{E} = \dot{F} = 0$  because the complexes are stationary. Summation gives (2.6). Part (ii) is a substitution: (2.7) gives  $p_i = B_{i-1}/t_{i-1}$ ,  $q_i = D_{i-1}/t_i$  and  $c_i p_i/(\gamma_i q_i) = t_i/t_{i-1}$ .  $\square$

Thus the positive equilibria, and their number in each class, depend on the  $6n$  rate constants only through the  $3n$  coefficients of  $A, B, D$ . The remaining  $3n$  degrees of freedom  $b_i, \beta_i, \gamma_i$  affect the dynamics, including stability, but not the equilibria. We call the coefficients the *geometry* and  $(b_i, \beta_i, \gamma_i)$  the *kinetics*. This separation is the organizing principle of the paper.

### 2.3 The scalar equation

Fix positive totals and put  $r = E_T/F_T$ ,

$$L(u) = B(u) - rD(u), \quad M(u) = B(u) - uD(u). \quad (2.8)$$

Dividing the first two equations of (2.6) gives  $r - u = suL(u)$ . Hence a positive equilibrium with  $u \neq r$  satisfies  $(r - u)L(u) > 0$ , and then

$$s(u) = \frac{r - u}{uL(u)}, \quad f(u) = \frac{F_T}{1 + s(u)uD(u)} = \frac{F_T L(u)}{M(u)}, \quad (2.9)$$

where we used  $L + (r - u)D = M$ ; in particular  $M(u) = L(u)(1 + suD(u))$  is nonzero and has the sign of  $L(u)$ . Conversely, for every  $u > 0$  with  $(r - u)L(u) > 0$  the formulas (2.9) define positive  $s, f$  such that (2.5) has enzyme totals  $E_T, F_T$ . On the open set  $\mathcal{U} = \{u > 0 : (r - u)L(u) > 0\}$  define the *substrate-total function*

$$\Phi(u) = s(u)A(u) + u s(u)f(u)(B(u) + D(u)). \quad (2.10)$$

The positive equilibria with totals  $(E_T, F_T, S_T)$  and  $u \neq r$  correspond bijectively to the solutions  $u \in \mathcal{U}$  of  $\Phi(u) = S_T$ . This reduction is the one used in [37], from which we take the following bound.

**Theorem 2.3** (Wang and Sontag [37, Theorem 3]). *For every  $n \geq 1$ , all positive rate constants and all positive totals, the system (1.1) has at most  $2n - 1$  positive equilibria with those totals.*

### 2.4 The restricted Jacobian

Put  $y = (S_1, \dots, S_n, C_1, \dots, C_n, Y_1, \dots, Y_n)$  and  $z = (S_0, E, F)$ , and order the totals as  $(S_T, E_T, F_T)$ . Then  $W = (W_y \ I_3)$  in the coordinates  $(y, z)$ , so  $\ker W$  is the graph of  $z = -W_y y$ , and  $y$  is a linear coordinate system on it. Let  $\mathcal{J}$  denote the matrix of  $DV|_{\ker W}$  in the coordinates  $y$ , that is,  $\mathcal{J} = V_{y,y} - V_{y,z}W_y$ , where  $V_y$  denotes the  $y$ -components of  $V$ . An equilibrium is *nondegenerate* if  $\det \mathcal{J} \neq 0$ , *hyperbolic* if  $\mathcal{J}$  has no eigenvalue on the imaginary axis, and a *sink* if  $\mathcal{J}$  is Hurwitz.

**Proposition 2.4** (Determinant formula). *For arbitrary positive rate constants, at any positive equilibrium with  $u = E/F \neq r = E_T/F_T$ ,*

$$\det \mathcal{J} = (-1)^{n+1} \left( \prod_{i=1}^n (b_i + c_i) \alpha_i \gamma_i \right) F^n u M(u) \Phi'(u), \quad (2.11)$$

where  $\Phi$  is formed with the enzyme totals of that equilibrium. In particular the equilibrium is nondegenerate if and only if  $\Phi'(u) \neq 0$ .

*Proof.* By the Schur complement with respect to the block  $I_3$ ,

$$\det \mathcal{J} = \det \begin{pmatrix} V_{y,y} & V_{y,z} \\ W_y & I_3 \end{pmatrix}.$$

Let  $g = (g_Z, g_C, g_Y)$  consist of the  $3n$  functions  $g_{Z,i} = c_i C_i - \gamma_i Y_i$ ,  $g_{C,i} = \dot{C}_i$ ,  $g_{Y,i} = \dot{Y}_i$ . By (2.1)–(2.2),  $g = UV_y$  for a constant unitriangular matrix  $U$ , since  $g_{Z,i}$  is  $\dot{S}_i$  plus components of  $V_y$

that come later in the ordering of  $y$ . Hence the first  $3n$  rows can be replaced by  $(g_y \ g_z)$  without changing the determinant. In the block form belonging to  $y = (S; C, Y)$ ,

$$g_y = \begin{pmatrix} 0 & * \\ * & \Delta \end{pmatrix}, \quad \Delta = \text{diag}(-(b_i + c_i); -(\beta_i + \gamma_i)),$$

and the Schur complement of  $\Delta$  is the Jacobian with respect to  $S_1, \dots, S_n$  of the reduced currents  $c_i p_i S_{i-1} E - \gamma_i q_i S_i F$ . It is lower bidiagonal with diagonal entries  $-\gamma_i q_i F$ . Therefore

$$\det g_y = \prod_i (b_i + c_i)(\beta_i + \gamma_i) \cdot (-1)^n \prod_i \gamma_i q_i F = (-1)^n F^n \prod_i (b_i + c_i) \alpha_i \gamma_i \neq 0.$$

In particular the zero set of  $g$  is locally the graph of a function  $y = \varphi(z)$ , in agreement with Proposition 2.2, and

$$\det \begin{pmatrix} g_y & g_z \\ W_y & I_3 \end{pmatrix} = \det g_y \cdot \det(I_3 - W_y g_y^{-1} g_z) = \det g_y \cdot \det \frac{\partial(S_T, E_T, F_T)}{\partial(S_0, E, F)},$$

where the last matrix is the derivative of the totals along the equilibrium manifold  $z \mapsto (\varphi(z), z)$ , because  $\varphi_z = -g_y^{-1} g_z$ . We compute it in the coordinates  $(u, s, f) = (E/F, S_0, F)$ , for which  $\det \partial(u, s, f)/\partial(S_0, E, F) = -1/F$ . From (2.6),

$$\det \frac{\partial(E_T, F_T)}{\partial(s, f)} = \det \begin{pmatrix} u f B & u(1 + sB) \\ f u D & 1 + s u D \end{pmatrix} = u f (B - u D) = u f M(u) \neq 0, \quad (2.12)$$

so near the given equilibrium the enzyme totals determine  $(s, f)$  as functions of  $u$ , namely (2.9), and the Schur complement of the block (2.12) in  $\partial(S_T, E_T, F_T)/\partial(u, s, f)$  is the derivative of  $S_T$  along that curve, which is  $\Phi'(u)$ . Hence  $\det \partial(S_T, E_T, F_T)/\partial(u, s, f) = u f M(u) \Phi'(u)$ , and collecting the factors gives (2.11).  $\square$

**Corollary 2.5.** *At a positive equilibrium with  $u < r$  and  $\Phi'(u) \neq 0$ , the number of positive real eigenvalues of  $\mathcal{J}$ , counted with multiplicity, is even if  $\Phi'(u) < 0$  and odd if  $\Phi'(u) > 0$ . In particular an equilibrium with  $u < r$  and  $\Phi'(u) > 0$  is unstable.*

*Proof.* Nonreal eigenvalues come in conjugate pairs, so  $\text{sign det } \mathcal{J} = (-1)^{e_-}$ , where  $e_-$  is the number of negative real eigenvalues, and  $3n - e_-$  has the parity of the number  $e_+$  of positive real eigenvalues. Thus  $\text{sign det } \mathcal{J} = (-1)^n (-1)^{e_+}$ . On the other hand  $M(u)$  has the sign of  $L(u)$ , hence of  $r - u$ , and (2.11) gives  $\text{sign det } \mathcal{J} = (-1)^{n+1} \text{sign } \Phi'(u)$ .  $\square$

### 3 A universal ceiling for attracting equilibria

This section proves Theorem 1.1(a). It is independent of the construction. We use the Brouwer degree  $\deg(g, \Omega, 0)$  of a continuous map on a bounded open set without zeros on the boundary, and the index  $\text{ind}(g, X)$  of an isolated zero [12, 31]. At a zero with invertible derivative the index is the sign of the determinant of the derivative. Fix positive totals  $T$ , use  $y$  as a coordinate system on the affine hull of  $\mathcal{P} = \mathcal{P}_T$ , and write  $g_\kappa$  for the vector field in these coordinates; its derivative at an equilibrium is  $\mathcal{J}$ . We always take degrees and indices of  $-g_\kappa$ , so that a sink has index  $\text{sign det}(-\mathcal{J}) = +1$ .

**Lemma 3.1.** (i)  $\deg(-g_\kappa, \text{int } \mathcal{P}, 0) = 1$  for every  $\kappa \in \mathbb{R}_{>0}^{6n}$ . (ii) The set of  $\kappa \in \mathbb{R}_{>0}^{6n}$  for which all equilibria in  $\mathcal{P}$  are nondegenerate is dense. (iii) For such  $\kappa$  the equilibria are finite in number and at most  $n$  of them have index  $+1$ .

*Proof.* (i) Let  $y_0 \in \text{int } \mathcal{P}$  and consider  $g^t(y) = (1-t)g_\kappa(y) + t(y_0 - y)$ ,  $0 \leq t \leq 1$ . On a face of  $\mathcal{P}$  where some species vanishes, the corresponding component of  $g_\kappa$  is nonnegative by Lemma 2.1 and that of  $y_0 - y$  is positive, so  $g^t$  has no zero there for  $t > 0$ ; for  $t = 0$  there is none by Lemma 2.1. (The species  $S_0, E, F$  are affine functions of  $y$ , and the same argument applies to their time derivatives.) By homotopy invariance,  $\deg(-g_\kappa, \text{int } \mathcal{P}, 0) = \deg(y - y_0, \text{int } \mathcal{P}, 0) = 1$ .

(ii) The vector field is linear in the rate constants:  $V_\kappa(X) = \Gamma \text{diag}(X^{\eta_1}, \dots, X^{\eta_{6n}})\kappa$ , where  $\Gamma$  is the stoichiometric matrix and  $X^{\eta_k}$  are the reactant monomials. At a positive  $X$  the monomials are positive and  $\Gamma$  has rank  $3n = \dim \ker W$  by Section 2. Hence  $(y, \kappa) \mapsto g_\kappa(y)$  is a submersion on  $\text{int } \mathcal{P} \times \mathbb{R}_{>0}^{6n}$ , and by the parametric transversality theorem [22, Ch. 2, §3] zero is a regular value of  $g_\kappa$  on  $\text{int } \mathcal{P}$  for almost every  $\kappa$ . By Lemma 2.1 there are no other equilibria.

(iii) Nondegenerate zeros are isolated, and an accumulation point of zeros would be a non-isolated zero in  $\text{int } \mathcal{P}$  or a zero on the boundary; so the zeros are finite in number. Let  $n_\pm$  be the numbers of zeros of index  $\pm 1$ . Additivity of the degree and (i) give  $n_+ - n_- = 1$ , Theorem 2.3 gives  $n_+ + n_- \leq 2n - 1$ , and therefore  $n_+ \leq n$ .  $\square$

The degree identity in (i), for dissipative networks without boundary equilibria, is the basis of the multistationarity criterion of [6]; see also [26]. Positive index does not imply stability, so (iii) alone does not bound the number of attractors in a degenerate system. The next lemma closes this gap.

**Lemma 3.2.** *Let  $X_* \in \text{int } \mathcal{P}$  be an equilibrium that is locally asymptotically stable relative to  $\mathcal{P}$ . Then  $X_*$  is an isolated zero of  $g_\kappa$  and  $\text{ind}(-g_\kappa, X_*) = +1$ .*

*Proof.* This is classical [31]; we include the short argument. Let  $\phi_t$  be the flow and  $\mathcal{B}$  a closed ball around  $X_*$  inside its basin of attraction and inside  $\text{int } \mathcal{P}$ . No point of  $\mathcal{B} \setminus \{X_*\}$  is an equilibrium or lies on a periodic orbit, because its orbit converges to  $X_*$ . Hence  $I - \phi_t$  has no zero on  $\partial\mathcal{B}$  for any  $t > 0$ , and  $\deg(I - \phi_t, \text{int } \mathcal{B}, 0)$  does not depend on  $t > 0$ . Stability and attraction imply that attraction is uniform on the compact set  $\mathcal{B}$ , so for large  $t$  we have  $\phi_t(\mathcal{B}) \subset \text{int } \mathcal{B}'$  for a smaller concentric ball  $\mathcal{B}'$ ; the homotopy  $I - \theta(\phi_t - X_*) - X_*$ ,  $0 \leq \theta \leq 1$ , then has no zero on  $\partial\mathcal{B}$  and shows that the degree is 1. On the other hand  $(I - \phi_t)/t \rightarrow -g_\kappa$  uniformly on  $\partial\mathcal{B}$  as  $t \downarrow 0$ , and  $g_\kappa$  does not vanish there, so  $\deg(-g_\kappa, \text{int } \mathcal{B}, 0) = 1$  as well.  $\square$

*Proof of Theorem 1.1(a).* Suppose that for some  $\kappa$  and  $T$  there are  $n + 1$  locally asymptotically stable equilibria. They are positive by Lemma 2.1. Choose pairwise disjoint closed balls  $\mathcal{B}_0, \dots, \mathcal{B}_n$  around them as in Lemma 3.2. Since  $g_\kappa \neq 0$  on the compact set  $\bigcup_k \partial\mathcal{B}_k$  and  $g_{\kappa'}$  depends continuously on  $\kappa'$ , we have  $\deg(-g_{\kappa'}, \text{int } \mathcal{B}_k, 0) = 1$  for all  $\kappa'$  close to  $\kappa$  and all  $k$ . By Lemma 3.1(ii) we may choose such a  $\kappa'$  for which all equilibria are nondegenerate. Then each  $\mathcal{B}_k$  contains at least one equilibrium of index +1, so there are at least  $n + 1$  of them, which contradicts Lemma 3.1(iii).  $\square$

The argument does not assume that a degenerate attractor persists under perturbation; it only uses the persistence of its index. The model-specific ingredients are the exclusion of boundary equilibria, the rank of the stoichiometric matrix, and Theorem 2.3. It follows that  $\mathcal{H}_n \leq \mathcal{C}_n \leq n$ .

## 4 Construction of $2n - 1$ equilibria

### 4.1 A locus on which the scalar equation factors

**Lemma 4.1.** *Suppose  $A(u) = (1 + u)D(u)$  and  $S_T = E_T + F_T$ , and normalize  $F_T = 2$ , so that  $E_T = 2r$  and  $S_T = 2(r + 1)$ . For  $u \in \mathcal{U}$  put  $\ell = B(u)/D(u) - r$ ,  $\eta = r - u$  and  $x = \sqrt{1 + 8u}$ . Then*

$$\Phi(u) - S_T = \frac{(1 + u)(\eta(\ell + \eta) - 2u\ell^2)}{u\ell(\ell + \eta)} = -\frac{2(1 + u)}{\ell(\ell + \eta)} \left( \ell - \frac{2\eta}{x - 1} \right) \left( \ell + \frac{2\eta}{x + 1} \right). \quad (4.1)$$

*Proof.* Since  $L = D\ell$  and  $M = D(\ell + \eta)$ , (2.9) gives  $s = \eta/(uD\ell)$  and  $f = 2\ell/(\ell + \eta)$ . Therefore

$$\Phi = \frac{(1 + u)\eta}{u\ell} + \frac{2\eta(\ell + r + 1)}{\ell + \eta}, \quad \Phi - 2(r + 1) = \frac{(1 + u)\eta}{u\ell} - \frac{2\ell(r + 1 - \eta)}{\ell + \eta} = (1 + u) \left( \frac{\eta}{u\ell} - \frac{2\ell}{\ell + \eta} \right),$$

because  $r + 1 - \eta = 1 + u$ . This is the first expression. For the second, expand the product and use  $x^2 - 1 = 8u$ :  $-2u(\ell^2 - \frac{4\eta}{x^2 - 1}\ell - \frac{4\eta^2}{x^2 - 1}) = -2u\ell^2 + \eta\ell + \eta^2$ .  $\square$

On  $\mathcal{U} \cap \{u < r\}$  we have  $\ell, \eta > 0$ , so only the first linear factor in (4.1) can vanish, and the equilibrium equation is equivalent to

$$\frac{B(u)}{D(u)} = r + \frac{2(r - u)}{x - 1}, \quad x = \sqrt{1 + 8u}. \quad (4.2)$$

The condition  $A = (1 + u)D$  says  $t_i = D_i + D_{i-1}$ , and  $S_T = E_T + F_T$  says that there is as much substrate as enzyme. They serve only to find a centre for the construction; Theorem 1.1(c) removes both.

### 4.2 The recurrence

Put  $m = n - 1$ . Let  $x_0, \dots, x_{2m}$  be real numbers greater than 1, *not necessarily distinct*, and write

$$u(x) = \frac{x^2 - 1}{8}, \quad u_j = u(x_j), \quad \Pi(x) = \prod_{j=0}^{2m} (x - x_j). \quad (4.3)$$

Start with  $N = 1$  and  $J = (x_0 - 1)/2$ , and for  $k = 0, \dots, m - 1$ , with  $(a, b) = (x_{2k+1}, x_{2k+2})$ , replace  $N, J$  simultaneously by

$$\begin{aligned} N_{\text{new}} &= [8u + (a + 1)(b + 1)]N + 2(a + b)J, \\ J_{\text{new}} &= 4(a + b)uN + [8u + (a - 1)(b - 1)]J. \end{aligned} \quad (4.4)$$

**Lemma 4.2.** *After  $m$  steps  $N = \sum_{i=0}^m N_i u^i$  and  $J = \sum_{i=0}^m J_i u^i$  have degree  $m$ , all coefficients  $N_i, J_i$  ( $0 \leq i \leq m$ ) are positive, and*

$$(x - 1)N(u(x)) - 2J(u(x)) = \Pi(x). \quad (4.5)$$

Moreover

$$N_m = 8^m, \quad J_m = 8^m \frac{\sum_j x_j - 1}{2}, \quad J_0 = J(0) = \frac{1}{2} \prod_j (x_j - 1). \quad (4.6)$$

*Proof.* Let  $N, J$  have positive coefficients in degrees  $0, \dots, k$  and none above. The coefficient of  $u^i$  in  $N_{\text{new}}$  is  $8N_{i-1} + (a+1)(b+1)N_i + 2(a+b)J_i$  and in  $J_{\text{new}}$  it is  $4(a+b)N_{i-1} + 8J_{i-1} + (a-1)(b-1)J_i$ , with  $N_{-1} = J_{-1} = N_{k+1} = J_{k+1} = 0$ . Since  $a, b > 1$ , both are positive for  $0 \leq i \leq k+1$ . The leading coefficients obey  $N_k \mapsto 8N_k$  and  $J_k \mapsto 4(a+b)N_k + 8J_k$ , which gives the first two formulas in (4.6). For (4.5), the initial pair gives  $x - x_0$ , and a direct expansion using  $8u = x^2 - 1$  shows

$$(x-1)N_{\text{new}} - 2J_{\text{new}} = (x-a)(x-b)[(x-1)N - 2J]$$

at  $u = u(x)$ : the coefficient of  $N$  is  $(x-1)(x^2 + ab + a + b) - (a+b)(x^2 - 1) = (x-1)(x-a)(x-b)$ , and the coefficient of  $J$  is  $2(a+b)(x-1) - 2(x^2 + ab - a - b) = -2(x-a)(x-b)$ . Setting  $x = 1$  in (4.5) gives  $J_0$ .  $\square$

Writing  $\Pi(x) = \Pi_e(x^2) + x\Pi_o(x^2)$  and replacing  $x$  by  $-x$  in (4.5) shows that, as functions of  $x^2 = 1 + 8u$ ,

$$N = \Pi_o, \quad J = -\frac{1}{2}(\Pi_e + \Pi_o). \quad (4.7)$$

So  $N$  and  $J$  depend only on the multiset  $\{x_j\}$ ; the recurrence is a convenient way to compute them and to see that their coefficients are positive.

### 4.3 Interlacing and coefficient ratios

**Lemma 4.3** (Interlacing). *Let  $m \geq 1$ . The polynomials  $N$  and  $J$  have only real, simple roots  $\nu_m < \dots < \nu_1$  and  $\zeta_m < \dots < \zeta_1$ , and*

$$\nu_m < \zeta_m < \dots < \nu_2 < \zeta_2 < \nu_1 < \zeta_1 < 0. \quad (4.8)$$

*Consequently*

$$\frac{N(u)}{J(u)} = \frac{N_m}{J_m} + \sum_{k=1}^m \frac{\sigma_k}{u - \zeta_k}, \quad \frac{J(u)}{N(u)} = \frac{J_m}{N_m} - \sum_{k=1}^m \frac{\varsigma_k}{u - \nu_k}, \quad \sigma_k, \varsigma_k > 0. \quad (4.9)$$

*Proof.* For  $y > 0$  evaluate (4.5) at  $x = iy$ , where  $u = -(1 + y^2)/8$  is real and runs over  $(-\infty, -\frac{1}{8})$ . Each factor is  $iy - x_j = -(x_j^2 + y^2)^{1/2}e^{-i\vartheta_j}$  with  $\vartheta_j = \arctan(y/x_j)$ . The number of factors is odd, so  $\Pi(iy) = \varrho(-\cos \vartheta + i \sin \vartheta)$  with  $\varrho = \prod_j (x_j^2 + y^2)^{1/2} > 0$  and  $\vartheta(y) = \sum_j \vartheta_j$ . Comparing real and imaginary parts in  $(iy-1)N - 2J = \Pi(iy)$  gives

$$N = \frac{\varrho \sin \vartheta}{y}, \quad J = \frac{\varrho}{2} \left( \cos \vartheta - \frac{\sin \vartheta}{y} \right). \quad (4.10)$$

The function  $\vartheta$  increases strictly and continuously from 0 to  $(2m+1)\pi/2$ , whether or not the  $x_j$  are distinct, so it takes each of the values  $\pi, 2\pi, \dots, m\pi$  exactly once, at points  $y_1 < \dots < y_m$ . These give  $m$  distinct roots  $\nu_k = -(1+y_k^2)/8 < -\frac{1}{8}$  of  $N$ , which are all its roots since  $\deg N = m$ . By (4.10),  $J(\nu_k) = (-1)^k \varrho/2$ . Hence  $J$  changes sign on each interval  $(\nu_{k+1}, \nu_k)$ , and since  $J(\nu_1) < 0 < J(0)$ , also on  $(\nu_1, 0)$ . This accounts for all  $m$  roots of  $J$  and proves (4.8). All roots are simple, so the partial-fraction expansions (4.9) exist with  $\sigma_k = N(\zeta_k)/J'(\zeta_k)$  and  $\varsigma_k = -J(\nu_k)/N'(\nu_k)$ . Exactly  $k-1$  roots of  $N$  and exactly  $k-1$  roots of  $J$  exceed  $\zeta_k$ , and both leading coefficients are positive; so  $N(\zeta_k)$  and  $J'(\zeta_k)$  both have sign  $(-1)^{k-1}$  and  $\sigma_k > 0$ . Similarly  $N'(\nu_k)$  has sign  $(-1)^{k-1}$  and  $J(\nu_k)$  has sign  $(-1)^k$ , so  $\varsigma_k > 0$ .  $\square$

**Lemma 4.4** (Coefficient ratios). *With  $J_{-1} = 0$ , the sequence  $J_{i-1}/J_i$  ( $0 \leq i \leq m$ ) is strictly increasing, and the sequence  $J_i/N_i$  ( $0 \leq i \leq m$ ) is nondecreasing.*

*Proof.* A polynomial with positive coefficients and only real roots satisfies Newton's inequalities  $J_i^2 > J_{i-1}J_{i+1}$  [24, §2.22]; this is the first claim. For the second, let  $m \geq 1$  and  $Q_k = N/(u - \nu_k) = \sum_{i=0}^{m-1} q_i u^i$ . Its roots are real and negative, so  $q_i > 0$  and, by Newton's inequalities again,  $q_{i-1}/q_i$  is nondecreasing in  $i$ . With  $q_{-1} = q_m = 0$  we have  $N_i = |\nu_k|q_i + q_{i-1}$ , hence

$$\frac{q_i}{N_i} = \frac{1}{|\nu_k| + q_{i-1}/q_i} \quad (0 \leq i \leq m-1), \quad \frac{q_m}{N_m} = 0,$$

which is nonincreasing in  $i$ . By (4.9),  $J = (J_m/N_m)N - \sum_k \varsigma_k Q_k$  with  $\varsigma_k > 0$ , so  $J_i/N_i$  is nondecreasing.  $\square$

#### 4.4 Conversion and coefficient positivity

For  $r > 0$  write  $N_r = N(r)$ ,  $J_r = J(r)$  and define

$$Q(u) = \frac{u J_r N(u) - r N_r J(u)}{u - r}, \quad D_{\text{raw}} = 4Q, \quad B_{\text{raw}} = r D_{\text{raw}} + (4r N_r - 2J_r)N - 2J_r J. \quad (4.11)$$

The numerator of  $Q$  vanishes at  $u = r$ , so  $Q$  is a polynomial, of degree  $m$  with leading coefficient  $J_r N_m$ . Comparing coefficients in  $(u - r)Q = u J_r N - r N_r J$  gives

$$Q_0 = N_r J_0, \quad Q_i = N_r J_i + \sum_{k < i} r^{k-i} (N_r J_k - J_r N_k) \quad (1 \leq i \leq m). \quad (4.12)$$

**Lemma 4.5.** *Let  $c_* = J_r$  and  $e_* = 4r N_r - J_r$ . Then, with  $u = u(x)$ ,*

$$(x - 1)(B_{\text{raw}}(u) - r D_{\text{raw}}(u)) + 2(u - r)D_{\text{raw}}(u) = (c_* x + e_*) \Pi(x). \quad (4.13)$$

*Proof.* We have  $B_{\text{raw}} - r D_{\text{raw}} = (e_* - c_*)N - 2c_* J$  and  $2(u - r)D_{\text{raw}} = 8c_* u N - 2(c_* + e_*)J$ , because  $8r N_r = 2(c_* + e_*)$ . With  $8u = x^2 - 1$  the left side becomes  $(x - 1)[e_* - c_* + c_*(x + 1)]N - 2(c_* x + e_*)J = (c_* x + e_*)[(x - 1)N - 2J]$ , and (4.5) applies.  $\square$

For  $1 \leq k \leq m$  let  $J^{(k)}(u) = J(u)/(u - \zeta_k)$ . Since all  $\zeta_i$  are negative,  $J^{(k)}$  has positive coefficients in degrees  $0, \dots, m - 1$ , and  $J^{(k)}(r) > 0$  for  $r > 0$ .

**Theorem 4.6** (Coefficient positivity). (i) *For every  $r > 0$ , all coefficients of  $Q$  and  $D_{\text{raw}}$  in degrees  $0, \dots, m$  are positive.*

(ii) *Let  $\rho = \sqrt{1 + 8r}$ . If  $\rho > \max_j x_j$  and  $4r + \rho \geq \sum_j x_j$ , then all coefficients of  $B_{\text{raw}}$  in degrees  $0, \dots, m$  are positive.*

*Proof.* For  $m = 0$  we have  $N = 1$ ,  $J = J_0$ ,  $Q = J_0$  and  $B_{\text{raw}} = 2(1 + J_0)(2r - J_0)$ . Here  $J_0 = (x_0 - 1)/2$ , and  $\rho > x_0$  gives  $2r = (\rho^2 - 1)/4 > (x_0 - 1)(x_0 + 1)/4 > J_0$ . Let  $m \geq 1$  and write  $\alpha_0 = N_m/J_m$ .

(i) By (4.9),  $uN/J = \alpha_0 u + \sum_k \sigma_k + \sum_k \sigma_k \zeta_k / (u - \zeta_k)$ . Hence

$$\begin{aligned} Q(u) &= J(u) J_r \frac{\frac{uN(u)}{J(u)} - \frac{rN_r}{J_r}}{u - r} = J(u) J_r \left( \alpha_0 + \sum_k \frac{\sigma_k |\zeta_k|}{(u - \zeta_k)(r - \zeta_k)} \right) \\ &= \alpha_0 J_r J(u) + \sum_{k=1}^m \sigma_k |\zeta_k| J^{(k)}(r) J^{(k)}(u). \end{aligned}$$

Every term has nonnegative coefficients and the first has positive coefficients in all degrees  $0, \dots, m$ .

(ii) From (4.9),  $N = \alpha_0 J + \sum_k \sigma_k J^{(k)}$ . Substituting this and the formula for  $Q$  into  $B_{\text{raw}} = 4rQ + 4rN_r N - 2J_r(N + J)$  and using  $J_r = (r + |\zeta_k|)J^{(k)}(r)$  gives

$$B_{\text{raw}} = \alpha_0 \left[ 4rN_r - J_r \left( \sum_j x_j + 1 - 4r \right) \right] J + \sum_{k=1}^m \sigma_k \left[ 4rN_r - 2J_r \left( 1 - \frac{2r|\zeta_k|}{r + |\zeta_k|} \right) \right] J^{(k)}, \quad (4.14)$$

where we used  $2 + 2/\alpha_0 = \sum_j x_j + 1$ , from (4.6). Evaluating (4.5) at  $x = \rho$ , where  $u = r$ , gives  $(\rho - 1)N_r - 2J_r = \Pi(\rho) > 0$ , that is,

$$J_r < \frac{1}{2}(\rho - 1)N_r. \quad (4.15)$$

Since  $4r = (\rho - 1)(\rho + 1)/2$ , each bracket in the sum in (4.14) is at least  $4rN_r - 2J_r > \frac{1}{2}(\rho - 1)^2 N_r > 0$ . If  $\sum_j x_j + 1 - 4r \leq 0$ , the first bracket is positive as well. Otherwise, by (4.15), it exceeds

$$\frac{1}{2}(\rho - 1)N_r \left[ (\rho + 1) - \left( \sum_j x_j + 1 - 4r \right) \right] = \frac{1}{2}(\rho - 1)N_r \left( 4r + \rho - \sum_j x_j \right) \geq 0.$$

Thus  $B_{\text{raw}}$  is a combination of  $J, J^{(1)}, \dots, J^{(m)}$  with positive coefficients, and  $J$  has positive coefficients in all degrees  $0, \dots, m$ .  $\square$

The hypotheses of (ii) hold for all sufficiently large  $r$ . For the equally spaced roots  $x_j = j + 2$  they hold for every  $r > (4n^2 - 1)/8$ , because then  $\rho > 2n = \max_j x_j$  and  $(\rho + 1)^2 > 4n^2 + 2n = 2(1 + \sum_j x_j)$ , which is the second condition. For the coalesced roots  $x_j = 3$  used below they hold for every  $r \geq 3n/2$ .

## 4.5 Common totals, the count, and nondegeneracy

Fix  $r$  as in Theorem 4.6(ii); note that  $\rho > x_j$  if and only if  $r > u_j$ . Normalize and choose the totals by

$$D = \frac{D_{\text{raw}}}{D_{\text{raw}}(0)}, \quad B = \frac{B_{\text{raw}}}{D_{\text{raw}}(0)}, \quad A = (1 + u)D, \quad (E_{\text{T}}, F_{\text{T}}, S_{\text{T}}) = (2r, 2, 2r + 2). \quad (4.16)$$

Then  $A(0) = D(0) = 1$ , all coefficients of  $A, B, D$  in the ranges required by (2.4) are positive, and Proposition 2.2(ii) provides rate constants for every choice of the kinetics  $(b_i, \beta_i, \gamma_i)$ . By Lemma 4.5, for every  $j$ ,

$$\frac{B(u_j)}{D(u_j)} = r + \frac{2(r - u_j)}{x_j - 1} > r. \quad (4.17)$$

For  $x = x_j$ ,  $u = u_j$  define

$$s = \frac{x - 1}{2uD(u)}, \quad f = \frac{4}{x + 1}, \quad \text{so that} \quad E = uf = \frac{x - 1}{2}, \quad (4.18)$$

and let  $X_j$  be the point (2.5) with these  $(u, s, f)$ . It is a positive equilibrium by Proposition 2.2(i). Its totals follow from (2.6): first  $suD = (x - 1)/2$ , so  $F_{\text{T}} = f(1 + suD) = 2$ ; next, by (4.17),  $suB = \frac{1}{2}r(x - 1) + (r - u)$ , so  $E_{\text{T}} = uf + f(\frac{1}{2}r(x - 1) + r - u) = \frac{1}{2}f r (x + 1) = 2r$ ; finally  $sA = (1 + u)(x - 1)/(2u) = 4(1 + u)/(x + 1) = (1 + u)f$ , that is,

$$\sum_{i=0}^n S_i = E + F, \quad (4.19)$$

and since the bound substrate is  $E_{\text{T}} + F_{\text{T}} - E - F$ , we get  $S_{\text{T}} = E_{\text{T}} + F_{\text{T}} = 2r + 2$ .

**Proposition 4.7** (Slopes). *For every  $j$ ,*

$$\Phi'(u_j) = -\frac{16(1+u_j)(c_*x_j + e_*)}{(r-u_j)(x_j+1)^2 D_{\text{raw}}(u_j)} \Pi'(x_j), \quad c_*x_j + e_* = 4rN_r + (x_j-1)J_r > 0. \quad (4.20)$$

*In particular, if the  $x_j$  are distinct and ordered increasingly, then  $\Phi'(u_j) \neq 0$  and  $\text{sign } \Phi'(u_j) = (-1)^{j+1}$ ; if  $x_j$  is a repeated root of  $\Pi$ , then  $\Phi'(u_j) = 0$ .*

*Proof.* By (4.17),  $u_j$  lies in  $\mathcal{U} \cap \{u < r\}$ , where Lemma 4.1 applies. Dividing (4.13) by  $(x-1)D_{\text{raw}}(u)$  gives

$$\Psi_1(u) := \ell - \frac{2\eta}{x-1} = \frac{(c_*x + e_*)\Pi(x)}{(x-1)D_{\text{raw}}(u)},$$

which vanishes at  $u_j$ , and  $\Phi - S_T = \Psi_2\Psi_1$  with  $\Psi_2 = -2(1+u)(\ell + 2\eta/(x+1))/(\ell(\ell+\eta))$ . Hence  $\Phi'(u_j) = \Psi_2(u_j)\Psi_1'(u_j)$ . At  $u_j$  we have  $\ell = 2\eta/(x-1)$ , so  $\ell + 2\eta/(x+1) = 4\eta x/(x^2-1)$ ,  $\ell + \eta = \eta(x+1)/(x-1)$ , and  $\Psi_2(u_j) = -4(1+u)x(x-1)/(\eta(x+1)^2)$ . Since  $\Pi(x_j) = 0$  and  $dx/du = 4/x$ ,  $\Psi_1'(u_j) = 4(c_*x + e_*)\Pi'(x)/(x(x-1)D_{\text{raw}}(u))$ . Multiplying gives (4.20). All factors other than  $\Pi'(x_j) = \prod_{k \neq j}(x_j - x_k)$  are positive, and  $\Pi'(x_j)$  has sign  $(-1)^{2m-j} = (-1)^j$  when the roots are distinct and ordered increasingly.  $\square$

**Theorem 4.8** (Maximal equilibrium count). *Let  $n \geq 1$ , let  $x_0 < \dots < x_{2n-2}$  be real numbers greater than 1, and let  $r$  satisfy  $r > \max_j u_j$  and  $4r + \sqrt{1+8r} \geq \sum_j x_j$ . For every choice of positive kinetics  $(b_i, \beta_i, \gamma_i)$ , the rate constants (2.7) formed from (4.16) are positive, and the system (1.1) with totals  $(2r, 2, 2r+2)$  has exactly the  $2n-1$  positive equilibria  $X_0, \dots, X_{2n-2}$ , with  $E/F = u_j$  at  $X_j$ . All of them are nondegenerate, and at the  $n-1$  equilibria with odd index the restricted Jacobian has an odd number of positive real eigenvalues. If the  $x_j$ ,  $r$  and the kinetics are rational, then so are all rate constants and all concentrations. Consequently  $\mathcal{E}_n = 2n-1$ , and exactly  $2n-1$  nondegenerate positive equilibria occur on a nonempty open subset of  $\mathbb{R}_{>0}^{6n+3}$ .*

*Proof.* The points  $X_j$  are positive equilibria of one and the same system with the same totals, and they are distinct because their ratios  $E/F = u_j$  are. Theorem 2.3 excludes any others. Nondegeneracy follows from Propositions 4.7 and 2.4, the statement about odd indices from Corollary 2.5, and rationality from the formulas. For the open set, apply the implicit function theorem to the  $3n$  equilibrium equations in the coordinates  $y$ , whose derivative is  $\mathcal{J}$ : each  $X_j$  continues to a smooth family of nondegenerate positive equilibria for parameters near the constructed ones, the continued equilibria remain distinct, and Theorem 2.3 again excludes others. The perturbed parameters need not satisfy  $A = (1+u)D$  or  $S_T = E_T + F_T$ .  $\square$

The map  $x \mapsto (x^2-1)/8$  is a bijection from  $(1, \infty)$  to  $(0, \infty)$ , so the ratios  $E/F$  at the  $2n-1$  equilibria can be prescribed arbitrarily. Whether the even-indexed equilibria are stable depends on the kinetics, which the equilibrium equations do not see. The rest of the proof of Theorem 1.1 consists in choosing the kinetics.

## 5 Kinetic freedom, coalescence, and the loaded slow system

From now on  $n \geq 2$ ,  $m = n-1$ , and the geometry is the one constructed in Section 4 for some roots  $x_j > 1$  and some admissible  $r$ .

## 5.1 Source-preserving kinetics

By Proposition 2.2(ii) we may choose positive numbers  $\theta_i, b_i, \beta_i$  ( $1 \leq i \leq n$ ) freely and set

$$\gamma_i = \theta_i, \quad c_i = \theta_i \frac{D_{i-1}}{B_{i-1}}, \quad a_i = (b_i + c_i) \frac{B_{i-1}}{t_{i-1}}, \quad \alpha_i = (\beta_i + \gamma_i) \frac{D_{i-1}}{t_i} \quad (5.1)$$

without changing any equilibrium (2.5). We call  $\theta_i$  the *catalytic scale* of site  $i$ . At an equilibrium the two catalytic fluxes of site  $i$  are equal, and we call their common value

$$\omega_i = c_i C_i = \gamma_i Y_i = \theta_i Y_i \quad (5.2)$$

the *catalytic current* of site  $i$ . At a given equilibrium, any positive vector  $\boldsymbol{\omega}$  can be realized by the choice  $\theta_i = \omega_i / Y_i$ .

Next, for  $0 < \varepsilon \leq 1$ , replace

$$a_i \mapsto \frac{a_i}{\varepsilon}, \quad b_i \mapsto \frac{b_i + c_i}{\varepsilon} - c_i, \quad \alpha_i \mapsto \frac{\alpha_i}{\varepsilon}, \quad \beta_i \mapsto \frac{\beta_i + \gamma_i}{\varepsilon} - \gamma_i. \quad (5.3)$$

The new constants are positive,  $p_i$  and  $q_i$  are unchanged, and the catalytic constants are untouched, so this is again of the form (5.1). The complex equations become

$$\varepsilon \dot{C}_i = a_i S_{i-1} E - (b_i + c_i) C_i, \quad \varepsilon \dot{Y}_i = \alpha_i S_i F - (\beta_i + \gamma_i) Y_i, \quad (5.4)$$

with the constants from before the replacement on the right. As  $\varepsilon \rightarrow 0$ , binding and unbinding become fast while catalysis keeps its time scale.

## 5.2 Loaded inventories

Let

$$\Lambda_i = S_i + C_{i+1} + Y_i \quad (0 \leq i \leq n), \quad C_{n+1} = Y_0 = 0, \quad (5.5)$$

be the total amount of substrate with exactly  $i$  phosphate groups, free or bound to either enzyme. Binding and unbinding do not change  $\Lambda_i$ , so with the net catalytic fluxes  $j_i = c_i C_i - \gamma_i Y_i$ ,

$$\dot{\Lambda}_i = j_i - j_{i+1} \quad (j_0 = j_{n+1} = 0), \quad \text{that is,} \quad \dot{\mathbf{\Lambda}} = \mathbf{R}^T \mathbf{j}, \quad (5.6)$$

where  $\mathbf{R}$  is the  $n \times (n+1)$  incidence matrix of the path on the levels  $0, \dots, n$ , with rows  $\mathbf{e}_i^T - \mathbf{e}_{i-1}^T$  ( $1 \leq i \leq n$ ). Also  $\sum_i \Lambda_i = S_T$ . In comparison with (2.1),  $Z_i = \sum_{k \geq i} \Lambda_k$ . The functions  $(\Lambda_1, \dots, \Lambda_n, C_1, \dots, C_n, Y_1, \dots, Y_n)$  are linear coordinates on each compatibility class. In them the right side of (5.6) is a linear function of  $(C, Y)$  that does not involve  $\varepsilon$ , and (5.4) does not involve  $\varepsilon$  on the right. The restricted Jacobian at an equilibrium therefore has the form

$$\mathcal{J}_\varepsilon = \begin{pmatrix} 0 & \mathbf{B}_0 \\ \varepsilon^{-1} \mathbf{C}_0 & \varepsilon^{-1} \mathbf{D}_0 \end{pmatrix} \quad (5.7)$$

with matrices  $\mathbf{B}_0$  ( $n \times 2n$ ),  $\mathbf{C}_0$  ( $2n \times n$ ),  $\mathbf{D}_0$  ( $2n \times 2n$ ) that do not depend on  $\varepsilon$ . (In (5.7) we use the new coordinates; this changes  $\mathcal{J}$  by a similarity.)

On the set where the right sides of (5.4) vanish, that is, at *fast-binding equilibrium*, write

$$\mathbf{S} = (S_0, \dots, S_n)^T, \quad \mathbf{c} = (C_1, \dots, C_n, 0)^T, \quad \mathbf{y} = (0, Y_1, \dots, Y_n)^T,$$

indexed by the levels  $0, \dots, n$ , so that  $\mathbf{\Lambda} = \mathbf{S} + \mathbf{c} + \mathbf{y}$ . The entry  $c_i = C_{i+1}$  of  $\mathbf{c}$  is the kinase-bound substrate of level  $i$ ; it should not be confused with the rate constant of the same name, which does not appear in this subsection.

**Lemma 5.1** (Mass matrix and feedback). *Consider a state at fast-binding equilibrium with enzyme totals  $E_T, F_T$ , and variations of it within the fast-binding equilibrium set that keep  $E_T$  and  $F_T$  fixed. In terms of the logarithmic variations  $\sigma_i = dS_i/S_i$ ,*

$$d\mathbf{\Lambda} = \mathbf{M}\boldsymbol{\sigma}, \quad \mathbf{M} = \text{diag}(\mathbf{S} + \mathbf{c} + \mathbf{y}) - \frac{\mathbf{c}\mathbf{c}^T}{E_T} - \frac{\mathbf{y}\mathbf{y}^T}{F_T}, \quad (5.8)$$

and if the state is an equilibrium with catalytic currents  $\boldsymbol{\omega}$ , then

$$d\mathbf{j} = \text{diag}(\boldsymbol{\omega})(-\mathbf{R} + \mathbf{1}\mathbf{h}^T)\boldsymbol{\sigma}, \quad \mathbf{h} = \frac{\mathbf{y}}{F_T} - \frac{\mathbf{c}}{E_T}. \quad (5.9)$$

The matrix  $\mathbf{M}$  is symmetric and positive definite.

*Proof.* At fast-binding equilibrium  $C_i = p_i S_{i-1} E$  and  $Y_i = q_i S_i F$ . Differentiating  $E_T = E + \sum_i C_i$  at fixed  $E_T$  gives  $dE(1 + \sum_i C_i/E) = -\sum_i C_i \sigma_{i-1}$ , that is,  $dE/E = -\mathbf{c}^T \boldsymbol{\sigma}/E_T$ ; likewise  $dF/F = -\mathbf{y}^T \boldsymbol{\sigma}/F_T$ . Then  $dC_{i+1} = C_{i+1}(\sigma_i + dE/E)$  and  $dY_i = Y_i(\sigma_i + dF/F)$ , and summing the three contributions to  $d\Lambda_i$  gives (5.8). At an equilibrium  $c_i C_i = \gamma_i Y_i = \omega_i$ , so  $dj_i = \omega_i[(\sigma_{i-1} + dE/E) - (\sigma_i + dF/F)]$ , which is (5.9). For positivity, the Cauchy–Schwarz inequality gives  $(\mathbf{c}^T \mathbf{a})^2 \leq (\sum_i c_i)(\sum_i c_i a_i^2)$  for every vector  $\mathbf{a}$ , and similarly for  $\mathbf{y}$ , so

$$\mathbf{a}^T \mathbf{M} \mathbf{a} \geq \sum_i S_i a_i^2 + \left(1 - \frac{\sum_i c_i}{E_T}\right) \sum_i c_i a_i^2 + \left(1 - \frac{\sum_i y_i}{F_T}\right) \sum_i y_i a_i^2 > 0 \quad (\mathbf{a} \neq 0),$$

because  $\sum_i c_i = E_T - E < E_T$ ,  $\sum_i y_i = F_T - F < F_T$  and all  $S_i > 0$ .  $\square$

The matrix  $\mathbf{M}$  converts changes of the free substrate concentrations into changes of the conserved inventories. Its rank-one terms express sequestration: raising one  $S_i$  binds enzyme and thereby lowers the complexes at all other levels. It cannot be replaced by  $\text{diag}(\mathbf{S})$ : in the regime used below most of the substrate is bound to the kinase.

**Proposition 5.2** (Loaded slow matrix). *At an equilibrium with catalytic currents  $\boldsymbol{\omega}$ , let*

$$\mathbf{K} = (-\mathbf{R} + \mathbf{1}\mathbf{h}^T)\mathbf{M}^{-1}\mathbf{R}^T \in \mathbb{R}^{n \times n}. \quad (5.10)$$

(i) *The block  $\mathbf{D}_0$  in (5.7) is similar to a symmetric negative definite matrix.* (ii) *The matrix  $-\mathbf{B}_0 \mathbf{D}_0^{-1} \mathbf{C}_0$  is similar to  $\mathbf{K} \text{diag}(\boldsymbol{\omega})$ .* (iii) *As  $\varepsilon \rightarrow 0$ ,  $2n$  eigenvalues of  $\mathcal{J}_\varepsilon$  have real parts tending to  $-\infty$ , and the other  $n$  converge, with multiplicities, to the eigenvalues of  $\mathbf{K} \text{diag}(\boldsymbol{\omega})$ .*

*Proof.* (i) With the inventories and the enzyme totals fixed,  $(C, Y)$  are the extents of the  $2n$  reversible binding reactions  $S_{i-1} + E \rightleftharpoons C_i$ ,  $S_i + F \rightleftharpoons Y_i$  with rate constants  $a_i, b_i + c_i$  and  $\alpha_i, \beta_i + \gamma_i$ . Let  $\Gamma_f$  be their  $(3n + 3) \times 2n$  stoichiometric matrix; its columns are independent because each complex occurs in exactly one of them. At an equilibrium the forward and backward rates of each of these reactions agree; call the common values  $\varphi_k > 0$ . The differential of the net rate of the  $k$ th reaction is  $\varphi_k$  times the differential of the logarithm of the ratio of its forward and backward rates, which is  $-(\Gamma_f^T \text{diag}(X)^{-1} dX)_k$ , where  $X$  is the vector of concentrations; and  $dX = \Gamma_f d(C, Y)$ . Hence  $\mathbf{D}_0 = -\text{diag}(\varphi) \Gamma_f^T \text{diag}(X)^{-1} \Gamma_f$ . This is a positive diagonal matrix times a symmetric negative definite matrix, hence similar to the symmetric negative definite matrix  $-\text{diag}(\varphi)^{1/2} \Gamma_f^T \text{diag}(X)^{-1} \Gamma_f \text{diag}(\varphi)^{1/2}$ ; compare [28].

(ii) Since  $\mathbf{D}_0$  is invertible, the equations (5.4) with zero left side define  $(C, Y)$  locally as a function of  $(\Lambda_1, \dots, \Lambda_n)$  with derivative  $-\mathbf{D}_0^{-1} \mathbf{C}_0$ , and  $-\mathbf{B}_0 \mathbf{D}_0^{-1} \mathbf{C}_0$  is the Jacobian of the reduced vector field  $\mathbf{\Lambda} \mapsto \mathbf{R}^T \mathbf{j}$ . By Lemma 5.1 this Jacobian is the restriction of  $\mathbf{R}^T \text{diag}(\boldsymbol{\omega})(-\mathbf{R} + \mathbf{1}\mathbf{h}^T)\mathbf{M}^{-1}$  to

the tangent space  $\{d\mathbf{\Lambda} : \mathbf{1}^T d\mathbf{\Lambda} = 0\}$  of the class. The map  $\mathbf{R}^T$  is an isomorphism of  $\mathbb{R}^n$  onto that space, and in the coordinates  $\mathbf{a}$  with  $d\mathbf{\Lambda} = \mathbf{R}^T \mathbf{a}$  the restriction is  $\text{diag}(\boldsymbol{\omega})\mathbf{K}$ , which is similar to  $\mathbf{K} \text{diag}(\boldsymbol{\omega})$ .

(iii) For  $\lambda$  in a compact set and  $\varepsilon$  small,

$$\det(\lambda - \mathcal{J}_\varepsilon) = \varepsilon^{-2n} \det(\varepsilon\lambda - \mathbf{D}_0) \det(\lambda - \mathbf{B}_0(\varepsilon\lambda - \mathbf{D}_0)^{-1}\mathbf{C}_0),$$

and the last factor converges uniformly to the characteristic polynomial of  $-\mathbf{B}_0\mathbf{D}_0^{-1}\mathbf{C}_0$ . By Hurwitz's theorem,  $n$  eigenvalues converge to its roots. The eigenvalues of  $\varepsilon\mathcal{J}_\varepsilon$  converge to those of its limit  $\begin{pmatrix} 0 & 0 \\ \mathbf{C}_0 & \mathbf{D}_0 \end{pmatrix}$ , namely  $n$  zeros and the  $2n$  negative eigenvalues of  $\mathbf{D}_0$ ; the latter correspond to  $2n$  eigenvalues of  $\mathcal{J}_\varepsilon$  of the form  $(\mu + o(1))/\varepsilon$  with  $\mu < 0$ . This is the standard two-time-scale decomposition [30, Ch. 2].  $\square$

### 5.3 Coalescence

Now set every auxiliary root equal to 3:  $x_0 = \dots = x_{2m} = 3$ ,  $\Pi(x) = (x-3)^{2n-1}$ , and let  $r \geq 3n/2$ . All results of Section 4 up to Proposition 4.7 remain valid. There is one constructed equilibrium  $X_\circ$ , with

$$u = 1, \quad E = F = 1, \quad s = \frac{1}{D(1)}, \quad (E_T, F_T, S_T) = (2r, 2, 2r+2), \quad (5.11)$$

and  $\Phi'(1) = 0$  by Proposition 4.7. By Proposition 2.4,

$$\det \mathcal{J} = 0 \quad \text{at } X_\circ, \quad \text{for every choice of the kinetics.} \quad (5.12)$$

**Lemma 5.3.** *At the coalesced equilibrium,  $\det \mathbf{K} = 0$ .*

*Proof.* Let  $u \mapsto X(u)$  be the curve of equilibria (2.5) with enzyme totals  $(2r, 2)$ , defined near  $u = 1$  by (2.9). Along it, fast-binding equilibrium and  $\mathbf{j} = 0$  hold identically and  $\sum_i \Lambda_i = \Phi(u)$ . Let  $\boldsymbol{\sigma}$  be the logarithmic derivative of  $(S_0, \dots, S_n)$  at  $u = 1$ . If  $\boldsymbol{\sigma} = 0$ , the proof of Lemma 5.1 gives  $dE = dF = 0$ , which contradicts  $E/F = u$ . By Lemma 5.1,  $\mathbf{M}\boldsymbol{\sigma} \neq 0$ ,  $\mathbf{1}^T \mathbf{M}\boldsymbol{\sigma} = \Phi'(1) = 0$  and  $(-\mathbf{R} + \mathbf{1}\mathbf{h}^T)\boldsymbol{\sigma} = 0$ . Hence  $\mathbf{M}\boldsymbol{\sigma} = \mathbf{R}^T \mathbf{a}$  with  $\mathbf{a} \neq 0$ , and  $\mathbf{K}\mathbf{a} = 0$ .  $\square$

The formulas (4.7) become explicit for coalesced roots. With  $x^2 = 1 + 8u$  and  $k = 2n - 1$ ,

$$N(u) = \frac{(x+3)^k + (x-3)^k}{2x}, \quad J(u) = \frac{(x-1)(x+3)^k - (x+1)(x-3)^k}{4x}. \quad (5.13)$$

Evaluating (5.13) and its derivative at  $x = 3$ , using  $du/dx = x/4$  and  $k \geq 3$ , gives

$$j := N(1) = J(1) = 36^{n-1}, \quad \frac{N'(1)}{j} = \frac{4n-6}{9}, \quad \frac{J'(1)}{j} = \frac{4n}{9}, \quad (5.14)$$

and (4.6) gives  $N_m = 8^{n-1}$ ,  $J_m = (3n-2)8^{n-1}$ .

## 6 A stable prefix for large kinase excess

Let  $\mathbf{K}(r)$  be the matrix (5.10) at the coalesced equilibrium, and let  $\mathbf{A}_r$  be its leading  $(n-1) \times (n-1)$  block, the *prefix*. It involves the sites  $1, \dots, n-1$ . In this section we prove that  $\mathbf{A}_r$  is Hurwitz for all sufficiently large  $r$ , for every fixed  $n \geq 2$ .

## 6.1 Limits of the coalesced state

By (4.12) and (4.11), as  $r \rightarrow \infty$ ,  $Q_i/N_r = J_i + O(1/r)$  and  $(B_{\text{raw}})_i/(4rN_r) = J_i + N_i + O(1/r)$ , because  $J_r/N_r$  is bounded. After the normalization (4.16),

$$D_i = \frac{J_i}{J_0} + O(1/r), \quad \frac{B_i}{r} = \frac{N_i + J_i}{J_0} + O(1/r),$$

and all these quantities are rational functions of  $r$ . By (2.5) and (5.11),  $Y_i = D_{i-1}/D(1)$ ,  $S_i = (D_i + D_{i-1})/D(1)$  and  $C_i = B_{i-1}/D(1)$ . Define

$$d_i = \frac{N_i + J_i}{J} \quad (0 \leq i < n), \quad \bar{y}_i = \frac{J_{i-1}}{J} \quad (1 \leq i \leq n), \quad \bar{y}_0 = \bar{y}_{n+1} = 0, \quad \bar{S}_i = \bar{y}_i + \bar{y}_{i+1}. \quad (6.1)$$

Then, as  $r \rightarrow \infty$ , the entries of  $\mathbf{y}, \mathbf{S}, \mathbf{c}$  satisfy

$$y_i \rightarrow \bar{y}_i, \quad S_i \rightarrow \bar{S}_i, \quad \frac{c_i}{r} \rightarrow d_i \quad (i < n), \quad c_n = 0, \quad (6.2)$$

with errors  $O(1/r)$ . By (5.14),

$$\begin{aligned} \sum_{i < n} d_i &= 2, \quad \sum_{i=0}^n \bar{y}_i = 1, \quad \mu := \frac{1}{2} \sum_{i < n} i d_i = \frac{4n-3}{9}, \\ v := \bar{y}_n &= \frac{J_n}{J} = (3n-2) \left(\frac{2}{9}\right)^{n-1} \in (0, 1). \end{aligned} \quad (6.3)$$

Here  $v < 1$  because  $J_n < J(1)$ . We also use three identities that hold exactly for every  $r$ : by (5.11) and (4.19),

$$\sum_i c_i = 2r - 1, \quad \sum_i y_i = 1, \quad \sum_i S_i = 2, \quad S_n = y_n, \quad (6.4)$$

the last because  $t_n = D_{n-1}$  and  $f = 1$ .

## 6.2 The singular limit of the mass matrix

By (5.8) and (6.2),  $\mathbf{M} = r\mathbf{M}_0 + \mathbf{M}_1(r)$ , where  $\mathbf{M}_1(r)$  converges as  $r \rightarrow \infty$  and

$$\mathbf{M}_0 = \begin{pmatrix} \text{diag}(d) - \frac{1}{2}dd^T & 0 \\ 0 & 0 \end{pmatrix}, \quad d = (d_0, \dots, d_{n-1})^T.$$

By the Cauchy–Schwarz inequality and  $\sum_i d_i = 2$ ,  $\mathbf{M}_0$  is positive semidefinite, and its kernel  $\mathcal{V}$  is spanned by  $\mathbf{1}_{<n} = (1, \dots, 1, 0)^T$  and  $\mathbf{e}_n$ . The inverse of  $\mathbf{M}$  is therefore of order one on  $\mathcal{V}$  and of order  $1/r$  on  $\mathcal{V}^\perp$ , and the limit of  $r\mathbf{M}^{-1}$  on  $\mathcal{V}^\perp$  involves the order-one part of  $\mathbf{M}$  on  $\mathcal{V}$ . The next lemma shows that this part is controlled by the exact identities (6.4) alone.

**Lemma 6.1** (Kernel compression). *As  $r \rightarrow \infty$ ,*

$$\begin{pmatrix} \mathbf{1}_{<n} & \mathbf{e}_n \end{pmatrix}^T \mathbf{M} \begin{pmatrix} \mathbf{1}_{<n} & \mathbf{e}_n \end{pmatrix} \rightarrow \begin{pmatrix} \frac{7}{2} - v - \frac{1}{2}v^2 & \frac{1}{2}v(v-1) \\ \frac{1}{2}v(v-1) & 2v - \frac{1}{2}v^2 \end{pmatrix}, \quad (6.5)$$

*and the limit is positive definite, with determinant  $v(7-4v)$ .*

*Proof.* By (5.8) and (6.4),  $\mathbf{M}\mathbf{1} = \mathbf{S} + \mathbf{c}(1 - \frac{2r-1}{2r}) + \mathbf{y}(1 - \frac{1}{2}) = \mathbf{S} + \mathbf{c}/(2r) + \mathbf{y}/2$ . Hence  $\mathbf{1}^T \mathbf{M} \mathbf{1} = 2 + \frac{2r-1}{2r} + \frac{1}{2} \rightarrow \frac{7}{2}$ ,  $\mathbf{1}^T \mathbf{M} \mathbf{e}_n = S_n + \frac{1}{2}y_n \rightarrow \frac{3}{2}v$ , and  $\mathbf{e}_n^T \mathbf{M} \mathbf{e}_n = S_n + y_n - \frac{1}{2}y_n^2 \rightarrow 2v - \frac{1}{2}v^2$ . With  $\mathbf{1}_{<n} = \mathbf{1} - \mathbf{e}_n$  these give (6.5). The diagonal entries are positive for  $0 < v < 1$ , and the determinant is  $(\frac{7}{2} - v - \frac{1}{2}v^2)(2v - \frac{1}{2}v^2) - \frac{1}{4}v^2(v-1)^2 = 7v - 4v^2$ .  $\square$

**Lemma 6.2** (Scaled inverse). *Let  $\chi \in \mathcal{V}^\perp$ , that is,  $\chi_n = 0$  and  $\sum_{i < n} \chi_i = 0$ . Then  $\tau^{(r)} := r\mathbf{M}^{-1}\chi$  converges as  $r \rightarrow \infty$  to the vector  $\tau$  with*

$$\tau_i = \frac{\chi_i}{d_i} + \tau_* \quad (i < n), \quad (6.6)$$

where the constants  $\tau_*$  and  $\tau_n$  are the unique solution of

$$\frac{3}{2}T + U + \frac{1}{2}(7 - 3v)\tau_* + \frac{3}{2}v\tau_n = 0, \quad (4 - v)\tau_n = T + (1 - v)\tau_*, \quad (6.7)$$

with  $T = \sum_{i < n} \bar{y}_i \chi_i / d_i$  and  $U = \sum_{i < n} \bar{y}_{i+1} \chi_i / d_i$ . Moreover, with  $\mathbf{h}_\infty = \lim_{r \rightarrow \infty} \mathbf{h}$ ,

$$\mathbf{h}_\infty^T \tau = 2\tau_n - \tau_* = \frac{10T + (2 + v)U}{2(7 - 4v)}. \quad (6.8)$$

*Proof. Convergence.* In an orthonormal basis adapted to  $\mathcal{V}^\perp \oplus \mathcal{V}$ ,

$$\mathbf{M} = \begin{pmatrix} r\mathbf{A}_0 + \mathbf{A}_1 & \mathbf{B}_1 \\ \mathbf{B}_1^T & \mathbf{C}_1 \end{pmatrix},$$

where  $\mathbf{A}_0$  is positive definite and does not depend on  $r$ , where  $\mathbf{A}_1, \mathbf{B}_1, \mathbf{C}_1$  converge as  $r \rightarrow \infty$ , and where the limit of  $\mathbf{C}_1$  is positive definite by Lemma 6.1. Solving  $\mathbf{M}\tau^{(r)} = r\chi$  with  $\chi = (\chi_\perp, 0)$  by elimination of the second block gives the  $\mathcal{V}^\perp$ -component  $(\mathbf{A}_0 + r^{-1}(\mathbf{A}_1 - \mathbf{B}_1\mathbf{C}_1^{-1}\mathbf{B}_1^T))^{-1}\chi_\perp$  and the  $\mathcal{V}$ -component  $-\mathbf{C}_1^{-1}\mathbf{B}_1^T$  times it. Both converge.

*Identification.* Dividing  $\mathbf{M}\tau^{(r)} = r\chi$  by  $r$  and letting  $r \rightarrow \infty$  gives  $\mathbf{M}_0\tau = \chi$ , that is,  $d_i\tau_i - \frac{1}{2}d_i(d^T\tau) = \chi_i$  for  $i < n$ , which is (6.6) with some constant  $\tau_*$ . Two further equations hold exactly for every  $r$ , because  $\mathbf{1}^T\chi = \mathbf{e}_n^T\chi = 0$ :

$$(\mathbf{M}\mathbf{1})^T\tau^{(r)} = 0, \quad (\mathbf{M}\mathbf{e}_n)^T\tau^{(r)} = 0.$$

By the proof of Lemma 6.1,  $\mathbf{M}\mathbf{1} \rightarrow \bar{\mathbf{S}} + \frac{1}{2}(d, 0) + \frac{1}{2}\bar{\mathbf{y}}$  and  $\mathbf{M}\mathbf{e}_n \rightarrow 2v\mathbf{e}_n - \frac{1}{2}v\bar{\mathbf{y}}$ . Inserting (6.6), the first equation becomes

$$\sum_{i < n} (\bar{y}_i + \bar{y}_{i+1} + \frac{1}{2}d_i + \frac{1}{2}\bar{y}_i) \left( \frac{\chi_i}{d_i} + \tau_* \right) + \frac{3}{2}v\tau_n = 0.$$

Since  $\sum_{i < n} \chi_i = 0$ ,  $\sum_{i < n} \bar{S}_i = 2 - v$ ,  $\sum_{i < n} d_i = 2$  and  $\sum_{i < n} \bar{y}_i = 1 - v$ , this is the first equation of (6.7). The second limit equation is  $2v\tau_n - \frac{1}{2}v(T + (1 - v)\tau_* + v\tau_n) = 0$ ; dividing by  $v/2$  gives the second equation of (6.7). The determinant of the system (6.7) in  $(\tau_*, \tau_n)$  is  $\frac{1}{2}(7 - 3v)(4 - v) + \frac{3}{2}v(1 - v) = 14 - 8v > 0$ , and Cramer's rule gives

$$\tau_* = -\frac{6T + (4 - v)U}{14 - 8v}, \quad \tau_n = \frac{2T - (1 - v)U}{14 - 8v}. \quad (6.9)$$

No next-order coefficient of  $\mathbf{c}$  enters.

*Feedback.* By (5.9) and (6.2),  $\mathbf{h}_\infty = \frac{1}{2}\bar{\mathbf{y}} - \frac{1}{2}(d, 0)$ . Hence  $\mathbf{h}_\infty^T\tau = \frac{1}{2}(T + (1 - v)\tau_* + v\tau_n) - \frac{1}{2} \cdot 2\tau_*$ , where we used  $\sum_{i < n} \chi_i = 0$  and  $\sum_{i < n} d_i = 2$ . By the second equation of (6.7) the first bracket equals  $(4 - v)\tau_n + v\tau_n = 4\tau_n$ , so  $\mathbf{h}_\infty^T\tau = 2\tau_n - \tau_*$ , and (6.9) gives (6.8).  $\square$

### 6.3 The limiting prefix

Let  $\mathbf{R}_0$  be the  $(n-1) \times n$  incidence matrix of the path on the levels  $0, \dots, n-1$ , and let

$$\mathbf{H} = \mathbf{R}_0 \operatorname{diag}(1/d_0, \dots, 1/d_{n-1}) \mathbf{R}_0^T \in \mathbb{R}^{(n-1) \times (n-1)}. \quad (6.10)$$

It is symmetric, positive definite and tridiagonal, with diagonal entries  $1/d_{i-1} + 1/d_i$  and off-diagonal entries  $-1/d_i$ .

**Lemma 6.3** (Limiting prefix). *As  $r \rightarrow \infty$ ,*

$$r\mathbf{A}_r \longrightarrow \mathbf{B}_* = -\mathbf{H} + \mathbf{1}\phi^T, \quad \phi_i = g_i - g_{i-1} \quad (1 \leq i \leq n-1), \quad (6.11)$$

where

$$g_i = \frac{10\bar{y}_i + (2+v)\bar{y}_{i+1}}{2(7-4v)d_i} = \frac{10J_{i-1} + (2+v)J_i}{2(7-4v)(N_i + J_i)} \quad (0 \leq i \leq n-1). \quad (6.12)$$

Every entry of  $\phi$  is strictly positive.

*Proof.* Column  $k \leq n-1$  of  $\mathbf{K}$  is  $(-\mathbf{R} + \mathbf{1}\mathbf{h}^T)\mathbf{M}^{-1}\boldsymbol{\chi}$  with  $\boldsymbol{\chi} = \mathbf{R}^T \mathbf{e}_k = \mathbf{e}_k - \mathbf{e}_{k-1} \in \mathcal{V}^\perp$ . By Lemma 6.2,  $r$  times this column converges to  $-\mathbf{R}\boldsymbol{\tau} + (\mathbf{h}_\infty^T \boldsymbol{\tau})\mathbf{1}$ . In the rows  $i \leq n-1$ ,  $(\mathbf{R}\boldsymbol{\tau})_i = \tau_i - \tau_{i-1} = \chi_i/d_i - \chi_{i-1}/d_{i-1}$ , since  $\tau_*$  cancels; this is the  $(i, k)$  entry of  $\mathbf{H}$ . For the feedback, (6.8) with  $\chi_k = 1$ ,  $\chi_{k-1} = -1$  gives  $T = \bar{y}_k/d_k - \bar{y}_{k-1}/d_{k-1}$  and  $U = \bar{y}_{k+1}/d_k - \bar{y}_k/d_{k-1}$ , hence  $\mathbf{h}_\infty^T \boldsymbol{\tau} = g_k - g_{k-1}$ .

For the sign, write

$$g_i = \frac{1}{2(7-4v)} \cdot \frac{J_i}{N_i + J_i} \cdot \left(10 \frac{J_{i-1}}{J_i} + 2 + v\right).$$

By Lemma 4.4, which applies to coalesced roots, the second factor is positive and nondecreasing in  $i$  and the third is positive and strictly increasing. Hence  $g_i$  is strictly increasing and  $\phi_i > 0$ .  $\square$

Thus the limiting transverse dynamics is a diffusion-like stable matrix  $-\mathbf{H}$  on a weighted path, perturbed by a rank-one *positive* feedback: every column of  $\mathbf{1}\phi^T$  is positive. Positive feedback can destabilize, and the question is whether its gain is below one.

**Lemma 6.4** (Strict feedback deficit). *Let  $\mathbf{z} = \mathbf{H}^{-1}\mathbf{1}$  and  $G = \phi^T \mathbf{z}$ . Then  $\mathbf{z} > 0$  entrywise,*

$$G = \frac{10[\frac{4}{3} - v(n-\mu)] + \frac{1}{3}(2+v)}{14-8v}, \quad 1-G = \frac{v(50n-45)}{9(14-8v)} > 0, \quad (6.13)$$

and  $\mathbf{B}_*$  is Hurwitz. Consequently  $\mathbf{A}_r$  is Hurwitz for all sufficiently large  $r$ .

*Proof.* The matrix  $\mathbf{H}$  is a nonsingular symmetric  $M$ -matrix: it is positive definite with nonpositive off-diagonal entries. Since it is irreducible,  $\mathbf{H}^{-1}$  is entrywise positive [2, Ch. 6], so  $\mathbf{z} > 0$ .

To compute  $G$ , let  $\psi = \operatorname{diag}(1/d)\mathbf{R}_0^T \mathbf{z} \in \mathbb{R}^n$  be the *level potential* of  $\mathbf{z}$ . Then  $\mathbf{R}_0\psi = \mathbf{H}\mathbf{z} = \mathbf{1}$ , so  $\psi_i - \psi_{i-1} = 1$ , and  $\sum_{i < n} d_i \psi_i = (\mathbf{R}_0\mathbf{1})^T \mathbf{z} = 0$ . With (6.3) this gives  $\psi_i = i - \mu$ . The map that sends  $\boldsymbol{\chi}$  to  $\mathbf{h}_\infty^T \boldsymbol{\tau}$  is linear, and  $\phi_k$  is its value at  $\boldsymbol{\chi} = \mathbf{R}_0^T \mathbf{e}_k$  (extended by  $\chi_n = 0$ ). Therefore  $G = \sum_k \phi_k z_k$  is its value at  $\boldsymbol{\chi} = \mathbf{R}_0^T \mathbf{z}$ , for which  $\chi_i/d_i = \psi_i$ . By (6.8),  $G = (10T + (2+v)U)/(14-8v)$  with

$$T = \sum_{i < n} \bar{y}_i(i - \mu) = \frac{1}{j} \sum_{k=0}^{n-2} J_k(k+1 - \mu) = \frac{J'(1)}{j} + (1 - \mu) - v(n - \mu) = \frac{4}{3} - v(n - \mu),$$

$$U = \sum_{i < n} \bar{y}_{i+1}(i - \mu) = \frac{J'(1)}{j} - \mu = \frac{1}{3},$$

by (5.14) and (6.3). This is the first formula in (6.13). With  $n - \mu = (5n + 3)/9$ ,

$$(14 - 8v)(1 - G) = 14 - 8v - \frac{40}{3} + 10v(n - \mu) - \frac{2}{3} - \frac{1}{3}v = v\left(\frac{10(5n+3)}{9} - \frac{25}{3}\right) = \frac{v(50n - 45)}{9}.$$

Now  $\mathbf{B}_* \mathbf{z} = -\mathbf{H} \mathbf{z} + \mathbf{1} (\phi^T \mathbf{z}) = -(1 - G) \mathbf{1} < 0$ . The matrix  $\mathbf{B}_*$  has nonnegative off-diagonal entries, because those of  $-\mathbf{H}$  are nonnegative and  $\phi > 0$ . After the similarity  $\text{diag}(\mathbf{z})^{-1} \mathbf{B}_* \text{diag}(\mathbf{z})$  it has nonnegative off-diagonal entries and strictly negative row sums, so all its Gershgorin discs lie in the open left half-plane. Thus  $\mathbf{B}_*$  is Hurwitz. The last claim follows from (6.11) and the continuity of eigenvalues.  $\square$

## 7 Lifting, splitting, and the proof of the main theorems

All choices in this section are made in the order

$$n \text{ fixed} \longrightarrow r \text{ large} \longrightarrow \omega_n > 0 \text{ small} \longrightarrow \varepsilon > 0 \text{ small} \longrightarrow \delta > 0 \text{ small.} \quad (7.1)$$

No scale or spectral gap uniform in  $n$  is required.

**Lemma 7.1** (A slow final link). *Let  $r \geq 3n/2$  be such that  $\mathbf{A}_r$  is Hurwitz. For all sufficiently small  $t > 0$ , the matrix  $\mathbf{K}(r) \text{diag}(1, \dots, 1, t)$  has an algebraically simple eigenvalue 0, and its other  $n - 1$  eigenvalues lie in the open left half-plane.*

*Proof.* For  $t = 0$  the last column vanishes, so the characteristic polynomial is  $\lambda \det(\lambda - \mathbf{A}_r)$ : a simple zero and  $n - 1$  stable roots. By continuity, for small  $t > 0$  there are  $n - 1$  eigenvalues in the open left half-plane and exactly one, counted with multiplicity, in a small disc around 0. By Lemma 5.3,  $\det(\mathbf{K} \text{diag}(1, \dots, 1, t)) = t \det \mathbf{K} = 0$ , so that eigenvalue is 0.  $\square$

**Lemma 7.2** (Full spectral lifting). *For every  $n \geq 2$  there are an admissible  $r$  and positive kinetics such that the restricted Jacobian at the coalesced equilibrium  $X_\circ$  has an algebraically simple eigenvalue 0 and  $3n - 1$  eigenvalues in the open left half-plane.*

*Proof.* Choose  $r$  by Lemma 6.4 and  $t$  by Lemma 7.1. Realize the currents  $\omega = (1, \dots, 1, t)$  at  $X_\circ$  by  $\theta_i = \omega_i / Y_i$ , take  $b_i = \beta_i = 1$  in (5.1), and apply (5.3). By Proposition 5.2(iii), for small  $\varepsilon$  the matrix  $\mathcal{J}_\varepsilon$  has  $2n$  eigenvalues with large negative real part,  $n - 1$  eigenvalues near the stable eigenvalues of  $\mathbf{K} \text{diag}(\omega)$ , and exactly one eigenvalue, counted with multiplicity, in a small disc around 0. By (5.12) that eigenvalue is 0.  $\square$

Geometric simplicity of the zero eigenvalue follows from the equilibrium geometry alone, but algebraic simplicity, which excludes a Jordan block, does not; here it is obtained together with transverse stability from the kinetics.

*Proof of Theorem 1.2.* Fix  $n \geq 2$  and the parameters  $r, t, \varepsilon$  of Lemma 7.2, and let  $\theta_i^\circ = \omega_i / Y_i(X_\circ)$  be the catalytic scales used there. For real  $\xi_0 < \dots < \xi_{2m}$  and small  $\delta > 0$  put  $x_j = 3 + \delta \xi_j$ . Since  $r \geq 3n/2$ , the hypotheses  $r > \max_j u_j$  and  $4r + \sqrt{1 + 8r} \geq \sum_j x_j$  of Theorem 4.8 hold with strict inequality at  $\delta = 0$ , hence for small  $\delta$ , at the same  $r$ ; the coefficients of  $N, J, Q, B, D$  are polynomial or rational in  $\delta$ . Define the kinetics of the split system by (5.1) with the frozen scales  $\theta_i = \theta_i^\circ$  and  $b_i = \beta_i = 1$ , followed by (5.3) with the same  $\varepsilon$ . For each  $\delta$  this is one rate vector  $\kappa(\delta)$ , common to all the equilibria  $X_0(\delta), \dots, X_{2m}(\delta)$  of Theorem 4.8, and as  $\delta \rightarrow 0$  the rate vector converges to that of Lemma 7.2 and every  $X_j(\delta)$  converges to  $X_\circ$ . All these objects are real-analytic in  $\delta$ .

Let  $\mathcal{J}_j(\delta)$  be the restricted Jacobian at  $X_j(\delta)$ . By continuity of the spectrum and Lemma 7.2, for small  $\delta$  it has  $3n - 1$  eigenvalues with real parts below a negative constant, and one eigenvalue  $\lambda_j(\delta)$  in a small disc around 0. The latter is real, since the nonreal eigenvalues of a real matrix occur in conjugate pairs, and it is simple, hence analytic in  $\delta$  [29, Ch. II]. Let  $\Theta_j(\delta)$  be the product of the other eigenvalues; then  $\det \mathcal{J}_j = \lambda_j \Theta_j$ , and  $\Theta_j(\delta) \rightarrow \Theta_{\circ}$ , the product of the nonzero eigenvalues at  $X_{\circ}$ , whose sign is  $(-1)^{3n-1} = (-1)^{n+1}$ . By Propositions 2.4 and 4.7,

$$\det \mathcal{J}_j(\delta) = (-1)^n \left( \prod_i (b_i + c_i) \alpha_i \gamma_i \right) F^n u_j M(u_j) \frac{16(1 + u_j)(c_* x_j + e_*)}{(r - u_j)(x_j + 1)^2 D_{\text{raw}}(u_j)} \delta^{2m} \prod_{k \neq j} (\xi_j - \xi_k),$$

where all quantities are those of the split system. As  $\delta \rightarrow 0$  every factor in front of  $\delta^{2m}$  converges to a positive limit that does not depend on  $j$ :  $F \rightarrow 1$ ,  $u_j \rightarrow 1$ ,  $x_j \rightarrow 3$ , and  $M(1) = L(1) + (r-1)D(1) > 0$ . Dividing by  $\Theta_j$  gives (1.3) with

$$a = \frac{4(2rN_r + J_r) M(1)}{(r-1) D_{\text{raw}}(1) |\Theta_{\circ}|} \prod_{i=1}^n (b_i + c_i) \alpha_i \gamma_i > 0, \quad (7.2)$$

where all quantities are evaluated for the coalesced system of Lemma 7.2. Finally  $\prod_{k \neq j} (\xi_j - \xi_k)$  has the sign  $(-1)^{2m-j} = (-1)^j$ .  $\square$

*Proof of Theorem 1.1(b) and (c).* Let  $n \geq 2$ . In the preceding proof choose rational  $\xi_j$ . For sufficiently small  $\delta > 0$ , the system with rate vector  $\kappa(\delta)$  and totals  $(2r, 2, 2r+2)$  has exactly the  $2n-1$  equilibria  $X_j(\delta)$  by Theorem 4.8; each of them has  $3n-1$  eigenvalues in the open left half-plane and a real eigenvalue  $\lambda_j \neq 0$ , so it is hyperbolic; and  $\lambda_j < 0$  exactly for the  $n$  even indices. Hence there are  $n$  sinks and  $n-1$  saddles with one unstable direction. This agrees with the degree count  $n_+ - n_- = 1$  of Section 3.

The conditions on  $r, t, \varepsilon$  and  $\delta$  in (7.1) are open, so these parameters can be chosen rational, and then all rate constants, totals and concentrations are rational, because they are rational functions of  $x_j, r, t, \varepsilon$  with rational coefficients. Hyperbolic equilibria and the numbers of their stable and unstable eigenvalues persist under small perturbations of all  $6n+3$  parameters, and by Theorem 2.3 no further equilibria appear. This gives the open set in (c).

For  $n = 1$ , let all six rate constants be 1. The state  $(S_0, S_1, E, F, C_1, Y_1) = (2, 2, 1, 1, 1, 1)$  is an equilibrium with totals  $(2, 2, 6)$ , it is the only one in its class by Theorem 2.3, and in the coordinates  $(S_1, C_1, Y_1)$

$$\mathcal{J} = \begin{pmatrix} -1 & 1 & 3 \\ -1 & -5 & -1 \\ 1 & 0 & -4 \end{pmatrix}, \quad \det(\lambda - \mathcal{J}) = \lambda^3 + 10\lambda^2 + 27\lambda + 10.$$

The Routh array has first column  $(1, 10, 26, 10)$ , so  $\mathcal{J}$  is Hurwitz [20, Ch. XV].

With part (a), proved in Section 3, and Theorem 4.8, this gives  $\mathcal{E}_n = 2n-1$  and  $n \leq \mathcal{H}_n \leq \mathcal{C}_n \leq n$  for every  $n \geq 1$ .  $\square$

## 8 Consequences of the proof

The results of this section are not needed for Theorem 1.1. They make the construction quantitative and more flexible.

## 8.1 The feedback deficit in closed form

**Proposition 8.1.** *For every  $n \geq 2$ , the stability margin  $1 - G_n$  of the limiting prefix in Lemma 6.4 is*

$$1 - G_n = \frac{(3n-2)(50n-45)(2/9)^{n-1}}{9[14-8(3n-2)(2/9)^{n-1}]}, \quad 1 - G_n \sim \frac{25}{21} n^2 \left(\frac{2}{9}\right)^{n-1} \quad (n \rightarrow \infty). \quad (8.1)$$

*Proof.* Insert  $v = (3n-2)(2/9)^{n-1}$  from (6.3) into (6.13); the asymptotic statement follows from  $v \rightarrow 0$  and  $3 \cdot 50/(9 \cdot 14) = 25/21$ .  $\square$

Table 1: The coefficient fraction  $v_n$  and the feedback deficit  $1 - G_n$  of the limiting prefix (exact values and decimal approximations).

| $n$       | 2                 | 3               | 4                    | 5                    | 6                     | 7                         | 8                          |
|-----------|-------------------|-----------------|----------------------|----------------------|-----------------------|---------------------------|----------------------------|
| $v_n$     | $\frac{8}{9}$     | $\frac{28}{81}$ | $\frac{80}{729}$     | $\frac{208}{6561}$   | $\frac{512}{59049}$   | $\frac{1216}{531441}$     | $\frac{2816}{4782969}$     |
| $1 - G_n$ | $\frac{220}{279}$ | $\frac{14}{39}$ | $\frac{6200}{43047}$ | $\frac{4264}{81171}$ | $\frac{4352}{246777}$ | $\frac{185440}{33437007}$ | $\frac{499840}{301225671}$ |
| decimal   | 0.789             | 0.359           | 0.144                | 0.0525               | 0.0176                | 0.00555                   | 0.00166                    |

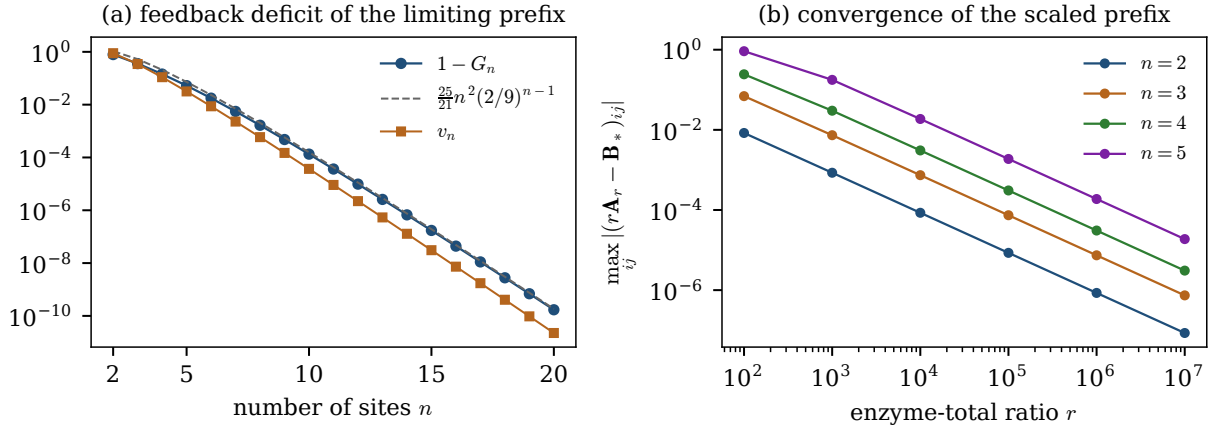


Figure 2: (a) The feedback deficit (8.1), its asymptote, and the coefficient fraction  $v_n$ . (b) The entrywise distance between  $r\mathbf{A}_r$ , computed exactly from the coalesced construction, and its limit  $\mathbf{B}_*$ ; the slope is  $-1$ , in agreement with the  $O(1/r)$  errors in Section 6.

The deficit is positive for every  $n$  but decays geometrically (Table 1, Figure 2(a)). This explains why the construction works for all  $n$  and is nevertheless poorly conditioned for large  $n$ . The decay is a property of this construction, with all roots coalesced at  $x = 3$  and  $A = (1 + u)D$ ; it is not a lower bound on the time scales or the resources that  $n$  stable states require.

## 8.2 Diagonal stability and independent catalytic currents

Lemma 6.4 used one vector of catalytic currents,  $(1, \dots, 1, t)$ . A diagonal Lyapunov matrix removes this restriction. Let  $\mathbf{z} = \mathbf{H}^{-1}\mathbf{1}$  and  $\mathbf{w} = \mathbf{H}^{-1}\phi$ ; both are entrywise positive.

**Proposition 8.2** (Diagonal Lyapunov matrix). *Let  $\mathbf{P} = \text{diag}(w_i/z_i)$  and  $\mathbf{Q}_* = \mathbf{B}_*^T \mathbf{P} + \mathbf{P} \mathbf{B}_*$ . Then  $\mathbf{Q}_*$  is negative definite. Consequently, for all sufficiently large  $r$ ,  $\mathbf{A}_r^T \mathbf{P} + \mathbf{P} \mathbf{A}_r$  is negative definite, and for every such  $r$  and every positive diagonal matrix  $\mathbf{\Omega}$  the matrix  $\mathbf{A}_r \mathbf{\Omega}$  is Hurwitz.*

*Proof.* Since  $\mathbf{H}$  is symmetric,  $\mathbf{1}^T \mathbf{w} = \mathbf{z}^T \phi = G$ . Hence  $\mathbf{B}_* \mathbf{z} = -(1 - G)\mathbf{1}$  and  $\mathbf{B}_*^T \mathbf{w} = -\phi + G\phi = -(1 - G)\phi$ . Because  $\mathbf{P} \mathbf{z} = \mathbf{w}$ ,

$$\mathbf{Q}_* \mathbf{z} = \mathbf{B}_*^T \mathbf{w} + \mathbf{P} \mathbf{B}_* \mathbf{z} = -(1 - G)(\phi + \mathbf{P} \mathbf{1}) < 0.$$

The matrix  $\mathbf{Q}_*$  is symmetric with nonnegative off-diagonal entries  $Q_{ik}$ , because  $\mathbf{B}_*$  has nonnegative off-diagonal entries and  $\mathbf{P}$  is a positive diagonal matrix. For such a matrix and any positive vector  $\mathbf{z}$ , expanding the squares gives the identity

$$\mathbf{a}^T \mathbf{Q}_* \mathbf{a} = \sum_i \frac{(\mathbf{Q}_* \mathbf{z})_i}{z_i} a_i^2 - \sum_{i < k} Q_{ik} z_i z_k \left( \frac{a_i}{z_i} - \frac{a_k}{z_k} \right)^2, \quad (8.2)$$

which is negative for  $\mathbf{a} \neq 0$ . Negative definiteness is an open condition, and  $r \mathbf{A}_r \rightarrow \mathbf{B}_*$ . Finally  $(\mathbf{A}_r \mathbf{\Omega})^T (\mathbf{\Omega} \mathbf{P}) + (\mathbf{\Omega} \mathbf{P}) (\mathbf{A}_r \mathbf{\Omega}) = \mathbf{\Omega} (\mathbf{A}_r^T \mathbf{P} + \mathbf{P} \mathbf{A}_r) \mathbf{\Omega}$  is negative definite, and  $\mathbf{\Omega} \mathbf{P}$  is positive definite, so  $\mathbf{A}_r \mathbf{\Omega}$  is Hurwitz by Lyapunov's theorem.  $\square$

**Corollary 8.3** (Independent prefix currents). *Let  $n \geq 2$ , let  $r$  be as in Proposition 8.2, and let  $\mathcal{K} \subset \mathbb{R}_{>0}^{n-1}$  be compact. There are  $t > 0$ ,  $\varepsilon > 0$  and  $\delta_0 > 0$  such that the conclusions of Theorems 1.1(b) and 1.2 hold for the construction of Section 7 with the catalytic currents  $(\omega_1, \dots, \omega_{n-1}, t)$  at the coalesced equilibrium, for every  $(\omega_1, \dots, \omega_{n-1}) \in \mathcal{K}$  and every  $0 < \delta \leq \delta_0$ .*

*Proof.* By Proposition 8.2 the prefix  $\mathbf{A}_r \text{diag}(\omega_1, \dots, \omega_{n-1})$  is Hurwitz for every point of  $\mathcal{K}$ , with a spectral bound that is uniform by compactness. The arguments of Lemmas 7.1 and 7.2 and of the proof of Theorem 1.2 use only the continuity of eigenvalues and the exact zero provided by (5.12) and Lemma 5.3. All matrices involved depend continuously on the point of  $\mathcal{K}$ , so  $t$ ,  $\varepsilon$  and  $\delta_0$  can be chosen uniformly on  $\mathcal{K}$ .  $\square$

The currents  $\omega_1, \dots, \omega_{n-1}$  are source-preserving coordinates; they are not independent perturbations of the original rate constants, which are covered by Theorem 1.1(c). No uniformity over unbounded sets of currents is claimed.

### 8.3 Readout and the separation–recovery trade-off

A natural observable is the total amount of fully phosphorylated substrate,  $S_n + Y_n$ . At the equilibrium of the construction of Section 4 with auxiliary coordinate  $x$ , formulas (2.5) and (4.18) with  $t_n = D_{n-1}$  give

$$S_n + Y_n = \mathcal{R}(x) := \frac{D_{n-1}}{2} \cdot \frac{u^{n-1}}{D(u)} \cdot \frac{(x-1)(x+5)}{x+1}, \quad u = \frac{x^2 - 1}{8}. \quad (8.3)$$

The function  $u^{n-1}/D(u) = 1/\sum_i D_i u^{i-(n-1)}$  is nondecreasing, and  $(x-1)(x+5)/(x+1) = x + 3 - 8/(x+1)$  has derivative  $1 + 8/(x+1)^2 > 0$ . Hence  $\mathcal{R}$  is strictly increasing: the readout orders the  $2n - 1$  equilibria in the same way as the ratio  $E/F$ , and sinks and saddles alternate along it. Also  $E = (x-1)/2$  and  $F = 4/(x+1)$  at these equilibria, so the total phosphorylation flux  $\sum_i \gamma_i Y_i$  is positive at each of them; they are nonequilibrium steady states sustained by the irreversible catalytic steps.

**Corollary 8.4.** *In the split family of Theorem 1.2,*

$$\mathcal{R}(x_j) - \mathcal{R}(x_k) = \mathcal{R}'_o(3) (\xi_j - \xi_k) \delta + O(\delta^2), \quad \frac{1}{|\lambda_j(\delta)|} = \frac{1 + O(\delta)}{a \prod_{k \neq j} |\xi_j - \xi_k|} \delta^{-(2n-2)},$$

where  $\mathcal{R}_o$  is the function (8.3) of the coalesced system and  $\mathcal{R}'_o(3) > 0$ .

*Proof.* At each  $\delta$  all equilibria share the polynomial  $D = D^{(\delta)}$ , so the dependence of  $D$  on  $\delta$  cancels from the difference to first order. The second formula is (1.3).  $\square$

Thus, within this near-coalescence family, separating the states by an amount  $\delta$  costs a linear relaxation time of order  $\delta^{-(2n-2)}$ . This is a statement about the family, not a speed limit for the network, and it suggests looking for well-separated roots when designing examples; Section 9.2 does so.

## 8.4 Labels, and a terminating design procedure

**Corollary 8.5.** *A single sequential distributive cycle admits  $q$  simultaneously stable positive equilibria, for one rate vector and one triple of totals, if and only if  $n \geq q$ . In particular  $2^b$  distinct stable equilibrium labels require  $n \geq 2^b$  sites.*

*Proof.* If  $n \geq q$ , Theorem 1.1(b) gives  $n \geq q$  sinks; the converse is Theorem 1.1(a).  $\square$

Coupled modules, transient codes and oscillatory phases are outside this statement, and it is not a statement about an information rate in the presence of noise.

*Remark 8.6 (Exact design).* For fixed  $n \geq 2$ , fix strictly ordered rational offsets  $\xi_0 < \dots < \xi_{2n-2}$  and use the candidates  $r = 2^a$ ,  $t = 2^{-b}$ ,  $\varepsilon = 2^{-c}$ , and  $\delta = 2^{-d}$ , with nonnegative integer indices, in the order (7.1). A source candidate is eligible only when  $r \geq 3n/2$  and both coalesced converted polynomials  $B_{\text{raw}}$  and  $D_{\text{raw}}$  have strictly positive coefficients through degree  $n - 1$  and no higher coefficients. Double  $r$  until an eligible candidate passes the original exact Routh test for  $\mathbf{A}_r$ , or the specified exact Lyapunov  $LDL^T$  test. Freeze the catalytic scales at this positive coalesced state, giving unit prefix currents and final current  $t$ . Halve  $t$ , starting at one, until the slow matrix has a simple zero and a Hurwitz deflation; halve  $\varepsilon$ , starting at one, until the coalesced restricted Jacobian has the same property. Finally halve  $\delta$ , starting at one. A distance candidate is eligible only when all prescribed roots  $x_j = 3 + \delta\xi_j$  exceed one and its converted  $B_{\text{raw}}, D_{\text{raw}}$  again satisfy the finite coefficient conditions above. Stop at the first eligible candidate whose original  $2n - 1$  exact Routh tests give the alternating pattern.

All eligibility tests use finite rational arithmetic. Large-source conversion positivity makes the first guard eventually true along the doubling sequence. At each admitted source, continuity at coalescence makes the last guard eventually true along the halving sequence. Thus these guards do not obstruct eventual success of the corresponding spectral tests. The positive coalesced state gives positive frozen catalytic factors;  $t > 0$  and  $0 < \varepsilon \leq 1$  persist under all later halvings. Every returned design therefore has positive rates and the same prescribed positive equilibrium family at the common totals. The termination argument uses the corresponding spectral lemmas and the exact spectral tests' necessity and soundness. No bound on the number of steps is claimed. Separately, Proposition 9.1 certifies explicit rational systems for  $n \leq 7$ ; those systems are not asserted to be outputs of this guarded asymptotic search.

## 9 Exact examples

### 9.1 Rational witnesses for $n \leq 7$

The proof of Theorem 1.1(b) is asymptotic in  $r$ ,  $t$ ,  $\varepsilon$  and  $\delta$ . For small  $n$ , direct rational tests certify the explicit witnesses below with moderate  $r$  and  $\delta$ , unit coalesced currents and no binding relaxation. These witnesses need not be outputs of the guarded asymptotic search.

**Proposition 9.1** (Computer-assisted). *Let  $2 \leq n \leq 7$ , and let  $(r, \delta)$  be  $(2, \frac{1}{2})$ ,  $(4, \frac{1}{2})$ ,  $(4, \frac{1}{2})$ ,  $(8, \frac{1}{4})$ ,  $(8, \frac{1}{4})$ ,  $(8, \frac{1}{4})$  for  $n = 2, \dots, 7$ . Let  $x_j = 3 + \delta(j - n + 1)$  for  $0 \leq j \leq 2n - 2$ , let the geometry be given by (4.16), and let the kinetics be given by (5.1) with  $b_i = \beta_i = 1$  and  $\theta_i = 1/Y_i(X_o)$ , where  $X_o$  is the coalesced equilibrium (5.11) for the same  $r$ ; no relaxation (5.3) is applied. Then the system with totals  $(2r, 2, 2r + 2)$  has exactly  $2n - 1$  positive equilibria; the  $n$  equilibria with even index are sinks, and at each of the  $n - 1$  equilibria with odd index exactly one eigenvalue of  $\mathcal{J}$  has positive real part and none lies on the imaginary axis.*

*Proof.* For each of the 48 equilibria the rational matrix  $\mathcal{J}$  was formed, its characteristic polynomial was computed exactly by the Faddeev–LeVerrier recursion in integer arithmetic, with the Cayley–Hamilton identity as a check, and the Routh array [20, Ch. XV] was formed in rational arithmetic. In every case all  $3n + 1$  entries of the first column are nonzero, and the number of sign changes is 0 for even  $j$  and 1 for odd  $j$ . The count of equilibria is Theorem 4.8.  $\square$

**Example 9.2** (Two sites). For  $n = 2$ ,  $r = 2$  and  $x = (\frac{5}{2}, 3, \frac{7}{2})$  one finds  $N = \frac{111}{4} + 8u$ ,  $J = \frac{15}{4} + 32u$ ,  $D = 1 + \frac{8672}{2625}u$ ,  $B = \frac{1287}{125} + \frac{2288}{875}u$ , the totals  $(E_T, F_T, S_T) = (4, 2, 6)$ , and the rate constants

| $i$ | $a_i$                   | $b_i$ | $c_i$               | $\alpha_i$              | $\beta_i$ | $\gamma_i$      |
|-----|-------------------------|-------|---------------------|-------------------------|-----------|-----------------|
| 1   | $\frac{19782}{1375}$    | 1     | $\frac{625}{1573}$  | $\frac{147000}{124267}$ | 1         | $\frac{45}{11}$ |
| 2   | $\frac{311808}{192049}$ | 1     | $\frac{4065}{2431}$ | $\frac{79}{34}$         | 1         | $\frac{45}{34}$ |

The three equilibria, in the order  $(S_0, S_1, S_2; E, F; C_1, C_2; Y_1, Y_2)$ , are

$$\begin{aligned} X_0 &= (\frac{250}{693}, \frac{1027}{1008}, \frac{271}{528}, \frac{3}{4}, \frac{8}{7}, \frac{39}{14}, \frac{13}{28}, \frac{125}{462}, \frac{271}{462}), \\ X_1 &= (\frac{2625}{11297}, 1, \frac{8672}{11297}, 1, 1, \frac{189}{79}, \frac{48}{79}, \frac{2625}{11297}, \frac{8672}{11297}), \\ X_2 &= (\frac{350}{2223}, \frac{869}{912}, \frac{4065}{3952}, \frac{5}{4}, \frac{8}{9}, \frac{77}{38}, \frac{55}{76}, \frac{875}{4446}, \frac{1355}{1482}). \end{aligned}$$

$X_0$  and  $X_2$  are sinks and  $X_1$  is a saddle. The eigenvalues of largest real part, computed in floating point, are approximately  $-0.0120$ ,  $+0.0050$  and  $-0.0084$ ; the remaining ones have real parts below  $-2$ .

### 9.2 A three-site benchmark with separated states

The witnesses above are close to coalescence. For a benchmark with well-separated states we take  $n = 3$ ,

$$x = (2, \frac{7}{2}, 5, \frac{13}{2}, 8), \quad r = 9, \quad (E_T, F_T, S_T) = (18, 2, 20),$$

for which  $N = \frac{10165}{4} + 1926u + 64u^2$ ,  $J = \frac{385}{2} + 3562u + 768u^2$  and

$$D = 1 + \frac{505005048}{38591245}u + \frac{48362752}{38591245}u^2, \quad B = \frac{111414528}{1102607} + \frac{5946508756}{38591245}u + \frac{236379904}{38591245}u^2.$$

The nine kinetic parameters  $(\theta_i, b_i, \beta_i)$  were chosen by a bounded numerical search that maximizes the smallest decay rate at the three even-indexed equilibria, subject to the ceilings  $a_i, \alpha_i \leq 2$  and

$b_i, c_i, \beta_i, \gamma_i \leq 2$  in model units; the ceilings remove the trivial improvement obtained by rescaling time. The result was rounded to rational numbers, and everything stated below refers to these exact rational rate constants, which are distributed with the source. Three root geometries were compared in this way; the smallest decay rates found were  $0.80 \times 10^{-4}$ ,  $0.98 \times 10^{-4}$  and  $1.26 \times 10^{-4}$  for  $x = (2, 3, 4, 5, 6)$ ,  $(\frac{3}{2}, \frac{5}{2}, \frac{7}{2}, \frac{9}{2}, \frac{11}{2})$  and the geometry selected here. These numbers are floating-point diagnostics, and the search makes no claim of optimality.

**Proposition 9.3** (Computer-assisted). *For the benchmark rate constants the class with totals  $(18, 2, 20)$  contains exactly five positive equilibria, listed in Table 2. The equilibria  $X_0, X_2, X_4$  are sinks, and  $X_1, X_3$  are hyperbolic saddles with one unstable direction.*

*Proof.* The rate constants realize the geometry above through (5.1), so Theorem 4.8 applies ( $r = 9 > \max_j u_j = \frac{63}{8}$  and  $4r + \sqrt{1 + 8r} > 36 > 25 = \sum_j x_j$ ). The stability statements are exact Routh computations as in Proposition 9.1.  $\square$

Table 2: The five equilibria of the three-site benchmark. Concentrations in the last two columns are in  $\mu\text{M}$  for the unit  $C_0 = 0.1 \mu\text{M}$ ; decimal entries are rounded values of exact rational numbers. “Unstable” is the number of eigenvalues of  $\mathcal{J}$  in the open right half-plane, from an exact Routh array.

| $j$ | $x_j$ | $u = E/F$ | $E$  | $F$  | $\Phi'(u_j)$ | $S_3 + Y_3$ ( $\mu\text{M}$ ) | unstable |
|-----|-------|-----------|------|------|--------------|-------------------------------|----------|
| 0   | 2     | 3/8       | 1/2  | 4/3  | −0.29235     | 0.003380                      | 0        |
| 1   | 7/2   | 45/32     | 5/4  | 8/9  | 0.02050      | 0.026743                      | 1        |
| 2   | 5     | 3         | 2    | 2/3  | −0.00772     | 0.072950                      | 0        |
| 3   | 13/2  | 165/32    | 11/4 | 8/15 | 0.01002      | 0.138019                      | 1        |
| 4   | 8     | 63/8      | 7/2  | 4/9  | −0.08439     | 0.216157                      | 0        |

**Declared units.** To read the benchmark in physical units we declare a concentration unit  $C_0 = 0.1 \mu\text{M}$  and a time unit  $T_0 = 10$  s. Bimolecular constants are divided by  $C_0 T_0$  and unimolecular constants by  $T_0$ . The totals become  $(E_T, F_T, S_T) = (1.8, 0.2, 2.0) \mu\text{M}$ , and the rate constants are those of Table 3. The association constants are at most  $2 \mu\text{M}^{-1}\text{s}^{-1}$  and the unimolecular constants at most  $0.2\text{s}^{-1}$ . The slowest of the three numerically computed decay rates corresponds to an e-folding time of about 22 hours. These units are declarations, not fits: the unimolecular constants span a factor of about 33,446, the kinase total is comparable to the substrate total, and no measured kinetic data were used. The benchmark is a reproducible test case, not a model of a particular kinase–phosphatase pair.

Table 3: Rate constants of the three-site benchmark in the declared units, rounded to six significant digits. The exact rational values in model units are distributed with the source and are authoritative.

| Site | $a_i$ ( $\mu\text{M}^{-1}\text{s}^{-1}$ ) | $b_i$ ( $\text{s}^{-1}$ ) | $c_i$ ( $\text{s}^{-1}$ ) | $\alpha_i$ ( $\mu\text{M}^{-1}\text{s}^{-1}$ ) | $\beta_i$ ( $\text{s}^{-1}$ ) | $\gamma_i$ ( $\text{s}^{-1}$ ) |
|------|-------------------------------------------|---------------------------|---------------------------|------------------------------------------------|-------------------------------|--------------------------------|
| 1    | 2.00000                                   | $5.96184 \times 10^{-6}$  | 0.00197333                | 0.171890                                       | 0.0427272                     | 0.199398                       |
| 2    | 1.86875                                   | $7.83183 \times 10^{-4}$  | 0.0162999                 | 1.99626                                        | 0.0268102                     | 0.191934                       |
| 3    | 0.217843                                  | 0.0126769                 | 0.0383203                 | 2.00000                                        | 0.0127038                     | 0.187296                       |

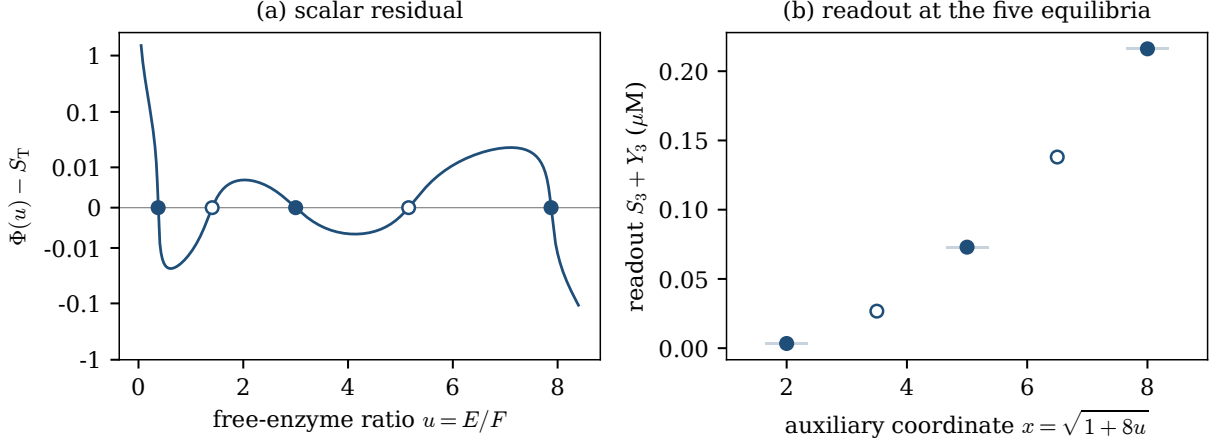


Figure 3: The three-site benchmark. (a) The residual  $\Phi(u) - S_T$  on an arcsinh scale; filled markers are sinks, open markers are saddles. (b) The readout  $S_3 + Y_3$  at the five equilibria; the shaded bars are the certified readout intervals of Proposition 9.4, including a measurement error of  $\pm 0.001 \mu\text{M}$ . The curves and markers are drawn from exact rational data; the counts are certified by the formulas and the Routh arrays, not by the plot.

### 9.3 A rigorous local certificate for independent parameter errors

Theorem 1.1(c) gives an open set of parameters without an estimate of its size. For the benchmark we certify an explicit, if very small, neighbourhood, in which all 18 rate constants and all three totals vary independently; in particular the perturbed systems need not lie in the source-preserving family (5.1), and their equilibria move.

The certificate uses model-unit parameters and concentrations; times and readouts are converted to physical units by  $T_0$  and  $C_0$ . Let  $q = (S_1, S_2, S_3, C_1, C_2, C_3, Y_1, Y_2, Y_3)$  be the model-unit coordinates on the class, let  $q_0$  be one of the three nominal sinks, and use the weighted deviations  $e = \text{diag}(q_0)^{-1}(q - q_0)$  from the equilibrium  $q_0$  of a perturbed parameter vector  $\theta$ . Let  $\mathcal{J}_0$  be the restricted Jacobian at  $q_0$  in the weighted coordinates.

**Proposition 9.4** (Computer-assisted). *Let  $\epsilon_{\text{rel}} = 1/671088640000 \approx 1.49 \times 10^{-12}$ , and let each of the 18 rate constants and the three totals of the benchmark vary independently within the relative distance  $\epsilon_{\text{rel}}$  of its nominal value. For each of the three nominal sinks  $X_0, X_2, X_4$ , with the radii  $\varrho_0$  and  $\varrho_{\text{in}}$  of Table 4:*

- (i) *the perturbed system has exactly one equilibrium  $q_\theta$  in the box  $\|\text{diag}(q_0)^{-1}(q - q_0)\|_\infty \leq \varrho_0$ ; it is positive and satisfies  $\|\text{diag}(q_0)^{-1}(q_\theta - q_0)\|_\infty \leq \varrho_0/4$ ;*
- (ii) *there is a rational symmetric positive definite matrix  $\mathbf{P}$ , the same for all admitted parameters, such that every solution with  $\|e(0)\|_2 \leq \varrho_{\text{in}}$  stays in that box and satisfies*

$$\|e(t)\|_2 \leq \frac{\varrho_0}{2} \exp\left(-\frac{c}{2M} \frac{t}{T_0}\right), \quad M = \|\mathbf{P}\|_\infty, \quad c = \frac{1}{\|\mathbf{Q}^{-1}\|_\infty} - \ell_{\text{loss}} > 0,$$

where  $\mathbf{Q} = -(\mathcal{J}_0^T \mathbf{P} + \mathbf{P} \mathcal{J}_0)$  and  $\ell_{\text{loss}}$  is an interval bound for the variation of  $\mathcal{J}^T \mathbf{P} + \mathbf{P} \mathcal{J}$  over the box and over all admitted parameters; in particular  $\|e(t)\|_2$  has decreased below  $\varrho_0/20$  after the time  $T_{10} = 60M/c$  seconds of Table 4;

- (iii) the readouts  $S_3 + Y_3$  at the three perturbed sinks, with a measurement error of  $\pm 0.001 \mu\text{M}$  added, lie in the pairwise disjoint intervals  $[0.00237970, 0.00437971]$ ,  $[0.07195005, 0.07395006]$  and  $[0.21515666, 0.21715672]$  ( $\mu\text{M}$ ).

*Proof.* All steps are carried out in rational or rational-interval arithmetic. A floating-point Lyapunov solve proposes  $\mathbf{P}$ , which is then rounded to a rational matrix; exact  $LDL^T$  factorizations verify that  $\mathbf{P}$  and  $\mathbf{Q}$  are positive definite. Since  $\mathbf{P}$  and  $\mathbf{Q}^{-1}$  are symmetric, their spectral norms are at most their  $\infty$ -norms, so  $\mathbf{P} \preceq M\mathbf{I}$  and  $\mathbf{Q} \succeq \|\mathbf{Q}^{-1}\|_\infty^{-1}\mathbf{I}$ .

(i) For the Newton-type map  $q \mapsto q - \mathcal{J}_0^{-1}g_\theta(q)$  (in weighted coordinates), interval evaluation over the box and the parameter set shows that its derivative has  $\infty$ -norm at most some  $\vartheta \leq \frac{1}{2}$  and that it moves  $q_0$  by at most  $(1 - \vartheta)\varrho_0/4$ ; by the contraction principle it has a unique fixed point in the box, within  $\varrho_0/4$  of  $q_0$ , and the fixed points of this map in the box are exactly the equilibria there. The same interval evaluation bounds all twelve concentrations away from zero. Here it matters that each reaction vector is multiplied by  $\mathcal{J}_0^{-1}$  (and, below, by  $\mathbf{P}$ ) *before* the interval evaluation: this keeps the exact cancellations between the reactions and improves the admissible  $\epsilon_{\text{rel}}$  by about eight orders of magnitude over a product of separate norm bounds.

(ii) For admitted  $\theta$  and  $q$  in the box, the segment from  $q_\theta$  to  $q$  lies in the box, and the mean-value form of the vector field gives  $\dot{e} = \bar{\mathcal{J}}e$ , where  $\bar{\mathcal{J}}$  is an average of restricted Jacobians over the segment. The interval bound gives  $\|(\bar{\mathcal{J}} - \mathcal{J}_0)^T\mathbf{P} + \mathbf{P}(\bar{\mathcal{J}} - \mathcal{J}_0)\|_2 \leq \ell_{\text{loss}}$ , so for  $V = e^T\mathbf{P}e$ ,

$$\dot{V} \leq -(\|\mathbf{Q}^{-1}\|_\infty^{-1} - \ell_{\text{loss}})\|e\|_2^2 = -c\|e\|_2^2 \leq -\frac{c}{M}V.$$

Let  $p_{\min}$  be a rational lower bound for the smallest eigenvalue of  $\mathbf{P}$ . The ellipsoid  $V \leq p_{\min}(\varrho_0/2)^2$  lies in the Euclidean ball of radius  $\varrho_0/2$  around  $q_\theta$ , hence in the box, because the centre has moved by at most  $\varrho_0/4$  in the  $\infty$ -norm; it is invariant; and it contains the ball  $\|e\|_2 \leq \varrho_{\text{in}} := (\varrho_0/2)\sqrt{p_{\min}/M}$  (rounded down). On it,  $\|e(t)\|_2^2 \leq V(t)/p_{\min} \leq (\varrho_0/2)^2 \exp(-ct/M)$  in model time. Since  $\log 10 < 3$ , a tenfold decrease of this envelope is reached by the model time  $6M/c$ , that is, by  $60M/c$  seconds.

(iii) The readout is a linear function of  $q$ , and (i) bounds the displacement of each sink.  $\square$

Table 4: The local certificate of Proposition 9.4. The column “earlier” is the bound obtained when the norms are rounded to powers of ten and half of the Lyapunov margin is discarded; the column  $T_{10}$  keeps the exact constants. Times are rounded upwards.

| Sink  | $\varrho_0$ | $\varrho_{\text{in}}$ | $M$       | $c$     | $T_{10}$ (s) | earlier (s)       |
|-------|-------------|-----------------------|-----------|---------|--------------|-------------------|
| $X_0$ | 1/8192000   | 1/16384000000         | 5846.11   | 0.98516 | 357,000      | $1.2 \times 10^7$ |
| $X_2$ | 1/16384000  | 1/327680000000        | 535109.89 | 0.97537 | 32,918,000   | $1.2 \times 10^9$ |
| $X_4$ | 1/2048000   | 1/409600000000        | 383168.49 | 0.98112 | 23,433,000   | $1.2 \times 10^9$ |

The certificate should be read for what it is. The parameter tolerance  $\epsilon_{\text{rel}} \approx 1.5 \times 10^{-12}$  and the neighbourhoods  $\varrho_{\text{in}}$  are far too small to represent laboratory tolerances, and the times  $T_{10}$  are upper bounds for an envelope inside an invariant ellipsoid, not predicted recovery times: the worst bound is about 381 days, whereas the numerically computed slowest e-folding time is about 22 hours. Much of the gap between the two reflects the conditioning of  $\mathbf{P}$  ( $M \approx 5 \times 10^5$ ) rather than the dynamics. Keeping the exact rational constants instead of rounded ones improved the worst bound 36-fold, from about 38 years, without changing the system, the parameter box or the neighbourhoods. What the proposition does establish is that a full-dimensional, explicitly described set of parameters supports three distinguishable, locally attracting states with quantified return.

## 10 Formal verification and reproducibility

The mathematical arguments in this paper are given above as conventional proofs, with exact computations identified separately. The available formal developments have two distinct verification scopes.

**Registered equilibrium attainment.** The theorem `PhosphorylationSharpness.sharp_lower_bound` states that for every  $n \geq 1$  there are positive rates and an injective family of  $2n - 1$  positive stationary states of the full mass-action vector field, all with one common positive triple of conserved totals. It is registered as [PALOMAR-2026-09-25-000020, version 1](#), at source commit `44cb5b18313448ede0f9e0df869c538602ffc66d`, with Lean `v4.35.0-rc2` and Mathlib revision `065356127b1d`. Its permitted axioms are `propext`, `Classical.choice` and `Quot.sound`. This registered statement covers attainment, rather than the upper bound, arbitrary prescribed ratios, nondegeneracy or stable-state capacity. The upper equilibrium bound is attributed to Wang and Sontag in Theorem [2.3](#).

**Extended capacity formalization.** The public [extended source development](#), at commit `19566faca29bf58f819725fe59a2f3b49395e042`, contains nineteen selected statements concerning the universal equilibrium and nonlinear stable-state ceilings, simultaneous rational sink–saddle attainment, open persistence, prescribed-root and splitting refinements, and benchmark and operational guarantees. Its principal declaration is `all_size_exact_capacity_with_hyperbolic_attainment_435` in the namespace `PhosphorylationStableCapacity`.

The recorded [full check](#) completed the Lean builds and exported the statements and proofs, but exhausted the runner’s memory during independent comparison. A complete independent comparison and kernel pass for that nineteen-statement release is therefore not established by that run, and this extended development has no public Palomar registration as of 15 October 2026. Earlier research receipts cover narrower components. The conventional arguments for Theorem [1.1](#) stand on their mathematical proofs in this paper; registration of the equilibrium-attainment component is not presented as verification of the whole manuscript. No Lean or numerical replay was performed to prepare this edition.

**Computational companion.** The [full computational archive](#) and [Lean source archive](#) are preserved with this paper at [doi:10.5281/zenodo.23438755](https://doi.org/10.5281/zenodo.23438755). The programs and saved data are available in the [versioned public computational source directory](#). The separate archive `capacities-computational-companion.zip` collects the programs and mathematical data and contains the exact data for the examples, the Python programs `phos_sharp.py`, `phos_capacity.py` and `check_paper.py`, and the saved computation report. It accompanies this manuscript as a separate file in the same Zenodo deposit. The checker uses exact rational arithmetic to reconstruct the equilibria, totals and Jacobians, test the spectral counts and reproduce the operational inequalities of Propositions [9.1](#), [9.3](#) and [9.4](#). Saved outputs are historical computational evidence. They do not replace the all-size proofs or establish a new formal verification result. The accompanying `formalization.zip` preserves the selected Lean source snapshots; the linked repositories retain their development history.

**Use of AI tools.** Generative AI was used extensively for mathematical development, manuscript drafting, computations and Lean formalization under human direction.

## 11 Discussion

The sequential distributive  $n$ -site cycle has equilibrium capacity  $2n - 1$  and stable-state capacity  $n$ , for every  $n$ . Both values are attained by one explicit rational system, and the stable ceiling holds without any nondegeneracy assumption. The reusable idea in the proof is the separation of geometry and kinetics: the equilibrium equations see only half of the parameters, so one can first build the richest possible equilibrium set, then coalesce it, and then use the remaining parameters to organize time scales so that the transverse spectrum becomes computable. In the present case the computation ends in a scalar gain with a closed form.

We close with limitations and open questions.

*Conditioning.* The proof is an existence proof with ordered limits. The margin (8.1) decays like  $n^2(2/9)^n$ , the splitting law (1.3) shows that near coalescence the slowest rate is of order  $\delta^{2n-2}$ , and the open set of Theorem 1.1(c) comes without a size. Proposition 9.1 and the benchmark show that for small  $n$  much better constants are available than the proof suggests. It would be interesting to find a construction of  $n$  sinks whose spectral gap and parameter tolerance decay at most polynomially in  $n$ , or to show that none exists.

*Enzyme levels.* In our examples  $S_T = E_T + F_T$  and the kinase is in large excess over the phosphatase, so that most of the substrate is bound to the kinase; this is far from the substrate-excess regime of Michaelis–Menten reductions, in which generically at most  $n + 1$  equilibria exist [37, Theorem 4]. How small  $E_T/S_T$  and  $F_T/S_T$  can be in a system with  $2n - 1$  equilibria, or with  $n$  sinks, is open. By the symmetry  $E \leftrightarrow F$ ,  $S_i \leftrightarrow S_{n-i}$  of (1.1) all results hold with the roles of the enzymes exchanged.

*Global dynamics.* Theorem 1.1 bounds the number of attracting equilibria, not the number of attractors. For  $n = 1$  every trajectory converges to the unique equilibrium [1]. Whether the two-site cycle admits periodic orbits is a well-known open question; see [10, 5, 9] for partial results and related mechanisms. Our ceiling neither uses nor implies the absence of oscillations for any  $n$ , and attracting periodic orbits, if present, are not counted by  $\mathcal{C}_n$ . We do not know the basins of the  $n$  sinks constructed here, and we do not claim that they attract almost all initial conditions.

*Thermodynamics and noise.* The catalytic steps in (1.1) are irreversible effective reactions. The constructed states carry a positive phosphorylation flux and therefore presuppose a maintained chemical driving force; a closed system with a finite supply of ATP cannot hold them indefinitely. A model with explicit fuel, or with reversible catalytic steps, is a different network and requires its own analysis. Likewise, the theorem concerns the deterministic rate equations. Whether the  $n$  states are retained for a useful time at finite copy numbers depends on the volume, the readout and the time horizon, and neither retention nor its failure is established here. For these reasons Corollary 8.5 should be read as a statement about stable equilibrium labels of a mechanism, not as a claim that a cell realizes or inherits them.

*Other mechanisms.* The method uses the monomial parametrization of the equilibria, a locus on which the scalar equation factors after a quadratic change of variable, and the path structure of the slow dynamics. It is natural to ask for analogues in cycles with several kinases or phosphatases, in mixed processive–distributive mechanisms, and in cascades, for which sharp bounds are not known. The ceiling of Section 3 applies verbatim to any mass-action network with compact compatibility classes and without boundary equilibria for which the number of positive equilibria in a class is at most  $2k - 1$  for all rate constants: such a network has at most  $k$  locally asymptotically stable equilibria in each class.

## References

- [1] D. Angeli and E. D. Sontag. Translation-invariant monotone systems, and a global convergence result for enzymatic futile cycles. *Nonlinear Anal. Real World Appl.*, 9(1):128–140, 2008. doi:[10.1016/j.nonrwa.2006.09.006](https://doi.org/10.1016/j.nonrwa.2006.09.006).
- [2] A. Berman and R. J. Plemmons. *Nonnegative Matrices in the Mathematical Sciences*, volume 9 of *Classics in Applied Mathematics*. SIAM, Philadelphia, 1994.
- [3] F. Bihan, A. Dickenstein, and M. Giaroli. Lower bounds for positive roots and regions of multistationarity in chemical reaction networks. *J. Algebra*, 542:367–411, 2020. doi:[10.1016/j.jalgebra.2019.10.002](https://doi.org/10.1016/j.jalgebra.2019.10.002); arXiv:[1807.05157](https://arxiv.org/abs/1807.05157).
- [4] P. Cohen. The regulation of protein function by multisite phosphorylation – a 25 year update. *Trends Biochem. Sci.*, 25(12):596–601, 2000. doi:[10.1016/S0968-0004\(00\)01712-6](https://doi.org/10.1016/S0968-0004(00)01712-6).
- [5] C. Conradi, E. Feliu, and M. Mincheva. On the existence of Hopf bifurcations in the sequential and distributive double phosphorylation cycle. *Math. Biosci. Eng.*, 17(1):494–513, 2020. doi:[10.3934/mbe.2020027](https://doi.org/10.3934/mbe.2020027); arXiv:[1905.08129](https://arxiv.org/abs/1905.08129).
- [6] C. Conradi, E. Feliu, M. Mincheva, and C. Wiuf. Identifying parameter regions for multistationarity. *PLoS Comput. Biol.*, 13(10):e1005751, 2017. doi:[10.1371/journal.pcbi.1005751](https://doi.org/10.1371/journal.pcbi.1005751); arXiv:[1608.03993](https://arxiv.org/abs/1608.03993).
- [7] C. Conradi, D. Flockerzi, and J. Raisch. Multistationarity in the activation of a MAPK: parametrizing the relevant region in parameter space. *Math. Biosci.*, 211(1):105–131, 2008. doi:[10.1016/j.mbs.2007.10.004](https://doi.org/10.1016/j.mbs.2007.10.004).
- [8] C. Conradi and M. Mincheva. Catalytic constants enable the emergence of bistability in dual phosphorylation. *J. R. Soc. Interface*, 11(95):20140158, 2014. doi:[10.1098/rsif.2014.0158](https://doi.org/10.1098/rsif.2014.0158).
- [9] C. Conradi and M. Mincheva. In distributive phosphorylation catalytic constants enable non-trivial dynamics. *J. Math. Biol.*, 89(2):20, 2024. doi:[10.1007/s00285-024-02114-8](https://doi.org/10.1007/s00285-024-02114-8); arXiv:[2308.08524](https://arxiv.org/abs/2308.08524).
- [10] C. Conradi, M. Mincheva, and A. Shiu. Emergence of oscillations in a mixed-mechanism phosphorylation system. *Bull. Math. Biol.*, 81(6):1829–1852, 2019. doi:[10.1007/s11538-019-00580-6](https://doi.org/10.1007/s11538-019-00580-6); arXiv:[1809.02886](https://arxiv.org/abs/1809.02886).
- [11] C. Conradi and A. Shiu. Dynamics of posttranslational modification systems: recent progress and future directions. *Biophys. J.*, 114(3):507–515, 2018. doi:[10.1016/j.bpj.2017.11.3787](https://doi.org/10.1016/j.bpj.2017.11.3787); arXiv:[1705.10913](https://arxiv.org/abs/1705.10913).
- [12] K. Deimling. *Nonlinear Functional Analysis*. Springer, Berlin, 1985.
- [13] A. Dickenstein. Biochemical reaction networks: an invitation for algebraic geometers. In *Mathematical Congress of the Americas*, volume 656 of *Contemp. Math.*, pages 65–83. Amer. Math. Soc., 2016. doi:[10.1090/conm/656/13076](https://doi.org/10.1090/conm/656/13076).
- [14] A. Dickenstein. Algebraic geometry tools in systems biology. *Notices Amer. Math. Soc.*, 67(11), 2020. doi:[10.1090/noti2188](https://doi.org/10.1090/noti2188).

- [15] E. Feliu, N. Kaihnsa, T. de Wolff, and O. Yürük. The kinetic space of multistationarity in dual phosphorylation. *J. Dynam. Differential Equations*, 34(2):825–852, 2022. doi:10.1007/s10884-020-09889-6; arXiv:2001.08285.
- [16] E. Feliu, N. Kaihnsa, T. de Wolff, and O. Yürük. Parameter region for multistationarity in  $n$ -site phosphorylation networks. *SIAM J. Appl. Dyn. Syst.*, 22(3):2024–2053, 2023. doi:10.1137/22M1504548; arXiv:2206.08908.
- [17] E. Feliu, A. D. Rendall, and C. Wiuf. A proof of unlimited multistability for phosphorylation cycles. *Nonlinearity*, 33(11):5629–5658, 2020. doi:10.1088/1361-6544/ab9a1e; arXiv:1904.02983.
- [18] E. Feliu and C. Wiuf. Enzyme-sharing as a cause of multi-stationarity in signalling systems. *J. R. Soc. Interface*, 9(71):1224–1232, 2012. doi:10.1098/rsif.2011.0664; arXiv:1101.5799.
- [19] D. Flockerzi, K. Holstein, and C. Conradi.  $N$ -site phosphorylation systems with  $2N - 1$  steady states. *Bull. Math. Biol.*, 76(8):1892–1916, 2014. doi:10.1007/s11538-014-9984-0; arXiv:1312.4774.
- [20] F. R. Gantmacher. *The Theory of Matrices*, volume 2. Chelsea, New York, 1959.
- [21] M. Giaroli, R. Rischter, M. Pérez Millán, and A. Dickenstein. Parameter regions that give rise to  $2\lfloor n/2 \rfloor + 1$  positive steady states in the  $n$ -site phosphorylation system. *Math. Biosci. Eng.*, 16(6):7589–7615, 2019. doi:10.3934/mbe.2019381; arXiv:1904.11633.
- [22] V. Guillemin and A. Pollack. *Differential Topology*. Prentice-Hall, Englewood Cliffs, NJ, 1974.
- [23] J. Gunawardena. Multisite protein phosphorylation makes a good threshold but can be a poor switch. *Proc. Natl. Acad. Sci. USA*, 102(41):14617–14622, 2005. doi:10.1073/pnas.0507322102.
- [24] G. H. Hardy, J. E. Littlewood, and G. Pólya. *Inequalities*. Cambridge University Press, 2nd edition, 1952.
- [25] J. Hell and A. D. Rendall. A proof of bistability for the dual futile cycle. *Nonlinear Anal. Real World Appl.*, 24:175–189, 2015. doi:10.1016/j.nonrwa.2015.02.004; arXiv:1404.0394.
- [26] J. Hofbauer. An index theorem for dissipative semiflows. *Rocky Mountain J. Math.*, 20(4):1017–1031, 1990. doi:10.1216/rmjm/1181073059.
- [27] K. Holstein, D. Flockerzi, and C. Conradi. Multistationarity in sequential distributed multisite phosphorylation networks. *Bull. Math. Biol.*, 75(11):2028–2058, 2013. doi:10.1007/s11538-013-9878-6; arXiv:1304.6661.
- [28] F. Horn and R. Jackson. General mass action kinetics. *Arch. Rational Mech. Anal.*, 47:81–116, 1972. doi:10.1007/BF00251225.
- [29] T. Kato. *Perturbation Theory for Linear Operators*. Classics in Mathematics. Springer, Berlin, 1995.
- [30] P. Kokotović, H. K. Khalil, and J. O’Reilly. *Singular Perturbation Methods in Control: Analysis and Design*, volume 25 of *Classics in Applied Mathematics*. SIAM, Philadelphia, 1999.
- [31] M. A. Krasnosel’skiĭ and P. P. Zabreĭko. *Geometrical Methods of Nonlinear Analysis*, volume 263 of *Grundlehren der mathematischen Wissenschaften*. Springer, Berlin, 1984.

- [32] N. I. Markevich, J. B. Hoek, and B. N. Kholodenko. Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. *J. Cell Biol.*, 164(3):353–359, 2004. doi:[10.1083/jcb.200308060](https://doi.org/10.1083/jcb.200308060).
- [33] M. Pérez Millán, A. Dickstein, A. Shiu, and C. Conradi. Chemical reaction systems with toric steady states. *Bull. Math. Biol.*, 74(5):1027–1065, 2012. doi:[10.1007/s11538-011-9685-x](https://doi.org/10.1007/s11538-011-9685-x); arXiv:[1102.1590](https://arxiv.org/abs/1102.1590).
- [34] C. Salazar and T. Höfer. Multisite protein phosphorylation – from molecular mechanisms to kinetic models. *FEBS J.*, 276(12):3177–3198, 2009. doi:[10.1111/j.1742-4658.2009.07027.x](https://doi.org/10.1111/j.1742-4658.2009.07027.x).
- [35] M. Thomson and J. Gunawardena. Unlimited multistability in multisite phosphorylation systems. *Nature*, 460:274–277, 2009. doi:[10.1038/nature08102](https://doi.org/10.1038/nature08102).
- [36] A. Torres and E. Feliu. Symbolic proof of bistability in reaction networks. *SIAM J. Appl. Dyn. Syst.*, 20(1):1–37, 2021. doi:[10.1137/20M1326672](https://doi.org/10.1137/20M1326672); arXiv:[1909.13608](https://arxiv.org/abs/1909.13608).
- [37] L. Wang and E. D. Sontag. On the number of steady states in a multiple futile cycle. *J. Math. Biol.*, 57(1):29–52, 2008. doi:[10.1007/s00285-007-0145-z](https://doi.org/10.1007/s00285-007-0145-z); arXiv:[0704.0036](https://arxiv.org/abs/0704.0036).