

Multistationarity of the Altan-Bonnet–Germain Kinetic-Proofreading Network: A Partial Answer to a Question of Rendall

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Abstract

In an Oberwolfach abstract of 2017, Rendall remarked that the kinetic-proofreading module of the Altan-Bonnet–Germain model of T-cell activation appears to have deficiency one even under the strongest simplifying assumptions, noted that it is unknown whether it admits multiple steady states, and asked about the asymptotics of solutions of the model. We give a partial answer for the module as published in SBML form (57 species, 158 irreversible reactions, deficiency 34), with mass-action kinetics and independent rate constants. Among 36 natural simplifications, those of deficiency one are exactly the four without CD8, with constant Lck and a single ZAP-70 docking level. For these, every positive stoichiometric class contains exactly one steady state, for all rate constants; the proof combines a flux-balance formula for the bound fraction with injectivity determinants. With two or three docking levels and constant kinases, and for the complete module, there are rate constants with several positive steady states. Exact rational certificates give, for each of these four reduced networks, a class with exactly three steady states, two locally exponentially stable and one unstable, and a class of the complete module with two locally exponentially stable steady states and at least one more. All 36 networks are persistent. The multistationary rate constants are reaction-specific, whereas Altan-Bonnet and Germain use one binding and one unbinding constant for all receptor states. Under that constraint the reduced networks with collapsed Michaelis–Menten steps are monostationary at every docking level; multistationarity of the complete module under this constraint, and global convergence in the deficiency-one case, remain open. This is an unrefereed note.

1 Introduction

Kinetic proofreading [Hop74] was proposed by McKeithan as a mechanism for ligand discrimination by the T-cell receptor (TCR) [McK95]: a bound receptor must pass through a sequence of modifications before it signals, and dissociation of the ligand resets it. Sontag used the Deficiency Zero Theorem to show that McKeithan’s model has, in every positive stoichiometric class, a unique positive steady state, which is globally asymptotically stable [Son01]. Altan-Bonnet and Germain built a much larger model of early T-cell signalling [ABG05], whose core is a kinetic-proofreading module with explicit Lck and ZAP-70 steps, CD8 and “memory” receptor states. François et al. later proposed a simplified phenotypic model [FVS⁺13]; as Rendall notes [Ren17], Sontag’s result covers its kinetic-proofreading module. For this model Rendall and Sontag proved that the system has a unique positive steady state for $N = 1, 2$ phosphorylation steps and three positive steady states for suitable parameters when $N = 3$ [RS17].

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In his report on this work at the 2017 Oberwolfach workshop *Reaction Networks and Population Dynamics* [Ren17], Rendall asked what can be said about the asymptotics of solutions of the Altan-Bonnet–Germain model. He noted that the situation appears to be much harder for this model, whose kinetic-proofreading module seems to have deficiency one even under the strongest simplifying assumptions, and wrote: “It is unknown whether it admits multiple steady states.” The question appears as record OWR-15436-004 of the UnsolvedMath dataset [ula26]: does the dynamical system admit multiple positive steady states, and what are the asymptotics of its solutions?

We study the module exactly as published, in the SBML file Protocol S4 of [ABG05], with mass-action kinetics. As usual in chemical reaction network theory, the rate constants are treated as independent positive parameters, and a network is *multistationary* if for some rate constants some positive stoichiometric class contains two distinct positive steady states. The source does not say which simplifying assumptions are meant. We therefore consider 36 simplifications obtained by removing CD8, truncating the ZAP-70 docking cascade after one, two or three docking levels, and treating Lck or ZAP-70 as constant (Section 2). The deficiency-one ones are the four networks $\mathcal{N}(1, m, z)$ without CD8, with constant Lck and one docking level; here $m \in \{\text{kept}, \text{coll}\}$ records whether the Michaelis–Menten intermediates of the Lck steps are kept or collapsed, and $z \in \{\text{const}, \text{dyn}\}$ whether ZAP-70 is constant or dynamic. Our main results are the following.

Theorem 1.1. *For each of the four deficiency-one networks $\mathcal{N}(1, m, z)$ and every choice of positive rate constants, every positive stoichiometric class contains exactly one steady state. It is positive and nondegenerate.*

Theorem 1.2. *Let $k \in \{2, 3\}$ and $m \in \{\text{kept}, \text{coll}\}$, and let $\mathcal{N}(k, m, \text{const})$ be the network without CD8, with Lck and ZAP-70 constant and k docking levels; it has deficiency k . There are positive rational rate constants and a positive class containing exactly three steady states, all positive. Two of them are rational and locally exponentially stable relative to the class, and the third is unstable. For $k = 3$ and $m = \text{kept}$ this is the Protocol S4 module with CD8 removed and both kinases held constant.*

Theorem 1.3. *For the complete module (57 species, 158 irreversible reactions, with Lck, ZAP-70 and CD8 dynamic) there are positive rational rate constants and a positive class containing two distinct rational positive steady states, both locally exponentially stable relative to the class. This class contains at least one further positive steady state.*

Theorem 1.4. *All 36 networks are conservative and persistent, for all positive rate constants: no positive class contains a steady state with a zero coordinate, and no solution with positive initial values has an ω -limit point on the boundary of the orthant.*

So in the reaction-network sense the answer is “no” for the deficiency-one simplifications and “yes” for the constant-kinase networks with two or three docking levels and for the complete module. The mechanism (Remark 5.1) needs a second memory state, which is reached through a rebinding competition that depends on the ligand concentration.

These answers concern independent rate constants. Our multistationary witnesses use TCR–pMHC binding and unbinding constants that differ by factors up to 10^4 between receptor states, whereas the file of Altan-Bonnet and Germain, like kinetic proofreading in general, uses one binding and one unbinding constant for all of them. Under that constraint the reduced networks with collapsed Michaelis–Menten steps are monostationary at every docking level (Proposition 7.1), and numerical evidence points the same way when the intermediates are kept; for the complete module the question is open (Problem 8.1). On the asymptotics, Theorem 1.4 gives persistence, and in the multistationary cases the long-time behaviour depends on the initial condition. Global convergence in the deficiency-one case was observed in all our simulations but is not proved (Problem 8.2).

2 The network and its simplifications

Notation. A reaction network with species set $\mathcal{X}$ and reactions $y \rightarrow y'$ (complexes $y, y' \in \mathbb{Z}_{\geq 0}^{\mathcal{X}}$) and rate constants $\kappa > 0$ defines the mass-action system $\dot{x} = f(x) = \sum_{y \rightarrow y'} \kappa_{y \rightarrow y'} x^y (y' - y)$. Let S be the span of the reaction vectors $y' - y$ and $s = \dim S$. A *positive class* is a set $P = (x^0 + S) \cap \mathbb{R}_{\geq 0}^{\mathcal{X}}$ with x^0 positive; it is forward invariant. Since f takes values in S , the Jacobian $J(x)$ maps $\mathbb{R}^{\mathcal{X}}$ into S , and we write $J(x)|_S$ for its restriction. A steady state is *nondegenerate* if $J|_S$ is invertible and *locally exponentially stable* (relative to its class) if $J|_S$ is Hurwitz. The characteristic polynomial of J is λ^d times that of $J|_S$, where $d = |\mathcal{X}| - s$, because J induces the zero map on $\mathbb{R}^{\mathcal{X}}/S$. The *deficiency* is $n - \ell - s$, with n complexes and ℓ linkage classes [Fei87]. The network is *conservative* if some $w \in \mathbb{R}_{> 0}^{\mathcal{X}}$ is orthogonal to S ; then all classes are compact. A *siphon* is a nonempty set Σ of species such that every reaction with a product in Σ has a reactant in Σ [ADS07].

The module. Protocol S4 of [ABG05] is a JDesigner SBML file with 57 species and 104 reactions. Its kinetic laws are of mass-action form; 54 of them have the form binding minus unbinding, and we read these as reversible reactions, which gives 158 irreversible reactions (all 104 reactions carry the attribute `reversible="false"`, although these 54 laws are net rates). In 90 of the 158 mass-action terms, which lie in 66 of the 104 laws, one reactant is written as a zero-valued local parameter instead of the species; there are 24 such alias names, and each always stands for the same species. Read literally, these 90 terms would vanish. This is an artefact of the export: we use the reactant and product lists of the reactions, which are the reactions shown in Fig. 2B of [ABG05], together with the explicit rate constants of the laws (11 distinct nonzero values). The file contains the core module of Fig. 2B of [ABG05]; the SHP-1 and ERK feedback modules are not part of it. We write P for pMHC (concentration L), T_0 for TCR and T_j ($j = 1, 2, 3$) for the *memory* receptor carrying j ZAP-70 molecules (SBML TCR_ZAP, TCR_2ZAP, TCR_3ZAP). The thirteen TCR–pMHC complexes are C_j, C'_j, C''_j for $j = 0, 1, 2$ and $C_3, C_3^{(1)}, C_3^{(2)}, C_3^{(3)}$ (SBML Complex, Complex_ITAM1_P, Complex_ITAM1_PP, Complex_ZAP, ..., Complex_3ZAP_3P); the index j is the number of docked ZAP-70. The CD8-free part consists of the following 37 SBML reactions (53 irreversible reactions).

- (R1) Binding and rebinding $T_j + P \rightleftharpoons C_j$, $j = 0, 1, 2, 3$.
- (R2) Nine Lck steps along $C_j \rightarrow C'_j \rightarrow C''_j$ ($j \leq 2$) and $C_3 \rightarrow C_3^{(1)} \rightarrow C_3^{(2)} \rightarrow C_3^{(3)}$, each of Michaelis–Menten form $X + \text{Lck} \rightleftharpoons N_X \rightarrow X^+ + \text{Lck}$ with an intermediate N_X (which carries pMHC).
- (R3) ZAP-70 docking $C''_j + \text{ZAP} \rightleftharpoons C_{j+1}$, $j = 0, 1, 2$.
- (R4) Dissociation $X \rightarrow T_j + P$ of each of the nine complexes X with j docked ZAP-70 other than C_j (for C_j it is the reverse of (R1)). The intermediates N_X do not dissociate.
- (R5) Loss of ZAP-70 by memory receptors, $T_j \rightarrow T_{j-1} + \text{ZAP}$, $j = 1, 2, 3$.

The remaining 67 of the 104 SBML reactions (105 of the 158 irreversible reactions) involve CD8: it binds pMHC, TCR, memory receptors and complexes, and the CD8-bound complexes have their own Lck steps (with CD8-associated Lck, so no free Lck is used), ZAP-70 docking and dissociation. The complete module has five conservation laws (numbers of TCR, pMHC, Lck, ZAP-70 and CD8 units), rank 52, 106 complexes, 20 linkage classes and deficiency 34. In the file, the four binding reactions $T_j + P \rightarrow C_j$ share one constant (TCRpMHC1Binding) and the thirteen dissociations share one constant (TCRpMHC1Debinding); the Lck steps share one binding, unbinding and catalytic constant, and the docking steps one binding and one unbinding constant (the latter value is also used in (R5)).

Simplifications. We combine four operations: (i) keep or remove CD8 (removal deletes all reactions involving a CD8-containing species); (ii) keep $k \in \{1, 2, 3\}$ docking levels (delete all reactions involving a receptor with more than k docked ZAP-70); (iii) keep Lck dynamic, treat it as constant (delete Lck from all complexes; the intermediates stay), or treat it as constant and collapse each Michaelis–Menten step with free Lck to $X \rightarrow X^+$ (the CD8-associated Lck steps keep their intermediates; collapsing them as well leaves every deficiency in Table 1 unchanged); (iv) keep ZAP-70 dynamic or treat it as constant. Treating a species as constant means that its concentration is absorbed into pseudo-first-order rate constants. This gives 36 networks; the complete module is the one with CD8, $k = 3$ and both kinases dynamic. We write $\mathcal{N}(k, m, z)$ for the network without CD8, with Lck constant, k docking levels, Michaelis–Menten intermediates $m \in \{\text{kept, coll}\}$ and ZAP-70 $z \in \{\text{const, dyn}\}$.

Proposition 2.1. *The deficiencies of the 36 networks are those of Table 1. The deficiency-one networks are exactly the four networks $\mathcal{N}(1, m, z)$. In each of them every linkage class has deficiency 0, so the Deficiency One Theorem [Fei87] does not apply.*

This is an exact rank computation over $\mathbb{Q}$ (see the Verification paragraph). For $\mathcal{N}(k, m, z)$ the deficiency equals k , the number of memory states.

The family keeps at least one docking level. With none ($k = 0$) there is no memory state and ZAP-70 takes part in no reaction. Without CD8 and with Lck constant the network is then $T_0 + P \rightleftharpoons C_0 \rightarrow C'_0 \rightarrow C''_0$ with the dissociations $C'_0, C''_0 \rightarrow T_0 + P$ (and the intermediates $N_{C_0}, N_{C'_0}$ unless they are collapsed). This is a McKeithan-type chain [McK95]; it is weakly reversible and has deficiency 0, so the Deficiency Zero Theorem [Fei87] applies to it, as to McKeithan’s model [Son01]. (For $k = 0$ the deficiency is 2 without CD8 and with Lck dynamic, and 4 or 6 with CD8, for Lck constant or dynamic.) So among the simplifications with at least one docking level the minimum deficiency is 1, and it is attained exactly by the four networks $\mathcal{N}(1, m, z)$. This is what supports our reading of Rendall’s remark as referring to these networks.

CD8	docking levels k	Lck dynamic	Lck constant	Lck constant, collapsed
yes	3	34	25	25
yes	2	23	17	17
yes	1	14	10	10
no	3	12	3	3
no	2	8	2	2
no	1	5	1	1

Table 1: Deficiencies of the 36 simplifications. Each entry holds for ZAP-70 dynamic and for ZAP-70 constant. The complete module is the entry 34.

3 Receptor networks

Definition 3.1. A *receptor network* has species $\mathcal{R} \cup \{P\}$, where the finite set $\mathcal{R}$ of receptor states is partitioned into free states F and bound states B , and reactions of three kinds: $X \rightarrow Y$ with $X, Y \in F$ or $X, Y \in B$; binding $X + P \rightarrow Y$ with $X \in F, Y \in B$; and dissociation $X \rightarrow Y + P$ with $X \in B, Y \in F$. We assume that the directed graph on $\mathcal{R}$ with an edge $X \rightarrow Y$ for each reaction is strongly connected.

If CD8 is absent and Lck and ZAP-70 are constant, every reaction moves one receptor between two states, so $\mathcal{N}(k, m, \text{const})$ is a receptor network with $F = \{T_0, \dots, T_k\}$. The graph is strongly

connected: every state leads to T_0 by dissociation and loss of ZAP-70, and from T_0 binding and the reactions (R2)–(R4) reach every state.

For fixed $L > 0$ let $Q(L)$ be the generator of the single-receptor Markov chain on $\mathcal{R}$: Q_{XY} is the rate constant of $X \rightarrow Y$, multiplied by L for binding, and $Q_{XX} = -\sum_{Y \neq X} Q_{XY}$. It is irreducible, so it has a unique stationary distribution $\pi(L) > 0$, which is a rational function of L . Let $\varphi(L) = \sum_{X \in B} \pi_X(L)$ be the stationary bound fraction. With $r \in \mathbb{R}^{\mathcal{R}}$ the receptor concentrations and $K(L) = Q(L)^T$, mass action gives

$$\dot{r} = K(L)r, \quad \dot{L} = -\mathbf{1}_B^T K(L)r.$$

The receptor total $\mathbf{1}^T r = R_{\text{tot}}$ and the pMHC total $L + \mathbf{1}_B^T r = L_{\text{tot}}$ are conserved. They span the conservation laws: by strong connectivity the receptor parts of the reaction vectors span $V = \{v : \mathbf{1}^T v = 0\}$, and every reaction vector (v, v_P) has $v_P = -\mathbf{1}_B^T v$. So $S = \{(v, -\mathbf{1}_B^T v) : v \in V\}$ and $s = |\mathcal{R}| - 1$.

Lemma 3.2. *The positive steady states in the class with totals $(R_{\text{tot}}, L_{\text{tot}})$ are exactly the points $(R_{\text{tot}} \pi(L), L)$ with $L > 0$ and*

$$g(L) := L + R_{\text{tot}} \varphi(L) - L_{\text{tot}} = 0,$$

and every such L lies in $(0, L_{\text{tot}})$. Consequently:

- (a) *if φ is nondecreasing on $(0, \infty)$, each positive class contains at most one positive steady state;*
- (b) *if $\varphi(L_a) > \varphi(L_b)$ for some $0 < L_a < L_b$, the class with $R_{\text{tot}} = (L_b - L_a)/(\varphi(L_a) - \varphi(L_b))$ and $L_{\text{tot}} = L_a + R_{\text{tot}} \varphi(L_a)$ contains the two positive steady states with free pMHC L_a and L_b .*

Proof. At a positive steady state $K(L)r = 0$, so r is a multiple of $\pi(L)$, and $\mathbf{1}^T r = R_{\text{tot}}$ gives $r = R_{\text{tot}} \pi(L)$; the pMHC law then gives $g(L) = 0$. Conversely, if $g(L) = 0$ with $L > 0$, the point $(R_{\text{tot}} \pi(L), L)$ is positive, lies in the class and is a steady state, since $\dot{r} = 0$ and hence $\dot{L} = 0$. As $\varphi > 0$, $L < L_{\text{tot}}$. In (a), g is strictly increasing. In (b), $g(L_a) = 0$ by definition and $g(L_b) = (L_b - L_a) - R_{\text{tot}}(\varphi(L_a) - \varphi(L_b)) = 0$. $\square$

Lemma 3.3. *At a positive steady state $(R_{\text{tot}} \pi(L), L)$ of a receptor network,*

$$\det(-J|_S) = \Delta(L) g'(L), \quad \Delta(L) = \det(-K(L)|_V) > 0.$$

In particular, if $g'(L) < 0$, then $J|_S$ has a real positive eigenvalue and the steady state is unstable.

Proof. Write $K = K(L)$ and $K_1 = K'(L)$ (K is affine in L), and let $r = R_{\text{tot}} \pi$. The map $(v, v_P) \mapsto v$ identifies S with V . In this coordinate, $J|_S$ acts by $v \mapsto Kv + (K_1 r) v_P = Kv - (K_1 r) \mathbf{1}_B^T v$. Both K and K_1 have zero column sums, so $KV \subseteq V$ and $u := K_1 r \in V$. The eigenvalues of $-Q$ lie in the closed right half-plane (Gershgorin), and 0 is a simple eigenvalue since Q is irreducible. As $\mathbb{R}^{\mathcal{R}} = \mathbb{R}\pi \oplus V$ with both summands K -invariant and $K\pi = 0$, the eigenvalues of $-K|_V$ are the nonzero eigenvalues of $-Q$. So $-K|_V$ is invertible, and $\Delta(L) > 0$ because non-real eigenvalues occur in conjugate pairs and real ones are positive. By the matrix determinant lemma,

$$\det(-J|_S) = \det(-K|_V + u \mathbf{1}_B^T) = \Delta(L)(1 + \mathbf{1}_B^T (-K|_V)^{-1} u).$$

Differentiating $K(L)\pi(L) = 0$ gives $K_1 \pi + K \pi' = 0$, and $\pi' \in V$ because $\mathbf{1}^T \pi = 1$. Hence $\pi' = (-K|_V)^{-1} K_1 \pi$ and $(-K|_V)^{-1} u = R_{\text{tot}} \pi'$, so the last factor is $1 + R_{\text{tot}} \varphi'(L) = g'(L)$. If $g'(L) < 0$, then $\det(-J|_S) < 0$; the non-real eigenvalues of $-J|_S$ contribute a positive factor, so $-J|_S$ has a negative real eigenvalue. A steady state whose linearization has an eigenvalue with positive real part is unstable. $\square$

4 Deficiency one

Proposition 4.1. *Let a receptor network have $F = \{T_0, T_1\}$, where the only reaction out of T_0 is $T_0 + P \rightarrow C_0$ (rate constant a_0) and the reactions out of T_1 are $T_1 \rightarrow T_0$ (rate constant μ) and $T_1 + P \rightarrow C_1$ (rate constant a_1), with $C_0, C_1 \in B$. Then there are numbers $\tau_0 > 0$, $\tau_1 \geq 0$ and $p_0, p_1 \in [0, 1]$, which depend only on the rate constants of reactions out of bound states, such that*

$$\frac{\varphi(L)}{1 - \varphi(L)} = \frac{\mu\tau_0 + a_1L((1 - p_1)\tau_0 + p_0\tau_1)}{\mu/(a_0L) + a_1(1 - p_1)/a_0 + p_0}.$$

In particular φ is strictly increasing on $(0, \infty)$.

Proof. Let G be the restriction of Q to $B \times B$. Every bound state leads to F , so the chain restricted to B is transient, $-G$ is invertible and $N = (-G)^{-1} \geq 0$ entrywise, with positive diagonal (N_{XY} is the expected time spent in Y before leaving B , starting from X). Let $d_i \in \mathbb{R}^B$ be the vector of dissociation rate constants into T_i ; then $-G\mathbf{1} = d_0 + d_1$. Put $\tau = N\mathbf{1}$ and $p = Nd_1$, so that $0 \leq p \leq N(d_0 + d_1) = \mathbf{1}$, and let $\tau_i = \tau_{C_i}$, $p_i = p_{C_i}$. These depend only on the rate constants of reactions out of B , and $\tau_0 \geq N_{C_0C_0} > 0$.

The only transitions from F into B are the two binding reactions, with stationary fluxes $J_0 = a_0L\pi_{T_0}$ and $J_1 = a_1L\pi_{T_1}$. The stationary equations on B read $\pi_B^T G + J_0 e_{C_0}^T + J_1 e_{C_1}^T = 0$, so $\pi_B^T = J_0 e_{C_0}^T N + J_1 e_{C_1}^T N$. Hence $\pi(B) = J_0\tau_0 + J_1\tau_1$, and the dissociation flux into T_1 is $J_0p_0 + J_1p_1$. The state T_1 is entered only by dissociation, so its stationary equation is $(\mu + a_1L)\pi_{T_1} = J_0p_0 + J_1p_1$, that is, $(\mu + a_1L(1 - p_1))\pi_{T_1} = J_0p_0$. Together with $\pi_{T_0} = J_0/(a_0L)$ this gives

$$\frac{\varphi}{1 - \varphi} = \frac{\pi(B)}{\pi_{T_0} + \pi_{T_1}} = \frac{\tau_0(\mu + a_1L(1 - p_1)) + a_1Lp_0\tau_1}{(\mu + a_1L(1 - p_1))/(a_0L) + p_0},$$

which is the stated formula. Its numerator is positive and nondecreasing in L , and its denominator is positive and strictly decreasing, since $\mu > 0$. $\square$

The hypotheses hold for $\mathcal{N}(1, m, \text{const})$, with both forms m of the Lck steps: $T_0 = \text{TCR}$ only binds pMHC, and $T_1 = \text{TCR_ZAP}$ either loses its ZAP-70 or rebinds pMHC. The only L -dependent choice is the one at T_1 between rebinding and loss of ZAP-70, and the proposition shows that this choice alone cannot make φ decrease.

The closest analogue of Theorem 1.1 that we know of is also a special case of Proposition 4.1. Bali and Rendall [BR25] analyse, among other phenotypic models, kinetic proofreading with sustained signalling, one of the models reviewed by Lever et al. [LMvdMD14]: a receptor released from the last complex C_N stays in a signalling state R^* , which reverts to the baseline state R at a fixed rate and can rebind pMHC directly into C_N . So R^* is a single memory state reached through rebinding. In [BR25] the ligand is a variable, and the total amounts of ligand and receptor are the conserved quantities. Bali and Rendall state that for this model, for kinetic proofreading with limited signalling and for the combination of the two, solutions converge to a unique steady state, and they use the Deficiency Zero Theorem for this. The sustained-signalling network is a receptor network with $F = \{R, R^*\}$ that satisfies the hypotheses of Proposition 4.1 ($T_0 = R$, $T_1 = R^*$, $C_1 = C_N$). So Proposition 4.1 and Lemma 3.2(a) show again that each positive class contains at most one positive steady state, and they do so for independent rate constants, whereas [BR25] uses one binding constant a and one unbinding constant δ ; with these shared constants the flux balance in the proof of Proposition 7.1 even gives $\varphi = aL/(aL + \delta)$. Remark 5.1 explains why multistationarity needs a second memory state.

For the variants with dynamic ZAP-70 we use injectivity. Let $W \in \mathbb{Q}^{d \times \mathcal{X}}$ be a basis of $S^\perp$ in reduced row echelon form, and let $M(x, \kappa)$ be the Jacobian of f with the rows at the pivot positions of W replaced by the rows of W . Its determinant is a polynomial in (x, κ) .

Theorem 4.2 ([CF05, FW12, WF13]). *If $\det M(x, \kappa)$ is a nonzero polynomial all of whose coefficients have the sign $(-1)^s$, then for every $\kappa > 0$ the map f is injective on the positive part of every positive class. In particular each positive class contains at most one positive steady state, and no degenerate one.*

network	species	reactions	s	monomials of $\det M$	signs
$\mathcal{N}(1, \text{coll}, \text{const})$	9	15	7	208	all negative
$\mathcal{N}(1, \text{kept}, \text{const})$	13	23	11	1472	all negative
$\mathcal{N}(1, \text{coll}, \text{dyn})$	10	15	7	400	all negative
$\mathcal{N}(1, \text{kept}, \text{dyn})$	14	23	11	3100	all negative

Table 2: The injectivity determinants of the deficiency-one networks (irreversible reactions counted separately). In all cases $(-1)^s = -1$.

Lemma 4.3. *Suppose every minimal siphon of a network contains the support of a nonnegative conservation law. Then no positive class contains a steady state with a zero coordinate.*

Proof. Let x be a steady state in a positive class P with zero set $Z \neq \emptyset$. For $i \in Z$ every reaction consuming i has rate zero at x ; since $f_i(x) = 0$, so has every reaction producing i , which therefore has a reactant in Z . Thus Z is a siphon. It contains a minimal siphon, and hence the support of a nonnegative conservation law $w \neq 0$. Then $w \cdot x = 0$, while $w \cdot x = w \cdot x^0 > 0$ for the positive point x^0 of P . $\square$

Proposition 4.4. *Each of the 36 networks is conservative, and each of its minimal siphons contains the support of one of its integer conservation laws that count TCR, pMHC, Lck, ZAP-70 or CD8 units.*

This is a finite computation: the minimal siphons (between two and five per network) are enumerated completely, and the inclusions and the conservation laws are checked in integer arithmetic. Together with the theorem of Angeli, De Leenheer and Sontag [ADS07] it proves Theorem 1.4: a conservative network in which every siphon contains the support of a nonnegative conservation law is persistent, and Lemma 4.3 gives the statement on boundary steady states.

Lemma 4.5 (cf. [CHW08, CFMW17]). *Let the network be conservative and let P be a positive class without steady states on its relative boundary. Then P contains a positive steady state. If the steady states in P are finitely many and all nondegenerate, then $\sum \text{sign det}(-J(x)|_S) = 1$, the sum running over them.*

Proof. Identify $x^0 + S$ with $\mathbb{R}^s$, let Ω be the relative interior of P , which is its set of positive points, and let $x^0 \in \Omega$. Consider $H_t(x) = t f(x) + (1 - t)(x^0 - x)$ for $t \in [0, 1]$ and $x \in P$; it takes values in S . Let $x \in \partial\Omega$, so $x_i = 0$ for some i . Then $f_i(x) \geq 0$, because every reaction consuming species i has rate zero at x , while $x_i^0 > 0$. Hence $(H_t(x))_i > 0$ for $t < 1$, and $H_1(x) = f(x) \neq 0$ by hypothesis. By homotopy invariance of the Brouwer degree, $\deg(f, \Omega, 0) = \deg(x^0 - \cdot, \Omega, 0) = \text{sign det}(-I_s) = (-1)^s \neq 0$. So f has a zero in Ω . If the zeros are finitely many and nondegenerate, the degree is $\sum \text{sign det}(J(x)|_S) = (-1)^s \sum \text{sign det}(-J(x)|_S)$. $\square$

Proof of Theorem 1.1. By Proposition 4.4 and Lemma 4.3, no positive class contains a boundary steady state, so by Lemma 4.5 every positive class contains a positive steady state. For $z = \text{const}$ uniqueness follows from Proposition 4.1 and Lemma 3.2(a), and nondegeneracy from Lemma 3.3, since $g' = 1 + R_{\text{tot}}\varphi' > 0$. For all four networks, Table 2 and Theorem 4.2 give uniqueness and nondegeneracy once more. $\square$

Feinberg's Deficiency One Algorithm [Fei95] might give another proof of the uniqueness part of Theorem 1.1 for those of the four networks that are regular in his sense; we have not checked this.

5 Two and three docking levels

Table 3 gives rate constants for $\mathcal{N}(2, \text{coll}, \text{const})$, which has twelve receptor states. Put $L_1 = 6/625$ and $L_3 = 410$. Then $\varphi(L_1) \approx 0.9545 > \varphi(L_3) \approx 0.0926$, and Lemma 3.2(b) gives the class

$$R_{\text{tot}} = \frac{24046314446258627834704160606502759}{50550731957549605604883273663125} \approx 475.687,$$

$$L_{\text{tot}} = \frac{9090297098225022304132298635407}{20020091864376081427676544025} \approx 454.059.$$

The steady states with $L = L_1$ and $L = L_3$ are rational, because $\pi(L)$ is rational at rational L .

reaction	rate	reaction	rate	reaction	rate
$T_0 + P \rightarrow C_0$	100	$C_0 \rightarrow C'_0$	1/100	$C'_0 \rightarrow T_0 + P$	1
$C_0 \rightarrow T_0 + P$	1/100	$C'_0 \rightarrow C''_0$	1	$C''_0 \rightarrow T_0 + P$	10
$T_1 + P \rightarrow C_1$	1	$C_1 \rightarrow C'_1$	100	$C'_1 \rightarrow T_1 + P$	1
$C_1 \rightarrow T_1 + P$	1	$C'_1 \rightarrow C''_1$	1	$C''_1 \rightarrow T_1 + P$	1/100
$T_2 + P \rightarrow C_2$	1/100	$C_2 \rightarrow C'_2$	1	$C'_2 \rightarrow T_2 + P$	1
$C_2 \rightarrow T_2 + P$	100	$C'_2 \rightarrow C''_2$	1	$C''_2 \rightarrow T_2 + P$	1
$C''_0 \rightarrow C_1$	1/10	$C''_1 \rightarrow C_2$	1	$T_1 \rightarrow T_0$	1/10
$C_1 \rightarrow C''_0$	1/100	$C_2 \rightarrow C''_1$	1/10	$T_2 \rightarrow T_1$	1/1000

Table 3: Rate constants of the witness for $\mathcal{N}(2, \text{coll}, \text{const})$.

Proof of Theorem 1.2. We describe the witness of Table 3; the other three networks are treated in the same way with the rate constants described below.

Three steady states. By the matrix-tree theorem [Hil66], $\pi_X(L)$ is proportional to the principal minor $w_X(L)$ of $-Q(L)$ obtained by deleting row and column X . The variable L occurs only in the rows of the free states, so w_X is a polynomial of degree at most $|F|$. Let $w_B = \sum_{X \in B} w_X$ and $w_F = \sum_{X \in F} w_X$, so that $\varphi = w_B/(w_B + w_F)$ and $g = p/(w_B + w_F)$ with $p(L) = (L - L_{\text{tot}})(w_B + w_F) + R_{\text{tot}}w_B$. We computed w_B and w_F exactly by interpolation at $|F| + 1$ points. The polynomial p has degree 4, is squarefree, and by Sturm's theorem has exactly three roots in $(0, L_{\text{tot}})$. They are L_1 , L_3 and a root $L_2 \approx 0.08672$ in $(99/10000, 11/125)$, where p changes sign from $+$ to $-$. So by Lemma 3.2 the class contains exactly three positive steady states, and by Proposition 4.4 and Lemma 4.3 no other steady states.

Stability. At L_1 and L_3 the Jacobian is an exact rational matrix. Its characteristic polynomial divided by λ^2 is the characteristic polynomial of $J|_S$; it has degree 11, and all entries of the first column of its Routh array are positive. So $J|_S$ is Hurwitz at both states. At L_2 we have $p'(L_2) < 0$, because the three roots are simple and p changes sign from $+$ to $-$ there; hence $g'(L_2) < 0$, and Lemma 3.3 shows that the middle steady state is unstable. Numerically $J|_S$ has there exactly one positive eigenvalue, about $4.94 \cdot 10^{-3}$, so numerically it is a saddle. As a check of Lemma 3.3, $\det(-J|_S) = \Delta g'$ was also verified exactly at the two rational states.

The other networks. Their rate constants are obtained from Table 3 as follows. For $m = \text{kept}$, each Lck step $X \rightarrow X^+$ of Table 3, with constant c , is replaced by $X \rightarrow N_X$ (constant c), $N_X \rightarrow X$ (constant 1) and $N_X \rightarrow X^+$ (constant 10^3). For $k = 3$, the reactions of the network with $k = 2$ and the same m keep their constants, the docking step $C''_2 \rightarrow C_3$ gets the constant 10^{-3} , and every

other reaction that involves a receptor state with three docked ZAP-70 gets the constant 1. In particular, in $\mathcal{N}(3, \text{kept}, \text{const})$ all three reactions $X \rightarrow N_X$, $N_X \rightarrow X$ and $N_X \rightarrow X^+$ of each of the three level-3 Lck steps have constant 1; the constant 10^3 is used only for the Lck steps of Table 3. This is the rate set from which the certificate of Theorem 1.3 was built. The four rate sets are the files `pow10_2_0_simple.json`, `pow10_2_1_embedded.json`, `pow10_3_0_embedded.json` and `pow10_3_1_embedded.json` in the folder `claimant/src` of the reproducibility package, for $(k, m) = (2, \text{coll})$, $(2, \text{kept})$, $(3, \text{coll})$ and $(3, \text{kept})$. Each file maps a key such as `k_J0f` to the base-10 exponent e of the rate constant 10^e . Here J0 is the SBML reaction identifier, `f` denotes the direction written in the file and `r` the reverse; for instance `k_J0f` has the value 2, the constant 100 of $T_0 + P \rightarrow C_0$. In the collapsed networks the step $X \rightarrow X^+$ carries the key of the catalytic reaction $N_X \rightarrow X^+ + \text{Lck}$. With the same L_1 , L_3 and the same bracket, every step above goes through: p has degree 4 or 5 and exactly three simple roots in $(0, L_{\text{tot}})$, and the Routh arrays have degrees 17, 16 and 25. The classes have $R_{\text{tot}} \approx 475.68\text{--}475.71$, $L_{\text{tot}} \approx 454.06\text{--}454.10$ and $L_2 \approx 0.08672\text{--}0.08686$. $\square$

Remark 5.1. In the witness, at L_1 about 95% of the receptors are in the long-lived complex C_0 , and at L_3 about 91% are in the memory state T_2 , which loses its ZAP-70 very slowly and is mostly unbound (it rebinds with constant 1/100, while C_2 dissociates with constant 100). A receptor released as T_1 either loses its ZAP-70 or rebinds and moves on to C_2 and T_2 ; the second outcome becomes likelier as L grows. So increasing L drives receptors into this low-bound-fraction trap. The mechanism uses different binding and unbinding constants for different receptor states; see Section 7.

6 The complete module

Proof of Theorem 1.3. Construction. We started from the rate constants of the witness for $\mathcal{N}(3, \text{kept}, \text{const})$ (the rate set described at the end of the proof of Theorem 1.2, with constant 1 for all reactions of the level-3 Lck steps), divided the Lck binding and ZAP-70 docking constants by 10^5 , and chose Lck and ZAP-70 totals 10^5 and a CD8 total 1. Reactions with free CD8 as a reactant got the constant 10^{-3} , ZAP-70 docking in CD8 complexes was also divided by 10^5 , and all other CD8 reactions got the constant 1. Newton’s method in 60-digit arithmetic, started at the two stable reduced states, gave two steady states in one class, with free pMHC about 0.0053887 and 213.94. We rounded them to 20 significant digits and restored exactly the relations that any two steady states must satisfy: for an intermediate N formed from X and free Lck, the ratio of its values in the two states equals the product of the ratios for X and Lck (for CD8 intermediates, the ratio for X). The five free species were then adjusted so that both points lie in the same class, and an exact rational solve for 86 of the 158 rate constants, the others kept fixed, made both points exact steady states. The largest relative change of a rate constant was $4.4 \cdot 10^{-14}$.

Exact checks. The certificate consists of 158 positive rational rate constants and two distinct positive rational vectors x^1, x^3 . In exact arithmetic, $f(x^1) = f(x^3) = 0$ and $x^3 - x^1 \in S$. The class totals of TCR, pMHC, Lck, ZAP-70 and CD8 are about 475.702, 454.101, 10^5 , 10^5 and 1. The rate constants range from 10^{-8} to 10^3 .

Stability. Eliminating the five free species by the conservation laws gives exact rational 52×52 matrices representing $J|_S$ at x^1 and x^3 . For each we rounded a numerical solution of the Lyapunov equation to an integer symmetric matrix P and verified exactly, by fraction-free elimination and Sylvester’s criterion, that $P \succ 0$ and $-(J^T P + P J) \succ 0$. Hence $J|_S$ is Hurwitz at both states; the largest real parts of the eigenvalues are about $-2.77 \cdot 10^{-3}$ and $-7.34 \cdot 10^{-5}$.

A third steady state. By Proposition 4.4 and Lemma 4.3 the class has no boundary steady states. If x^1 and x^3 were its only steady states, Lemma 4.5 would give $1 + 1 = 1$. $\square$

Inheritance results such as those of Banaji and Pantea [BP18] might give another route to Theorem 1.3; we have not checked their hypotheses. We did not certify the intermediate networks with dynamic kinases or CD8.

7 Shared rate constants and asymptotics

Proposition 7.1. *Consider $\mathcal{N}(k, \text{coll}, \text{const})$ for some $k \in \{1, 2, 3\}$. Suppose all binding reactions $T_j + P \rightarrow C_j$ have the same rate constant a and all dissociation reactions have the same rate constant δ ; the other rate constants are arbitrary. Then $\varphi(L) = aL/(aL + \delta)$, and every positive class contains exactly one steady state.*

Proof. Each free state has exactly one binding reaction and each bound state exactly one dissociation reaction. Summing the stationary equations over B shows that the stationary flux from F to B equals the flux from B to F , that is, $aL\pi(F) = \delta\pi(B)$. So φ is increasing, and by Lemma 3.2(a) each positive class contains at most one positive steady state. By Proposition 4.4 and Lemma 4.3, no positive class contains a steady state with a zero coordinate. So Lemma 4.5 gives a positive steady state in every positive class, and it is the only steady state there. $\square$

The Altan-Bonnet–Germain file has exactly this structure (binding 10^4 , unbinding 8888), so the reduced networks with collapsed Michaelis–Menten steps are monostationary at its parameter values, whatever the Lck and ZAP-70 levels. The same sharing is the defining feature of kinetic proofreading [McK95]; in [ABG05] the ligand quality enters only through the lifetime of the TCR–pMHC interaction. With the intermediates kept, the argument fails because the intermediates carry pMHC but cannot dissociate. For $\mathcal{N}(k, \text{kept}, \text{const})$, $k = 2, 3$, we looked numerically for a decrease of φ when the seven Altan-Bonnet–Germain parameter groups are shared (binding, unbinding, the Lck triple and the ZAP-70 pair). A suitable objective is the smallest log-odds slope

$$\sigma = \min_L \frac{d \log(\varphi/(1 - \varphi))}{d \log L},$$

which is negative exactly when φ decreases somewhere, and which equals 1 for the collapsed networks by Proposition 7.1. The logarithmic slope of φ itself tends to 0 at both ends of the range of L , so minimizing it drives a search to those ends. Earlier searches with that objective (4500 random parameter vectors and 24 local optimizations) found no negative values beyond rounding error, which says little. In the search of the second referee round (see the Verification paragraph), σ was computed by finite differences on 161 points with $L \in [10^{-8}, 10^8]$. Over 3000 random parameter vectors with rates in $[10^{-4}, 10^4]$ and 40 Nelder–Mead runs in the box $[10^{-6}, 10^6]$ for each k , the smallest values of σ were 0.7143 for $k = 2$ and 0.7045 for $k = 3$. So in this search the log-odds slope stayed above 0.7. This is evidence, not proof. In contrast, the witnesses of Theorems 1.2 and 1.3 use binding constants 100, 1, 1/100 and unbinding constants from 1/100 to 100 for different receptor states.

On the asymptotics, Theorem 1.4 excludes extinction and boundary equilibria, and in the settings of Theorems 1.2 and 1.3 the limit behaviour depends on the initial condition. For the deficiency-one networks we sampled 600 random rate vectors and totals (rate constants in $[10^{-3}, 10^3]$): in every case $J|_S$ was Hurwitz at the steady state and a trajectory from a random initial point converged to it. This is numerical evidence only.

8 Concluding remarks

Two questions remain open.

Problem 8.1. *Is the complete module (or $\mathcal{N}(k, \text{kept}, \text{const})$, $k = 2, 3$) monostationary for all rate constants with the Altan-Bonnet–Germain sharing pattern, and in particular at the parameter values of [ABG05]?*

Problem 8.2. *Is the unique positive steady state of $\mathcal{N}(1, m, z)$ globally asymptotically stable in its class, for all rate constants? In the multistationary cases, what are the basins of attraction, and can periodic orbits occur?*

Verification. The claims were checked as follows.

1. The finder’s programs (Python, exact rational arithmetic) parse the SBML file, compute Table 1, build the witnesses of Theorem 1.2 and check $f = 0$, the classes, the sign changes of g and the Routh arrays. They compute three of the four determinants of Table 2 as fraction-free determinants over $\mathbb{Z}[x, \kappa]$, check Proposition 4.1 on random rational instances and the nonnegativity of all 1150 coefficients of the numerator of φ' for $\mathcal{N}(1, \text{coll}, \text{const})$, and construct the certificate of Theorem 1.3 with its Lyapunov matrices. No proof rests on their exploratory floating-point scans.
2. The author’s audit programs compute w_B , w_F and p of Section 5 exactly with their own Sturm sequences, verify $\det(-J|_S) = \Delta g'$ at the rational states, check Proposition 4.4 for all 36 networks, and check $\varphi = aL/(aL + \delta)$ for $k = 1, 2, 3$, all exactly. The determinant for $\mathcal{N}(1, \text{kept}, \text{const})$ was computed with the finder’s determinant code, and the shared-constant search for $k = 2$ reran the scripts of one of the first-round referees. For this revision, a further program of the author, independent of the finder’s and the referees’ parsers, counts the alias terms and the CD8 reactions of Section 2 and computes the deficiencies for $k = 0$; another checks symbolically that the model described after Proposition 4.1 reproduces the response function that [BR25] give for sustained signalling.
3. In a first referee round, two independent referees checked the proofs and wrote their own code; one of them also reran all programs. That referee parsed the SBML file with its own parser (the finder’s certificate check uses the finder’s parser), re-verified Theorem 1.3 with its own conservation laws and stability proof (a reaction-vector basis of S , a rigorous rounding bound, exact LDL^T factorizations), redid the witnesses with Hurwitz determinants, counted roots by Sturm’s theorem, enumerated the injectivity coefficients by the Cauchy–Binet formula (a multistationary control fails, as it should), and checked the siphon condition by branch and prune. The other referee used a different parser and recomputed the certificate and 50-digit eigenvalues. Their adversarial checks found Proposition 7.1 and the first numerical evidence after it.
4. In a second round, a further independent referee checked every proof line by line and redid the computations with its own code, written from the text of the paper in exact rational or integer arithmetic: its own SBML parser and network construction (Table 1, Proposition 2.1, the case $k = 0$ and the counts of Section 2), the injectivity coefficients by Laplace expansion (with a failing multistationary control), the siphons by SAT and complete enumeration, the witnesses of Theorem 1.2 by fraction-free elimination over $\mathbb{Q}[L]$ with its own Sturm and Routh routines, Proposition 4.1 with the 1150 coefficients, Proposition 7.1, and the certificate of Theorem 1.3 with its own reaction matching and a separate rigorous stability proof (dyadic rounding, exact balancing and an exact perturbation bound). It also rebuilt the rate constants of the certificate from the construction in Section 6, which showed that the rule for the level-3 Lck steps in the proof of Theorem 1.2 had to be stated precisely, and it ran the log-odds search of Section 7. It did not rerun the half of the 600-sample scan of Section 7 with dynamic ZAP-70.

Scope and priority. Theorems 1.1 to 1.3 answer Rendall’s question [Ren17] in the reaction-network sense, with independent rate constants. That the deficiency-one simplifications of Proposition 2.1 are the ones Rendall had in mind is our interpretation; the source does not specify them. The interpretation is supported by the case without docking: removing ZAP-70 docking altogether ($k = 0$) gives, without CD8 and with Lck constant, a McKeithan-type network of deficiency 0 (Section 2). Our family therefore keeps at least one docking level, and the minimum deficiency 1 is attained exactly by the networks $\mathcal{N}(1, m, z)$. For rate constants with the sharing pattern of [ABG05], the question is answered only for the reduced networks with collapsed Michaelis–Menten steps (Proposition 7.1), and the asymptotics are only partly settled (Problem 8.2). The coupled model with the SHP-1 and ERK feedback modules was not studied. In a TCR signalling model with kinetic proofreading and these two feedbacks, Lipniacki et al. found bistability caused by the competition between them [LHFH08], and Owens et al. analysed a detailed model of this kind [OTGT10]; our results concern the kinetic-proofreading module without the feedbacks. Closely related work includes the analysis of McKeithan’s model by Sontag [Son01], of the model of François et al. by Rendall and Sontag [RS17], Kreusser and Rendall on Lck [KR21], and Bali and Rendall’s comparison of phenotypic models [BR25], whose sustained-signalling model is the closest analogue of Theorem 1.1 that we know of (see the discussion after Proposition 4.1). Other related work includes Yu and Sontag on nonmonotonic dose response in a kinetic-proofreading model [YS24], Vassena on structural conditions for saddle-node bifurcations and structural obstructions to a simple zero eigenvalue [Vas23, Vas24], Franco and Velázquez on stochastic kinetic proofreading [FV25, FV26], Bali, Rendall and Quapp on catch–slip bonds [BRQ26], and Rey Barreiro, Faro and Villaverde on the use of models to predict and control T-cell activation [RFV26]. We searched arXiv, Crossref, zbMATH and Europe PMC (including a keyword screen of the 381 records that Europe PMC lists as citing [ABG05]), checked all works that OpenCitations lists as citing [RS17] or the Oberwolfach report [Ren17], and ran a few web searches. We found no treatment of the multistationarity of the Altan-Bonnet–Germain module. OpenAlex list queries and Semantic Scholar were not available to us (rate limits); single OpenAlex lookups were. This negative search is not a proof of priority.

AI-assisted tools were used to search the problem corpus and the literature, to find the certificates and the arguments, to write and run the verification programs, and to help prepare this manuscript. The author has reviewed the proofs, the computations and the sources and is responsible for all claims. The note has not been peer reviewed.

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