Sharp equilibrium and stable-state capacities of multisite phosphorylation

Abstract

The sequential distributive n-site phosphorylation cycle, in which one kinase and one phosphatase act on a substrate with n ordered sites, is the standard mass-action model of multisite protein modification. Wang and Sontag proved that it has at most $2n-1$ positive equilibria in each compatibility class, and the best general multistability result, due to Feliu, Rendall and Wiuf, provides $\lfloor n/2 \rfloor + 1$ asymptotically stable equilibria. We determine both capacities exactly. For every $n \geq 1$, and for one common list of positive rate constants and one compatibility class, the maximal number of positive equilibria is $2n-1$ and the maximal number of locally asymptotically stable equilibria is n. The upper bound n is proved by degree theory and parametric transversality and includes nonhyperbolic attractors. The lower bounds are attained simultaneously by rational data: $2n-1$ hyperbolic equilibria, of which n are sinks and $n-1$ are saddles with a one-dimensional unstable manifold, and the same configuration persists on a nonempty open subset of the $(6n+3)$-dimensional space of rate constants and totals. The equilibria are produced by an explicit positive polynomial recurrence together with an interlacing argument. Stability is obtained by separating equilibrium geometry from kinetic time scales: after coalescing the equilibria, fast binding reduces the dynamics to a slow system on substrate inventories that retain the enzyme-bound substrate, and for a large kinase excess its transverse part converges to a path matrix with a rank-one positive feedback whose gain is strictly less than one by an explicit margin. We also obtain a diagonal Lyapunov certificate, the exact splitting law of the critical eigenvalue, exact rational witnesses for $n \leq 7$, and a three-site benchmark with a rigorous local robustness and recovery certificate. The algebraic core of the argument is formally verified in Lean 4.

1 Introduction

1.1 Background

Reversible phosphorylation at several sites is one of the most common mechanisms by which cells regulate protein function [4, 35]. 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

$$\begin{aligned} S_{i-1} + E \underset{b_i}{\overset{a_i}{\rightleftharpoons}} C_i &\xrightarrow{c_i} S_i + E, \\ S_i + F \underset{\beta_i}{\overset{\alpha_i}{\rightleftharpoons}} Y_i &\xrightarrow{\gamma_i} S_{i-1} + F, \qquad i = 1, \dots, n, \end{aligned} \tag{1.1}$$

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,

$$E_{\mathrm{T}} = E + \sum_{i=1}^{n} C_i, \qquad F_{\mathrm{T}} = F + \sum_{i=1}^{n} Y_i, \qquad S_{\mathrm{T}} = \sum_{i=0}^{n} S_i + \sum_{i=1}^{n} (C_i + Y_i). \tag{1.2}$$

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 [33]. This started a long line of work on the equilibria of (1.1); see [24, 7, 37, 19, 28, 8, 26, 6, 3, 16, 38] and the surveys [11, 14, 15]. 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 [34].

Three results frame the present work. Wang and Sontag [39] 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 [20] 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. Whether $2n-1$ is attained for larger n has been recorded as open [18, 17]. Feliu, Rendall and Wiuf [18] 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 [22], and numerical evidence for growth of the number of stable states with n goes back to [37]. 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 $\kappa \in \mathbb{R}^{6n}_{>0}$ and totals $T = (E_{\mathrm{T}}, F_{\mathrm{T}}, S_{\mathrm{T}}) \in \mathbb{R}^{3}_{>0}$ 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

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

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 $n \geq 1$,*

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

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 $\mathbb{R}^{6n+3}_{>0}$, 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 $n \leq 4$ [20]. For $n = 1$ the unique equilibrium is globally attracting [1], and for $n = 2$ two stable equilibria occur [26]; 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 $X_0, \ldots, X_{2n-2}$. 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 $\xi_0 < \cdots < \xi_{2n-2}$ be real numbers. In the family of systems constructed in Section 7, depending on a parameter $\delta > 0$ and having equilibria $X_0(\delta), \ldots, X_{2n-2}(\delta)$ 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*

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

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 δ, 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 ± 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

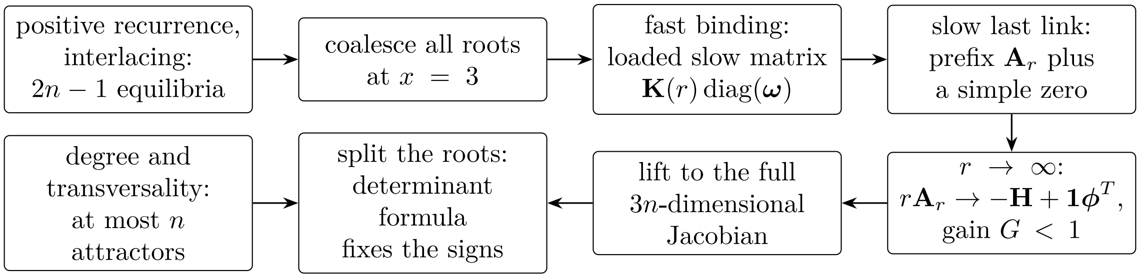

Figure 1: Structure of the proof. The ceiling (bottom left) is independent of the construction. The parameters are chosen in the order n, r, last catalytic current, binding time scale, root separation.

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 $E_{\mathrm{T}} = uf(1+sB(u))$, $F_{\mathrm{T}} = f(1+suD(u))$, $S_{\mathrm{T}} = sA(u)+usf(B(u)+D(u))$ 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 scalar equation $\Phi(u) = S_{\mathrm{T}}$. On the locus $A = (1+u)D$, $S_{\mathrm{T}} = E_{\mathrm{T}} + F_{\mathrm{T}}$, the numerator of $\Phi - S_{\mathrm{T}}$ 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 $(x-1)(B - rD) = 2(r-u)D$, $r = E_{\mathrm{T}}/F_{\mathrm{T}}$. 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 $\mathbf{K}\ \mathrm{diag}(\boldsymbol{\omega})$, where $\boldsymbol{\omega}$ is the vector of catalytic currents and $\mathbf{K} = (-\mathbf{R} + \mathbf{1}\boldsymbol{h}^T)\mathbf{M}^{-1}\mathbf{R}^T$ is formed from the path incidence matrix $\mathbf{R}$, a positive definite "mass matrix" $\mathbf{M}$, and a feedback vector $\boldsymbol{h}$ 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 $\mathbf{B}_* = -\mathbf{H} + \mathbf{1}\boldsymbol{\phi}^T$, where $\mathbf{H}$ is the Dirichlet-type matrix of a weighted path and $\boldsymbol{\phi}$ is entrywise positive. This is a positive-feedback perturbation of a stable matrix, and its stability is decided by the scalar gain $G = \boldsymbol{\phi}^T\mathbf{H}^{-1}\mathbf{1}$. The moments of the coalesced polynomials give

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

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 [18]; see also [26]. 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 existence of $2n-1$ positive equilibria for every n, as a statement about the vector field of (1.1), is formally verified in Lean 4 [12] with Mathlib [36], and so are the algebraic identities on which the stability proof turns: the coalesced product identity, the source-preserving changes of kinetics, the factorization of the slow characteristic polynomial, the elimination that yields the feedback vector, and the closed form and positivity of $1-G$. The analytic parts of the proof (degree theory, interlacing, matrix stability, spectral perturbation) are proved in the conventional way only; Section 10 states the boundary precisely. All numbers in the paper are reproduced in exact rational arithmetic by a script distributed with the source.

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 $W \colon \mathbb{R}^{3n+3} \to \mathbb{R}^3$ be the linear map (1.2). The totals are conserved, so V takes values in $\ker W$. For positive $T = (E_\mathrm{T}, F_\mathrm{T}, S_\mathrm{T})$ 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 $T \in \mathbb{R}^3_{>0}$. 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 $E_\mathrm{T}=0$; 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 $S_\mathrm{T}=0$. Then the complexes are positive as well. □

2.2 Parametrization of the positive equilibria

For given rate constants put

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

and define three polynomials with positive coefficients,

$$\begin{gathered} A(u) = \sum_{i=0}^{n} t_i u^i, \qquad B(u) = \sum_{i=0}^{n-1} B_i u^i, \qquad D(u) = \sum_{i=0}^{n-1} D_i u^i, \\ B_{i-1} = p_i t_{i-1}, \qquad D_{i-1} = q_i t_i \qquad (1 \le i \le n). \end{gathered} \tag{2.4}$$

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*

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

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

$$E_\mathrm{T} = uf\big(1+sB(u)\big), \qquad F_\mathrm{T} = f\big(1+suD(u)\big), \qquad S_\mathrm{T} = sA(u) + usf\big(B(u)+D(u)\big). \tag{2.6}$$

(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,β_i,γ_i $(1 \le i \le n)$, the rate constants*

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

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 $c_i p_i/(\gamma_i q_i) = t_i/t_{i-1}$. □

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, β_i, γ_i affect the dynamics, including stability, but not the equilibria. We call the coefficients the *geometry* and (b_i, β_i, γ_i) the *kinetics*. This separation is the organizing principle of the paper.

2.3 The scalar equation

Fix positive totals and put $r = E_{\mathrm{T}}/F_{\mathrm{T}}$,

$$L(u) = B(u) - rD(u), \qquad M(u) = B(u) - uD(u). \tag{2.8}$$

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

$$s(u) = \frac{r-u}{uL(u)}, \qquad f(u) = \frac{F_{\mathrm{T}}}{1 + s(u)uD(u)} = \frac{F_{\mathrm{T}} L(u)}{M(u)}, \tag{2.9}$$

where we used $L + (r-u)D = M$; in particular $M(u) = L(u)\big(1 + suD(u)\big)$ 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 $E_{\mathrm{T}}, F_{\mathrm{T}}$. On the open set $\mathcal{U} = \{u > 0 : (r-u)L(u) > 0\}$ define the *substrate-total function*

$$\Phi(u) = s(u)A(u) + u\, s(u) f(u)\big(B(u) + D(u)\big). \tag{2.10}$$

The positive equilibria with totals $(E_{\mathrm{T}}, F_{\mathrm{T}}, S_{\mathrm{T}})$ and $u \neq r$ correspond bijectively to the solutions $u \in \mathcal{U}$ of $\Phi(u) = S_{\mathrm{T}}$. This reduction is the one used in [39], from which we take the following bound.

Theorem 2.3 (Wang and Sontag [39, 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 $(S_{\mathrm{T}}, E_{\mathrm{T}}, F_{\mathrm{T}})$. 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 $u = E/F \neq r = E_{\mathrm{T}}/F_{\mathrm{T}}$,*

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

where Φ 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 $y = (S; C, Y)$,

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

and the Schur complement of Δ 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

$$\det \begin{pmatrix} g_y & g_z \\ W_y & I_3 \end{pmatrix} = \det g_y \cdot \det\big(I_3 - W_y g_y^{-1} g_z\big) = \det g_y \cdot \det \frac{\partial(S_{\mathrm{T}}, E_{\mathrm{T}}, F_{\mathrm{T}})}{\partial(S_0, E, F)},$$

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),

$$\det \frac{\partial(E_{\mathrm{T}}, F_{\mathrm{T}})}{\partial(s, f)} = \det \begin{pmatrix} ufB & u(1 + sB) \\ fuD & 1 + suD \end{pmatrix} = uf\,(B - uD) = uf\,M(u) \neq 0, \tag{2.12}$$

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 $\partial(S_{\mathrm{T}}, E_{\mathrm{T}}, F_{\mathrm{T}})/\partial(u, s, f)$ is the derivative of S_{T} along that curve, which is $\Phi'(u)$. Hence $\det \partial(S_{\mathrm{T}}, E_{\mathrm{T}}, F_{\mathrm{T}})/\partial(u, s, f) = ufM(u)\Phi'(u)$, and collecting the factors gives (2.11). ☐

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 $\operatorname{sign} \det \mathcal{J} = (-1)^{e_-}$, where e_- is the number of negative real eigenvalues, and $3n - e_-$ has the parity of the number e_+ of positive real eigenvalues. Thus $\operatorname{sign} \det \mathcal{J} = (-1)^n (-1)^{e_+}$. On the other hand $M(u)$ has the sign of $L(u)$, hence of $r - u$, and (2.11) gives $\operatorname{sign} \det \mathcal{J} = (-1)^{n+1} \operatorname{sign} \Phi'(u)$. ☐

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 $\operatorname{ind}(g, X)$ of an isolated zero [13, 32]. 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_κ 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 $\operatorname{sign}\det(-\mathcal{J}) = +1$.

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

Proof. (i) Let $y_0 \in \operatorname{int}\mathcal{P}$ and consider $g^t(y) = (1-t)g_\kappa(y) + t(y_0 - y)$, $0 \le t \le 1$. On a face of $\mathcal{P}$ where some species vanishes, the corresponding component of g_κ 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, $\deg(-g_\kappa, \operatorname{int}\mathcal{P}, 0) = \deg(y - y_0, \operatorname{int}\mathcal{P}, 0) = 1$.

(ii) The vector field is linear in the rate constants: $V_\kappa(X) = \Gamma \operatorname{diag}(X^{\eta_1}, \ldots, X^{\eta_{6n}})\kappa$, where Γ is the stoichiometric matrix and X^{η_k} are the reactant monomials. At a positive X the monomials are positive and Γ has rank $3n = \dim\ker W$ by Section 2. Hence $(y, \kappa) \mapsto g_\kappa(y)$ is a submersion on $\operatorname{int}\mathcal{P} \times \mathbb{R}^{6n}_{>0}$, and by the parametric transversality theorem [23, Ch. 2, §3] zero is a regular value of g_κ on $\operatorname{int}\mathcal{P}$ for almost every κ. 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 $\operatorname{int}\mathcal{P}$ or a zero on the boundary; so the zeros are finite in number. Let $n_\pm$ be the numbers of zeros of index ± 1. Additivity of the degree and (i) give $n_+ - n_- = 1$, Theorem 2.3 gives $n_+ + n_- \le 2n - 1$, and therefore $n_+ \le n$. ☐

The degree identity in (i), for dissipative networks without boundary equilibria, is the basis of the multistationarity criterion of [6]; see also [27]. 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* $X_* \in \operatorname{int}\mathcal{P}$ *be an equilibrium that is locally asymptotically stable relative to* $\mathcal{P}$*. Then* X_* *is an isolated zero of* g_κ *and* $\operatorname{ind}(-g_\kappa, X_*) = +1$*.*

Proof. This is classical [32]; we include the short argument. Let ϕ_t be the flow and $\mathcal{B}$ a closed ball around X_* inside its basin of attraction and inside $\operatorname{int}\mathcal{P}$. 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 $\deg(I - \phi_t, \operatorname{int}\mathcal{B}, 0)$ 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 $\phi_t(\mathcal{B}) \subset \operatorname{int}\mathcal{B}'$ for a smaller concentric ball $\mathcal{B}'$; the homotopy $I - \theta(\phi_t - X_*) - X_*$, $0 \le \theta \le 1$, then has no zero on $\partial\mathcal{B}$ and shows that the degree is 1. On the other hand $(I - \phi_t)/t \to -g_\kappa$ uniformly on $\partial\mathcal{B}$ as $t \downarrow 0$, and g_κ does not vanish there, so $\deg(-g_\kappa, \operatorname{int}\mathcal{B}, 0) = 1$ as well. ☐

Proof of Theorem 1.1(a)*.* Suppose that for some κ and T there are $n+1$ locally asymptotically stable equilibria. They are positive by Lemma 2.1. Choose pairwise disjoint closed balls $\mathcal{B}_0, \ldots, \mathcal{B}_n$ 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 κ', we have $\deg(-g_{\kappa'}, \operatorname{int}\mathcal{B}_k, 0) = 1$ for all κ' close to κ and all k. By Lemma 3.1(ii) we may choose such a κ' 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). ☐

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 $\mathcal{H}_n \le \mathcal{C}_n \le n$.

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 $S_{\mathrm{T}} = E_{\mathrm{T}} + F_{\mathrm{T}}$, and normalize $F_{\mathrm{T}} = 2$, so that $E_{\mathrm{T}} = 2r$ and $S_{\mathrm{T}} = 2(r+1)$. For $u \in \mathcal{U}$ put $\ell = B(u)/D(u) - r$, $\eta = r - u$ and $x = \sqrt{1+8u}$. Then*

$$\Phi(u) - S_{\mathrm{T}} = \frac{(1+u)\big(\eta(\ell+\eta) - 2u\ell^2\big)}{u\,\ell\,(\ell+\eta)} = -\frac{2(1+u)}{\ell(\ell+\eta)}\Big(\ell - \frac{2\eta}{x-1}\Big)\Big(\ell + \frac{2\eta}{x+1}\Big). \tag{4.1}$$

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

$$\Phi = \frac{(1+u)\eta}{u\ell} + \frac{2\eta(\ell+r+1)}{\ell+\eta}, \qquad \Phi - 2(r+1) = \frac{(1+u)\eta}{u\ell} - \frac{2\ell(r+1-\eta)}{\ell+\eta} = (1+u)\Big(\frac{\eta}{u\ell} - \frac{2\ell}{\ell+\eta}\Big),$$

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

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

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

The condition $A = (1+u)D$ says $t_i = D_i + D_{i-1}$, and $S_{\mathrm{T}} = E_{\mathrm{T}} + F_{\mathrm{T}}$ 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

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

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

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

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 $(0 \le i \le m)$ are positive, and*

$$(x-1)\,N(u(x)) - 2\,J(u(x)) = \Pi(x). \tag{4.5}$$

Moreover

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

Proof. Let N, J have positive coefficients in degrees $0, \dots, k$ and none above. The coefficient of u^i in N_{new} is $8N_{i-1}+(a+1)(b+1)N_i+2(a+b)J_i$ and in J_{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 $0 \le i \le k+1$. 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

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

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 $\Pi(x) = \Pi_{\text{e}}(x^2) + x\,\Pi_{\text{o}}(x^2)$ and replacing x by $-x$ in (4.5) shows that, as functions of $x^2 = 1 + 8u$,

$$N = \Pi_{\text{o}}, \qquad J = -\tfrac{1}{2}\big(\Pi_{\text{e}} + \Pi_{\text{o}}\big). \tag{4.7}$$

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 $m \ge 1$. The polynomials N and J have only real, simple roots $\nu_m < \cdots < \nu_1$ and $\zeta_m < \cdots < \zeta_1$, and*

$$\nu_m < \zeta_m < \cdots < \nu_2 < \zeta_2 < \nu_1 < \zeta_1 < 0. \tag{4.8}$$

Consequently

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

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

$$N = \frac{\varrho \sin\vartheta}{y}, \qquad J = \frac{\varrho}{2}\Big(\cos\vartheta - \frac{\sin\vartheta}{y}\Big). \tag{4.10}$$

The function ϑ 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 $y_1 < \cdots < y_m$. 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 (ν_{k+1}, ν_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 ζ_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 $(0 \le i \le m)$ is strictly increasing, and the sequence J_i/N_i $(0 \le i \le m)$ is nondecreasing.*

Proof. A polynomial with positive coefficients and only real roots satisfies Newton's inequalities $J_i^2 > J_{i-1}J_{i+1}$ [25, §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

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

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

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

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 $(u-r)Q = uJ_rN - rN_rJ$ gives

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

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

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

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

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

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

$$Q(u) = J(u)J_r\,\frac{\frac{uN(u)}{J(u)} - \frac{rN_r}{J_r}}{u-r} = J(u)J_r\Big(\alpha_0 + \sum_k \frac{\sigma_k|\zeta_k|}{(u-\zeta_k)(r-\zeta_k)}\Big)$$
$$= \alpha_0 J_r\, J(u) + \sum_{k=1}^{m} \sigma_k |\zeta_k|\, J^{(k)}(r)\, J^{(k)}(u).$$

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

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

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

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,

$$J_r < \tfrac{1}{2}(\rho-1)N_r. \tag{4.15}$$

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 $\sum_j x_j + 1 - 4r \le 0$, the first bracket is positive as well. Otherwise, by (4.15), it exceeds

$$\tfrac{1}{2}(\rho-1)N_r\Big[(\rho+1) - \Big(\sum_j x_j + 1 - 4r\Big)\Big] = \tfrac{1}{2}(\rho-1)N_r\Big(4r + \rho - \sum_j x_j\Big) \;\ge 0.$$

Thus B_{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 $r \ge 3n/2$.

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

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

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, β_i, γ_i). By Lemma 4.5, for every j,

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

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

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

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 $F_{\mathrm{T}} = f(1 + suD) = 2$; next, by (4.17), $suB = \frac{1}{2}r(x-1) + (r-u)$, so $E_{\mathrm{T}} = uf + f\big(\frac{1}{2}r(x-1) + r - u\big) = \frac{1}{2}f\,r\,(x+1) = 2r$; finally $sA = (1+u)(x-1)/(2u) = 4(1+u)/(x+1) = (1+u)f$, that is,

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

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

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

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

In particular, if the x_j are distinct and ordered increasingly, then $\Phi'(u_j)\neq 0$ and $\operatorname{sign}\Phi'(u_j) = (-1)^{j+1}$; if x_j is a repeated root of Π, 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 $(x-1)D_{\mathrm{raw}}(u)$ gives

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

which vanishes at u_j, and $\Phi - S_{\mathrm{T}} = \Psi_2\Psi_1$ 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 $dx/du = 4/x$, $\Psi_1'(u_j) = 4(c_*x+e_*)\Pi'(x)/(x(x-1)D_{\mathrm{raw}}(u))$. 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. □

Theorem 4.8 (Maximal equilibrium count)**.** *Let $n\geq 1$, let $x_0<\cdots<x_{2n-2}$ 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,β_i,γ_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 $\mathbb{R}^{6n+3}_{>0}$.*

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 $S_{\mathrm{T}} = E_{\mathrm{T}}+F_{\mathrm{T}}$. □

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 θ_i, b_i, β_i $(1 \le i \le n)$ freely and set

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

without changing any equilibrium (2.5). We call θ_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

$$\omega_i = c_i C_i = \gamma_i Y_i = \theta_i Y_i \tag{5.2}$$

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 $0 < \varepsilon \le 1$, replace

$$a_i \mapsto \frac{a_i}{\varepsilon}, \qquad b_i \mapsto \frac{b_i + c_i}{\varepsilon} - c_i, \qquad \alpha_i \mapsto \frac{\alpha_i}{\varepsilon}, \qquad \beta_i \mapsto \frac{\beta_i + \gamma_i}{\varepsilon} - \gamma_i. \tag{5.3}$$

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

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

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

5.2 Loaded inventories

Let

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

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

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

where $\mathbf{R}$ is the $n \times (n+1)$ incidence matrix of the path on the levels $0, \dots, n$, with rows $\boldsymbol{e}_i^T - \boldsymbol{e}_{i-1}^T$ $(1 \le i \le n)$. Also $\sum_i \Lambda_i = S_{\mathrm{T}}$. In comparison with (2.1), $Z_i = \sum_{k \ge i} \Lambda_k$. 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 ε, and (5.4) does not involve ε on the right. The restricted Jacobian at an equilibrium therefore has the form

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

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 ε. (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

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

indexed by the levels $0, \dots, n$, so that $\boldsymbol{\Lambda} = \boldsymbol{S} + \boldsymbol{c} + \boldsymbol{y}$. The entry $c_i = C_{i+1}$ of $\boldsymbol{c}$ 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 $E_\mathrm{T}, F_\mathrm{T}$, and variations of it within the fast-binding equilibrium set that keep E_T and F_T fixed. In terms of the logarithmic variations $\sigma_i = dS_i/S_i$,*

$$d\boldsymbol{\Lambda} = \mathbf{M}\boldsymbol{\sigma}, \qquad \mathbf{M} = \mathrm{diag}(\boldsymbol{S}+\boldsymbol{c}+\boldsymbol{y}) - \frac{\boldsymbol{c}\boldsymbol{c}^T}{E_\mathrm{T}} - \frac{\boldsymbol{y}\boldsymbol{y}^T}{F_\mathrm{T}}, \tag{5.8}$$

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

$$d\boldsymbol{j} = \mathrm{diag}(\boldsymbol{\omega})(-\mathbf{R}+\mathbf{1}\boldsymbol{h}^T)\boldsymbol{\sigma}, \qquad \boldsymbol{h} = \frac{\boldsymbol{y}}{F_\mathrm{T}} - \frac{\boldsymbol{c}}{E_\mathrm{T}}. \tag{5.9}$$

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 $E_\mathrm{T} = E + \sum_i C_i$ at fixed E_T gives $dE\,(1+\sum_i C_i/E) = -\sum_i C_i\sigma_{i-1}$, that is, $dE/E = -\boldsymbol{c}^T\boldsymbol{\sigma}/E_\mathrm{T}$; likewise $dF/F = -\boldsymbol{y}^T\boldsymbol{\sigma}/F_\mathrm{T}$. 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 $c_iC_i = \gamma_i Y_i = \omega_i$, so $dj_i = \omega_i\big[(\sigma_{i-1} + dE/E) - (\sigma_i + dF/F)\big]$, which is (5.9). For positivity, the Cauchy–Schwarz inequality gives $(\boldsymbol{c}^T\boldsymbol{a})^2 \le (\sum_i c_i)(\sum_i c_i a_i^2)$ for every vector $\boldsymbol{a}$, and similarly for $\boldsymbol{y}$, so

$$\boldsymbol{a}^T\mathbf{M}\boldsymbol{a} \;\ge\; \sum_i S_i a_i^2 + \Big(1 - \frac{\sum_i c_i}{E_\mathrm{T}}\Big)\sum_i c_i a_i^2 + \Big(1 - \frac{\sum_i y_i}{F_\mathrm{T}}\Big)\sum_i y_i a_i^2 \;>0 \qquad (\boldsymbol{a}\neq 0),$$

because $\sum_i c_i = E_\mathrm{T} - E < E_\mathrm{T}$, $\sum_i y_i = F_\mathrm{T} - F < F_\mathrm{T}$ and all $S_i > 0$. □

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 $\mathrm{diag}(\boldsymbol{S})$: 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*

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

(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 $\mathbf{K}\,\mathrm{diag}(\boldsymbol{\omega})$.* (iii) *As $\varepsilon \to 0$, $2n$ eigenvalues of $\mathcal{J}_\varepsilon$ have real parts tending to $-\infty$, and the other n converge, with multiplicities, to the eigenvalues of $\mathbf{K}\,\mathrm{diag}(\boldsymbol{\omega})$.*

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 Γ_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 kth reaction is φ_k times the differential of the logarithm of the ratio of its forward and backward rates, which is $-(\Gamma_f^T\,\mathrm{diag}(X)^{-1}dX)_k$, where X is the vector of concentrations; and $dX = \Gamma_f\,d(C,Y)$. Hence $\mathbf{D}_0 = -\,\mathrm{diag}(\varphi)\,\Gamma_f^T\,\mathrm{diag}(X)^{-1}\Gamma_f$. This is a positive diagonal matrix times a symmetric negative definite matrix, hence similar to the symmetric negative definite matrix $-\,\mathrm{diag}(\varphi)^{1/2}\Gamma_f^T\,\mathrm{diag}(X)^{-1}\Gamma_f\,\mathrm{diag}(\varphi)^{1/2}$; compare [29].

(ii) Since $\mathbf{D}_0$ is invertible, the equations (5.4) with zero left side define (C, Y) locally as a function of $(\Lambda_1, \ldots, \Lambda_n)$ 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 $\boldsymbol{\Lambda} \mapsto \mathbf{R}^T\boldsymbol{j}$. By Lemma 5.1 this Jacobian is the restriction of $\mathbf{R}^T\,\mathrm{diag}(\boldsymbol{\omega})(-\mathbf{R}+\mathbf{1}\boldsymbol{h}^T)\mathbf{M}^{-1}$ 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 $\boldsymbol{a}$ with $d\mathbf{\Lambda} = \mathbf{R}^T\boldsymbol{a}$ the restriction is $\operatorname{diag}(\boldsymbol{\omega})\mathbf{K}$, which is similar to $\mathbf{K}\operatorname{diag}(\boldsymbol{\omega})$.

(iii) For λ in a compact set and ε 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 $\left(\begin{smallmatrix} 0 & 0 \\ \mathbf{C}_0 & \mathbf{D}_0 \end{smallmatrix}\right)$, 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 [31, Ch. 2]. $\square$

5.3 Coalescence

Now set every auxiliary root equal to 3: $x_0 = \cdots = x_{2m} = 3$, $\Pi(x) = (x-3)^{2n-1}$, 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

$$u = 1, \qquad E = F = 1, \qquad s = \frac{1}{D(1)}, \qquad (E_{\mathrm{T}}, F_{\mathrm{T}}, S_{\mathrm{T}}) = (2r, 2, 2r+2), \tag{5.11}$$

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

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

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 $\boldsymbol{j} = 0$ hold identically and $\sum_i \Lambda_i = \Phi(u)$. Let $\boldsymbol{\sigma}$ be the logarithmic derivative of $(S_0, \ldots, S_n)$ 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 $(-\mathbf{R} + \mathbf{1}\boldsymbol{h}^T)\boldsymbol{\sigma} = 0$. Hence $\mathbf{M}\boldsymbol{\sigma} = \mathbf{R}^T\boldsymbol{a}$ with $\boldsymbol{a} \neq 0$, and $\mathbf{K}\boldsymbol{a} = 0$. $\square$

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

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

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

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

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 $1, \ldots, n-1$. 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 $r \to \infty$, $Q_i/N_r = J_i + O(1/r)$ and $(B_{\mathrm{raw}})_i/(4rN_r) = J_i + N_i + O(1/r)$, because J_r/N_r is bounded. After the normalization (4.16),

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

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

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

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

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

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

$$\begin{gathered} \sum_{i<n} d_i = 2, \qquad \sum_{i=0}^{n} \bar{y}_i = 1, \qquad \mu := \frac{1}{2}\sum_{i<n} i\, d_i = \frac{4n-3}{9}, \\ v := \bar{y}_n = \frac{J_m}{\jmath} = (3n-2)\Big(\frac{2}{9}\Big)^{n-1} \in (0,1). \end{gathered} \tag{6.3}$$

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

$$\sum_i c_i = 2r - 1, \qquad \sum_i y_i = 1, \qquad \sum_i S_i = 2, \qquad S_n = y_n, \tag{6.4}$$

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 $r \to \infty$ and

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

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 $\boldsymbol{e}_n$. 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 $r \to \infty$,*

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

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

Proof. By (5.8) and (6.4), $\mathbf{M1} = \boldsymbol{S} + \boldsymbol{c}\,(1 - \frac{2r-1}{2r}) + \boldsymbol{y}\,(1 - \frac{1}{2}) = \boldsymbol{S} + \boldsymbol{c}/(2r) + \boldsymbol{y}/2$. Hence $\mathbf{1}^T\mathbf{M1} = 2 + \frac{2r-1}{2r} + \frac{1}{2} \to \frac{7}{2}$, $\mathbf{1}^T\mathbf{M}\boldsymbol{e}_n = S_n + \frac{1}{2}y_n \to \frac{3}{2}v$, and $\boldsymbol{e}_n^T\mathbf{M}\boldsymbol{e}_n = S_n + y_n - \frac{1}{2}y_n^2 \to 2v - \frac{1}{2}v^2$. With $\mathbf{1}_{<n} = \mathbf{1} - \boldsymbol{e}_n$ these give (6.5). The diagonal entries are positive for $0 < v < 1$, and the determinant is $(\frac{7}{2} - v - \frac{1}{2}v^2)(2v - \frac{1}{2}v^2) - \frac{1}{4}v^2(v-1)^2 = 7v - 4v^2$. □

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

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

where the constants τ_* and τ_n are the unique solution of

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

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 $\boldsymbol{h}_\infty = \lim_{r\to\infty} \boldsymbol{h}$,

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

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 $r \to \infty$, and where the limit of $\mathbf{C}_1$ is positive definite by Lemma 6.1. Solving $\mathbf{M}\boldsymbol{\tau}^{(r)} = r\boldsymbol{\chi}$ with $\boldsymbol{\chi} = (\boldsymbol{\chi}_\perp, 0)$ by elimination of the second block gives the $\mathcal{V}^{\perp}$-component $\big(\mathbf{A}_0 + r^{-1}(\mathbf{A}_1 - \mathbf{B}_1\mathbf{C}_1^{-1}\mathbf{B}_1^T)\big)^{-1}\boldsymbol{\chi}_\perp$ and the $\mathcal{V}$-component $-\mathbf{C}_1^{-1}\mathbf{B}_1^T$ times it. Both converge.

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

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

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

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

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 $2v\,\tau_n - \frac{1}{2}v\big(T + (1-v)\tau_* + v\tau_n\big) = 0$; dividing by $v/2$ gives the second equation of (6.7). The determinant of the system (6.7) in (τ_*, τ_n) is $\frac{1}{2}(7-3v)(4-v) + \frac{3}{2}v(1-v) = 14 - 8v > 0$, and Cramer's rule gives

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

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

Feedback. By (5.9) and (6.2), $\boldsymbol{h}_\infty = \frac{1}{2}\bar{\boldsymbol{y}} - \frac{1}{2}(d,0)$. Hence $\boldsymbol{h}_\infty^T\boldsymbol{\tau} = \frac{1}{2}\big(T + (1-v)\tau_* + v\tau_n\big) - \frac{1}{2}\cdot 2\tau_*$, 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 $\boldsymbol{h}_\infty^T\boldsymbol{\tau} = 2\tau_n - \tau_*$, 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

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

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 $r\to\infty$,*

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

where

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

Every entry of $\boldsymbol{\phi}$ is strictly positive.

Proof. Column $k\le n-1$ of $\mathbf{K}$ is $(-\mathbf{R}+\mathbf{1}\boldsymbol{h}^T)\mathbf{M}^{-1}\boldsymbol{\chi}$ with $\boldsymbol{\chi} = \mathbf{R}^T\boldsymbol{e}_k = \boldsymbol{e}_k - \boldsymbol{e}_{k-1} \in \mathcal{V}^\perp$. By Lemma 6.2, r times this column converges to $-\mathbf{R}\boldsymbol{\tau} + (\boldsymbol{h}_\infty^T\boldsymbol{\tau})\mathbf{1}$. In the rows $i\le n-1$, $(\mathbf{R}\boldsymbol{\tau})_i = \tau_i - \tau_{i-1} = \chi_i/d_i - \chi_{i-1}/d_{i-1}$, since τ_* 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 $\boldsymbol{h}_\infty^T\boldsymbol{\tau} = g_k - g_{k-1}$.

For the sign, write

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

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 $\mathbf{1}\boldsymbol{\phi}^T$ is positive. Positive feedback can destabilize, and the question is whether its gain is below one.

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

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

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 $\boldsymbol{z} > 0$.

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

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

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

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

Now $\mathbf{B}_* \boldsymbol{z} = -\mathbf{H}\boldsymbol{z} + \mathbf{1}\,(\boldsymbol{\phi}^T \boldsymbol{z}) = -(1-G)\mathbf{1} < 0$. The matrix $\mathbf{B}_*$ has nonnegative off-diagonal entries, because those of $-\mathbf{H}$ are nonnegative and $\boldsymbol{\phi} > 0$. After the similarity $\operatorname{diag}(\boldsymbol{z})^{-1}\mathbf{B}_* \operatorname{diag}(\boldsymbol{z})$ 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. □

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

All choices in this section are made in the order

$$n \text{ fixed} \;\longrightarrow\; r \text{ large} \;\longrightarrow\; \omega_n > 0 \text{ small} \;\longrightarrow\; \varepsilon > 0 \text{ small} \;\longrightarrow\; \delta > 0 \text{ small}. \tag{7.1}$$

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 $\mathbf{K}(r)\operatorname{diag}(1, \ldots, 1, t)$ 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, $\det\big(\mathbf{K}\operatorname{diag}(1, \ldots, 1, t)\big) = t \det \mathbf{K} = 0$, so that eigenvalue is 0. □

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 $\boldsymbol{\omega} = (1, \ldots, 1, t)$ 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 ε the matrix $\mathcal{J}_\varepsilon$ has $2n$ eigenvalues with large negative real part, $n-1$ eigenvalues near the stable eigenvalues of $\mathbf{K}\operatorname{diag}(\boldsymbol{\omega})$, and exactly one eigenvalue, counted with multiplicity, in a small disc around 0. By (5.12) that eigenvalue is 0. □

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, ε of Lemma 7.2, and let $\theta_i^\circ = \omega_i / Y_i(X_\circ)$ be the catalytic scales used there. For real $\xi_0 < \cdots < \xi_{2m}$ 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 δ, at the same r; the coefficients of N, J, Q, B, D are polynomial or rational in δ. 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 ε. For each δ this is one rate vector $\kappa(\delta)$, common to all the equilibria $X_0(\delta), \ldots, X_{2m}(\delta)$ of Theorem 4.8, and as $\delta \to 0$ 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 δ.

Let $\mathcal{J}_j(\delta)$ be the restricted Jacobian at $X_j(\delta)$. By continuity of the spectrum and Lemma 7.2, for small δ 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 δ [30, Ch. II]. Let $\Theta_j(\delta)$ be the product of the other eigenvalues; then $\det \mathcal{J}_j = \lambda_j \Theta_j$, and $\Theta_j(\delta) \to \Theta_\circ$, 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,

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

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

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

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 $(-1)^{2m-j} = (-1)^j$. □

Proof of Theorem 1.1(b) *and* (c). Let $n \geq 2$. In the preceding proof choose rational ξ_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, ε and δ 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, ε 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)

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

The Routh array has first column $(1, 10, 26, 10)$, so $\mathcal{J}$ is Hurwitz [21, 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 $n \geq 1$. □

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*

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

Proof. Insert $v=(3n-2)(2/9)^{n-1}$ from (6.3) into (6.13); the asymptotic statement follows from $v\to 0$ 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

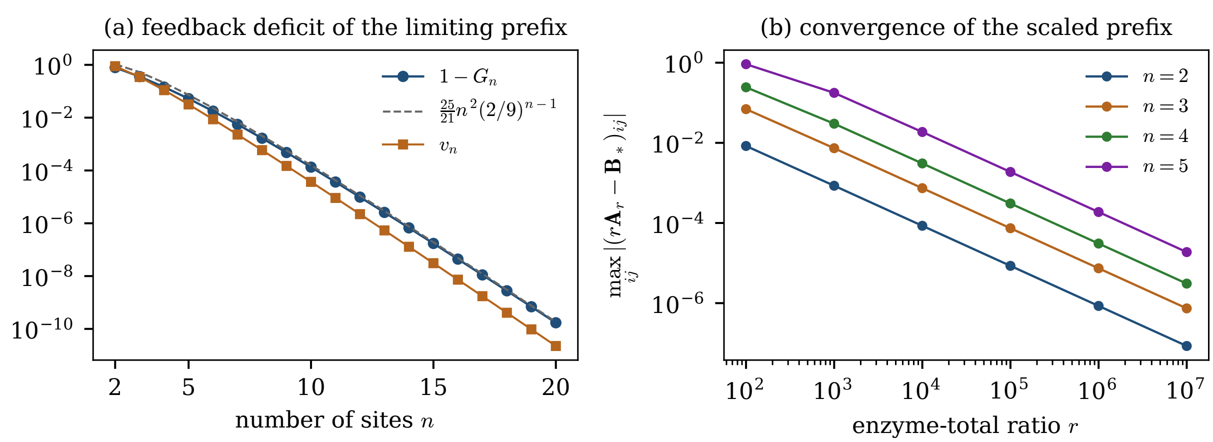

Figure 2: (a) The feedback deficit (8.1), its asymptote, and the coefficient fraction v_n. (b) The entrywise distance between $r\mathbf{A}_r$, computed exactly from the coalesced construction, and its limit $\mathbf{B}_*$; the slope is -1, in agreement with the $O(1/r)$ errors in Section 6.

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 $\boldsymbol{z}=\mathbf{H}^{-1}\mathbf{1}$ and $\boldsymbol{w}=\mathbf{H}^{-1}\boldsymbol{\phi}$; both are entrywise positive.

Proposition 8.2 (Diagonal Lyapunov matrix)**.** *Let* $\mathbf{P} = \operatorname{diag}(w_i/z_i)$ *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, $\mathbf{1}^T\boldsymbol{w} = \boldsymbol{z}^T\boldsymbol{\phi} = G$. Hence $\mathbf{B}_*\boldsymbol{z} = -(1-G)\mathbf{1}$ and $\mathbf{B}_*^T\boldsymbol{w} = -\boldsymbol{\phi} + G\boldsymbol{\phi} = -(1-G)\boldsymbol{\phi}$. Because $\mathbf{P}\boldsymbol{z} = \boldsymbol{w}$,

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

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 $\boldsymbol{z}$, expanding the squares gives the identity

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

which is negative for $\boldsymbol{a} \neq 0$. Negative definiteness is an open condition, and $r\mathbf{A}_r \to \mathbf{B}_*$. 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. □

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 $\mathbf{A}_r \operatorname{diag}(\omega_1, \dots, \omega_{n-1})$ 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, ε and δ_0 can be chosen uniformly on $\mathcal{K}$. □

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

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

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,*

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

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

Proof. At each δ all equilibria share the polynomial $D = D^{(\delta)}$, so the dependence of D on δ 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 δ 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 the choices (7.1) can be made by a terminating procedure in rational arithmetic: double r until $\mathbf{A}_r$ passes an exact Routh test (or until $-(\mathbf{A}_r^T\mathbf{P}+\mathbf{P}\mathbf{A}_r)$ passes an exact LDL^T test); halve t until the slow matrix has a simple zero and a Hurwitz deflation; halve ε until the coalesced restricted Jacobian has the same property; halve δ until all $2n-1$ exact Routh tests give the alternating pattern. Each loop terminates by the corresponding lemma. No bound on the number of steps is claimed. Proposition 9.1 reports the outcome for $n \leq 7$.

9 Exact examples

9.1 Rational witnesses for $n \leq 7$

The proof of Theorem 1.1(b) is asymptotic in r, t, ε and δ. For small n the procedure of Remark 8.6 stops at once: no slow final link and no fast binding are needed, and r and δ are moderate.

Proposition 9.1 (Computer-assisted)**.** *Let $2 \leq n \leq 7$, and let (r, δ) 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 $n = 2, \ldots, 7$. 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 $\theta_i = 1/Y_i(X_\circ)$, where $X_\circ$ 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 [21, 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 $(E_{\mathrm{T}},F_{\mathrm{T}},S_{\mathrm{T}})=(4,2,6)$, and the rate constants

i	a_i	b_i	c_i	α_i	β_i	γ_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

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

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 $n=3$,

$$x=(2,\tfrac{7}{2},5,\tfrac{13}{2},8),\qquad r=9,\qquad (E_{\mathrm{T}},F_{\mathrm{T}},S_{\mathrm{T}})=(18,2,20),$$

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

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

The nine kinetic parameters (θ_i,b_i,β_i) were chosen by a bounded numerical search that maximizes the smallest decay rate at the three even-indexed equilibria, subject to the ceilings $a_i,\alpha_i\le 2$ and $b_i,c_i,\beta_i,\gamma_i\le 2$ 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 0.80×10^{-4}, 0.98×10^{-4} and 1.26×10^{-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. $\square$

Declared units. To read the benchmark in physical units we declare a concentration unit $C_0=0.1\,\mu$M and a time unit $T_0=10$ s. Bimolecular constants are divided by C_0T_0 and unimolecular constants by T_0. The totals become $(E_{\mathrm{T}},F_{\mathrm{T}},S_{\mathrm{T}})=(1.8,\ 0.2,\ 2.0)\,\mu$M, and the rate constants are those of Table 3. The association constants are at most $2\,\mu\mathrm{M}^{-1}\mathrm{s}^{-1}$ and the unimolecular constants at most $0.2\,\mathrm{s}^{-1}$. The slowest of the three numerically computed decay rates corresponds to an

Table 2: The five equilibria of the three-site benchmark. Concentrations in the last two columns are in μM for the unit $C_0 = 0.1\,\mu$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	$\Phi'(u_j)$	$S_3 + Y_3$ (μM)	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

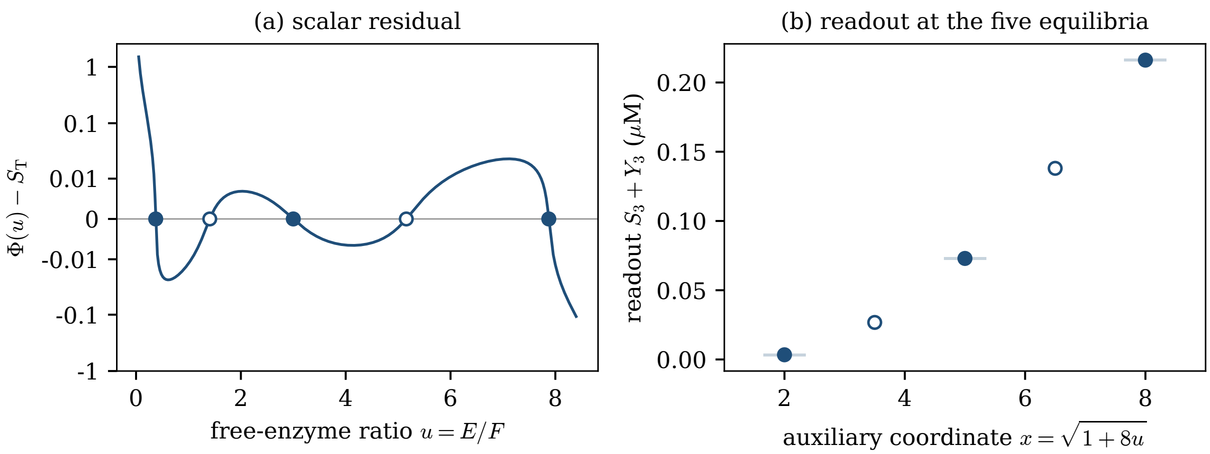

Figure 3: The three-site benchmark. (a) The residual $\Phi(u) - S_{\mathrm{T}}$ on an arcsinh scale; filled markers are sinks, open markers are saddles. (b) The readout $S_3 + Y_3$ at the five equilibria; the shaded bars are the certified readout intervals of Proposition 9.4, including a measurement error of $\pm 0.001\,\mu$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.

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.

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.

Let $q = (S_1, S_2, S_3, C_1, C_2, C_3, Y_1, Y_2, Y_3)$ be the coordinates on the class, let q_0 be one of the three nominal sinks, and use the weighted deviations $e = \mathrm{diag}(q_0)^{-1}(q - q_\theta)$ from the equilibrium q_θ of a perturbed parameter vector θ. Let $\mathcal{J}_0$ be the restricted Jacobian at q_0 in the weighted

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 (μM^{-1}s^{-1})	b_i (s^{-1})	c_i (s^{-1})	α_i (μM^{-1}s^{-1})	β_i (s^{-1})	γ_i (s^{-1})
1	2.00000	5.96184×10^{-6}	0.00197333	0.171890	0.0427272	0.199398
2	1.86875	7.83183×10^{-4}	0.0162999	1.99626	0.0268102	0.191934
3	0.217843	0.0126769	0.0383203	2.00000	0.0127038	0.187296

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 ϵ_{rel} of its nominal value. For each of the three nominal sinks X_0, X_2, X_4, with the radii ϱ_0 and ϱ_{in} of Table 4:*

(i) *the perturbed system has exactly one equilibrium q_θ in the box $\|\operatorname{diag}(q_0)^{-1}(q - q_0)\|_\infty \leq \varrho_0$; it is positive and satisfies $\|\operatorname{diag}(q_0)^{-1}(q_\theta - q_0)\|_\infty \leq \varrho_0/4$;*

(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*

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

where $\mathbf{Q} = -(\mathcal{J}_0^T\mathbf{P} + \mathbf{P}\mathcal{J}_0)$ and ℓ_{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 $\pm 0.001\,\mu$M added, lie in the pairwise disjoint intervals* $[0.00237970, 0.00437971]$, $[0.07195005, 0.07395006]$ *and* $[0.21515666, 0.21715672]$ *(μM).*

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 ∞-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 ∞-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 ϵ_{rel} by about eight orders of magnitude over a product of separate norm bounds.

(ii) For admitted θ and q in the box, the segment from q_θ 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 $V \le p_{\min}(\varrho_0/2)^2$ lies in the Euclidean ball of radius $\varrho_0/2$ around q_θ, hence in the box, because the centre has moved by at most $\varrho_0/4$ in the ∞-norm; it is invariant; and it contains the ball $\|e\|_2 \le \varrho_{\text{in}} := (\varrho_0/2)\sqrt{p_{\min}/M}$ (rounded down). On it, $\|e(t)\|_2^2 \le V(t)/p_{\min} \le (\varrho_0/2)^2 \exp(-ct/M)$ 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. □

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	ϱ_0	ϱ_{in}	M	c	T_{10} (s)	earlier (s)
X_0	1/8192000	1/16384000000	5846.11	0.98516	357,000	1.2×10^7
X_2	1/16384000	1/327680000000	535109.89	0.97537	32,918,000	1.2×10^9
X_4	1/2048000	1/40960000000	383168.49	0.98112	23,433,000	1.2×10^9

The certificate should be read for what it is. The parameter tolerance $\epsilon_{\text{rel}} \approx 1.5 \times 10^{-12}$ and the neighbourhoods ϱ_{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 development in Lean 4 [12] with Mathlib [36] has two parts. All modules compile without warnings under Lean `v4.30.0` with warnings treated as errors, contain no `sorry`, and depend only on the axioms `propext`, `Classical.choice` and `Quot.sound`.

The equilibrium count. The modules `Recurrence`, `Conversion`, `Source`, `Assembly` and `Resolution` of the namespace `PhosphorylationSharpness` define the state space, the six rate constants per site, the mass-action vector field of (1.1) componentwise, and the three totals, and prove

```
theorem sharp_lower_bound (n : ℕ) (hn : 1 ≤ n) : Attains n (2*n-1)
```

where `Attains n k` states that there exist positive rate constants, positive totals and an injective family of k positive states at which every component of the vector field vanishes and the three totals take the given values. The statement contains no reduced equation and no numerical input. The proof follows Section 4 for the roots $x_j = j + 2$, except that positivity of D_{raw} and B_{raw} is proved for all sufficiently large r by an elementary estimate based on (4.12) instead of Theorem 4.6. A further module, `PublicationAlgebra`, verifies (4.5) and coefficient positivity for arbitrary lists of roots greater than one, the determinant (2.12), the factorization (4.1), and the sign of the prefactor in (4.20).

The algebra of the stability proof. The namespace `PhosphorylationStableCapacity` contains four modules. `StructuralAlgebra` proves the coalesced identity $(x-1)N - 2J = (x-3)^{2m+1}$ for every m (`coalesced_identity`); that the relaxation (5.3) preserves the complex balances and positivity (`relaxation_balance`, `relaxation_positive`); that a matrix with vanishing last column has characteristic polynomial λ times that of its leading block (`slow_link_charpoly`), as used in Lemma 7.1; the elimination (6.7)⇒(6.8) (`loaded_feedback_elimination`); the monotonicity step behind $\phi_i > 0$ (`feedback_ratio_step`); the reduction of $\mathbf{B}_*\boldsymbol{z}$ to the scalar gain (`feedback_vector`); and the identity and positivity (6.13) for every natural number $n \geq 2$ and every $0 < v < 1$ (`feedback_gain_identity`, `feedback_gap_positive`, `feedback_gain_lt_one`). `KineticFreedom` proves, for the vector field of the first part, that the retuning (5.1) keeps all rate constants positive and preserves every equilibrium satisfying the three balance relations (`retune_positive`, `retune_equilibrium`). `Assembly` combines these into the statement that, given the matrix identities $\mathbf{H}\boldsymbol{z} = \mathbf{1}$ and the value of $\boldsymbol{\phi}^T\boldsymbol{z}$, every entry of $\mathbf{B}_*\boldsymbol{z}$ is negative (`large_r_block_certificate`). `DiagonalAlgebra` verifies the pairwise identity behind (8.2), the congruence used for $\mathbf{A}_r\boldsymbol{\Omega}$, and the sign of $\mathbf{Q}_*\boldsymbol{z}$.

What is not formalized. Theorem 1.1 as a whole is not a formal theorem. The following parts are proved only in the conventional way: the ceiling of Section 3 (degree theory, transversality, and the quoted Theorem 2.3); the interlacing lemma, Theorem 4.6 and Lemma 4.4; the determinant and slope formulas; the derivation of $\mathbf{M}$, $\boldsymbol{h}$ and $\mathbf{K}$ and the limits of Section 6, whose *outputs* are the hypotheses of the formal algebraic statements; the M-matrix and Gershgorin arguments; and all spectral perturbation arguments of Section 7. A compiled conditional identity does not prove its analytic premises. Propositions 9.1, 9.3 and 9.4 are exact computations that are not formalized.

Reproduction. The source package contains the Lean modules, the exact data of the benchmark, and a Python script, `check_paper.py`, which uses only the standard library and rational arithmetic. It verifies the construction for $n \leq 6$ (equilibrium equations, totals, the identity (4.19)) for distinct and for coalesced roots; Lemma 4.3 by Sturm sequences, Lemma 4.4, and Theorem 4.6 at the boundary of its range, on random root configurations with and without repeated roots; the moments (5.14) and (6.3), the positivity of $\boldsymbol{\phi}$, the gain formula (6.13), Table 1, and the definiteness of $\mathbf{Q}_*$, for $n \leq 8$; the exact identities of Lemma 6.1, the singularity of $\mathbf{K}$, and the $O(1/r)$ convergence $r\mathbf{A}_r \to \mathbf{B}_*$ for $n \leq 5$; the determinant formula (2.11) and the readout formula (8.3); the base case $n = 1$; Proposition 9.1 (for $n \leq 4$ by default and for $n \leq 7$ with the option `--full`); Example 9.2; and every number in Section 9.2, including an independent replay of the interval bounds of Proposition 9.4. These computations test the exposition and the implementation. The proofs of Theorems 1.1 and 1.2 do not depend on them.

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 δ^{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 $S_{\mathrm{T}} = E_{\mathrm{T}} + F_{\mathrm{T}}$ 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 [39, Theorem 4]. How small $E_{\mathrm{T}}/S_{\mathrm{T}}$ and $F_{\mathrm{T}}/S_{\mathrm{T}}$ 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.

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