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CRITERIA: A network decomposition and elementary flux mode translation-based tool for computing equilibria of biochemical systems

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Abstract

Analytically deriving equilibria, which often govern the long-term behavior of biochemical reaction networks, is essential for understanding cellular decision-making and robustness, yet remains computationally challenging for large and complex systems. The COMPILES framework of Hernandez et al. addresses this problem via network decomposition but is limited by a computationally intensive translation step and by its restriction to networks with zero kinetic deficiency. We introduce CRITERIA (Computing paRametrized posITive EquilibRIA), a new framework that overcomes these limitations through two key advances. First, it replaces the translation mechanism of COMPILES with a more efficient graph-theoretic formulation based on the work of Johnston and Burton, which generates a reaction-to-reaction graph from elementary flux modes and identifies directed cycles of the constructed graph via a binary linear program. Second, CRITERIA computes equilibria on a single unified translated network rather than solving subnetworks independently, thereby eliminating interdependencies that previously required extensive symbolic manipulation. Across a benchmark set of 26 biochemical models, CRITERIA achieves consistent and often substantial speed improvements while expanding applicability beyond the restricted class of zero kinetic deficiency systems. We demonstrate the biological utility of the framework by analyzing multistationarity, which underlies cellular decision-making, and absolute concentration robustness, a key mechanism for maintaining stable biochemical outputs, in the EnvZ–OmpR signaling pathway and a large-scale CRISPRi toggle switch, respectively. By improving both scalability and applicability, CRITERIA enables a systematic equilibrium analysis in biochemical networks of realistic size and complexity, providing a practical tool for studying long-term dynamical behavior in systems biology.

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Author summary

Understanding how biochemical systems settle into stable states, such as how protein concentrations reach equilibrium, is central to explaining cellular behavior and designing synthetic biological circuits. However, existing analytical tools for computing these equilibria, such as COMPILES, are limited by computational bottlenecks and can only be applied to a restricted class of reaction networks. In this work, we introduce CRITERIA (Computing paRametrized posITive EquilibRIA), a new computational framework that makes equilibrium analysis more efficient and broadly applicable. CRITERIA uses a graph-based approach built on elementary flux modes to streamline key steps in the computation. It also changes how the problem is solved by combining subnetworks into a single system before computing equilibria, which avoids complicated symbolic calculations required in previous methods. We demonstrate the usefulness of CRITERIA by studying biologically important systems, including the EnvZ-OmpR signaling pathway and a synthetic CRISPRi circuit. Our approach enables faster and more scalable analysis, allowing researchers to better understand how complex biochemical networks behave over time.

Introduction

An equilibrium (or steady state) of a system refers to a state in which the concentrations of species in the system remain constant over time [1,16]. The study of equilibria is fundamental to understanding biochemical systems, which are typically modeled using ordinary differential equations based on mass-action kinetics, since they often characterize the long-term behavior of these systems [2,15]. They reveal important dynamical properties of the system, such as multistationarity, which refers to the capacity of the system to admit multiple positive equilibria, and absolute concentration robustness, where the concentration of a particular species remains the same in every positive equilibrium regardless of initial conditions.

Despite their importance in understanding biochemical systems, the mathematical study of positive equilibria remains challenging because of the high dimensionality of the underlying dynamical systems, the significant nonlinearities they exhibit, and the large number of parameters, many of which are typically unknown. Hence, recent research has focused on network-based approaches for deriving analytic positive equilibria of biochemical systems that are valid independently of specific parameter values [2–4,18,22].

Several approaches have been developed towards this goal. In [31–33], the set of steady states in mass-action systems is characterized using tools from algebraic geometry. Specifically, the authors showed that the set of positive steady states can be characterized as an algebraic variety, allowing techniques such as Gröbner bases to be used to study positive equilibria. Another line of research exploits the structure of particular classes of biochemical reaction networks. In [4,34], the rational

parametrization theorem was established for a class of multisite post-translational modification systems, which states that every positive steady states of these systems admits a rational parametrization in terms of a collection of free parameters and the reaction rate constants. The authors also provided a procedure for obtaining this parametrization. More recently, a general framework was developed for explicitly constructing positive equilibrium parametrizations for broader classes of biochemical reaction networks [22]. Their approach uses deficiency theory [16], generalized mass-action systems [17], and network translation [18]. Rather than using tools from algebraic geometry, the method draws on techniques from linear algebra.

Building upon this theoretical framework, a publicly-available computational package built in MATLAB called COMPILES (COMPutIng anaLytic stEady States) was developed to facilitate this process [2]. Chemical reaction network (CRN) theory is the main analytical framework used by COMPILES, wherein the structural properties of being *weakly reversible* (WR) and *deficiency zero* (DZ) are exploited to derive analytic equilibria. A network is WR if each of its reactions belongs to a directed cycle, and is said to be DZ if its deficiency, which is a nonnegative integer that measures the linear dependency of its reactions, is equal to zero [24].

The method in COMPILES begins with *network decomposition*, a divide and conquer approach to efficiently handle large networks, by decomposing the network into its *independent subnetworks* [2,24,27]. This is a special kind of network decomposition with the property that the rank of the parent network is equal to the sum of the ranks of its subnetworks. This is then followed by *network translation*, a process through which each subnetwork that initially does not possess WR and DZ is transformed into one that satisfies these properties while preserving the original dynamics of the system [18,23]. The translation scheme implemented in COMPILES follows the method in [23], in which pre- and post-translational filters are applied to identify, from a large number of candidates, translated networks with WR and DZ. Network translation produces a *generalized chemical reaction network* (GCRN), which consists of two associated structures: *stoichiometric CRN* and *kinetic-order CRN*. The stoichiometric CRN has vertices corresponding to the translated complexes (i.e., reaction nodes), whereas the kinetic-order CRN retains vertices corresponding to the original complexes of the original CRN. Once the stoichiometric and kinetic-order CRNs have been successfully translated, equilibria are then parametrized per subnetwork based on [22]. Finally, the equilibria of each subnetwork are merged to obtain the equilibria of the entire network. This is guaranteed because independent decomposition has the property that the intersection of the set of positive equilibria of the subnetworks is equal to that of the entire network. [24,27]. In [2], a sufficient condition was established to ensure that this property is maintained for any choice of rate constants.

Despite its potential, COMPILES has several issues that make the analysis of biochemical reaction networks via equilibrium parametrization challenging. First, the network translation scheme employed by COMPILES constitutes a major bottleneck in the overall procedure. Although filters are incorporated to construct networks that satisfy WR and DZ, the exhaustive strategy of generating all possible candidate network translates imposes a significant computational overhead, making COMPILES impractical for larger systems. Second, there is a merging issue in COMPILES resulting in interdependencies in the derived equilibrium parametrization. In other words, this merging issue manifests itself in the presence of multiple species within each other's equilibrium expressions. For instance, COMPILES obtains an equilibrium parametrization of the following species in the MAPK model [5] with their corresponding functional forms as follows

$$X_{pp} = f(X_{pp}M, X_p^*M, X_p, \sigma_1, \sigma_2), \qquad X_{pp}M = g(X_{pp}, X_p^*M, X_p), \qquad X_p = h(X_{pp}, \sigma_2)$$

where only X_p^*M, σ_1, and σ_2 are classified by COMPILES as free parameters. It can be observed that both the species X_{pp} and $X_{pp}M$ appear in each other's equilibrium expressions. Although COMPILES identifies equilibrium relations among the species, its output is not immediately an explicit equilibrium parametrization because some species remain recursively defined in terms of other species. As a result, the equilibrium concentrations cannot be computed directly from the chosen free parameters without further algebraic manipulation. Merging equilibrium expressions requires extensive symbolic manipulation, which

may cause MATLAB to fail in obtaining a closed-form solution free of interdependencies. Although not intrinsic to COMPILES, it nonetheless limits the method's applicability to more complex biochemical networks. Lastly, COMPILES only solves the equilibria of reaction networks whose kinetic deficiency (i.e., the deficiency of the kinetic-order CRN) is zero.

In this paper, we present an enhanced framework for computing the equilibria (i.e., deriving the analytic steady states) of biochemical systems by addressing the limitations of COMPILES as discussed above. We develop a MATLAB-based computational package called CRITERIA (Computing paRametrized posITive EquilibRIA) to facilitate this improved process. Here, we replace the translation scheme of COMPILES with the method developed in [5] to construct WR and DZ translations using an elementary flux mode-based approach. We also modify the order of steps in COMPILES to resolve the merging issue. In particular, rather than solving for the positive equilibria of each subnetwork independently, we first merge the translated (and, if there are any, untranslated) subnetworks into a single network and then derive the equilibrium parametrization of the whole network. Refer to Fig 1 for a more detailed discussion of the overall procedure of CRITERIA illustrated using a simple example. Finally, we incorporate the additional conditions required to parametrize the equilibria of networks with positive kinetic deficiency as established in [22].

To demonstrate how CRITERIA improves COMPILES, we compare the runtime performance of both computational frameworks across a wide range of models. We further demonstrate the utility of the enhanced procedure by examining critical dynamical properties of complex biochemical systems, including absolute concentration robustness (ACR) in the full CRISPRi toggle switch model and the capacity for multistationarity in the EnvZ-OmpR signaling pathway.

Results

An elementary flux mode-based approach for constructing weakly reversible and deficiency zero network translation

One of the improvements made to resolve a key issue of COMPILES is the replacement of its network translation scheme with a more efficient procedure. In [5], a computational method was developed to construct a WR and DZ translation via an elementary flux mode-based approach. *Elementary flux modes* (EFMs) are flux-balanced pathways in the network that cannot be simplified in the sense that it is not possible to remove a subset of active reactions from an EFM and still be able to build a flux-balanced path using only the remaining active reactions [5,6] (see the Methods section for more details).

The approach proposed in [5] consists of three main steps:

1. From the given CRN, compute the set of EFMs $\mathcal{E} = \{e_1, ..., e_p\}$.
2. Construct a reaction-to-reaction graph $G^{\mathcal{R}} = \left(V^{\mathcal{R}}, E^{\mathcal{R}}\right)$ which is common source compatible (CS-compatible) and elementary flux mode compatible (EM-compatible) with the given CRN.
3. Determine the translation complexes to produce the WR and DZ network translation.

To illustrate the process, we consider the histidine kinase system below [21]. Here, the histidine kinase (X) can autophosphorylate (X_p) and can transfer the phosphate group to a response regulator (Y) yielding (Y_p), which undergoes autodephosphorylation.

$$X \xrightarrow{k_1} X_p \qquad X_p + Y \underset{k_3}{\overset{k_2}{\rightleftarrows}} X + Y_p \qquad Y_p \xrightarrow{k_4} Y$$

Step T1. Since EFMs represent flux-balanced pathways in the network, it is necessary to track the net production and consumption of species in the CRN. This information is codified in the *stoichiometric matrix N* (see the Methods section for

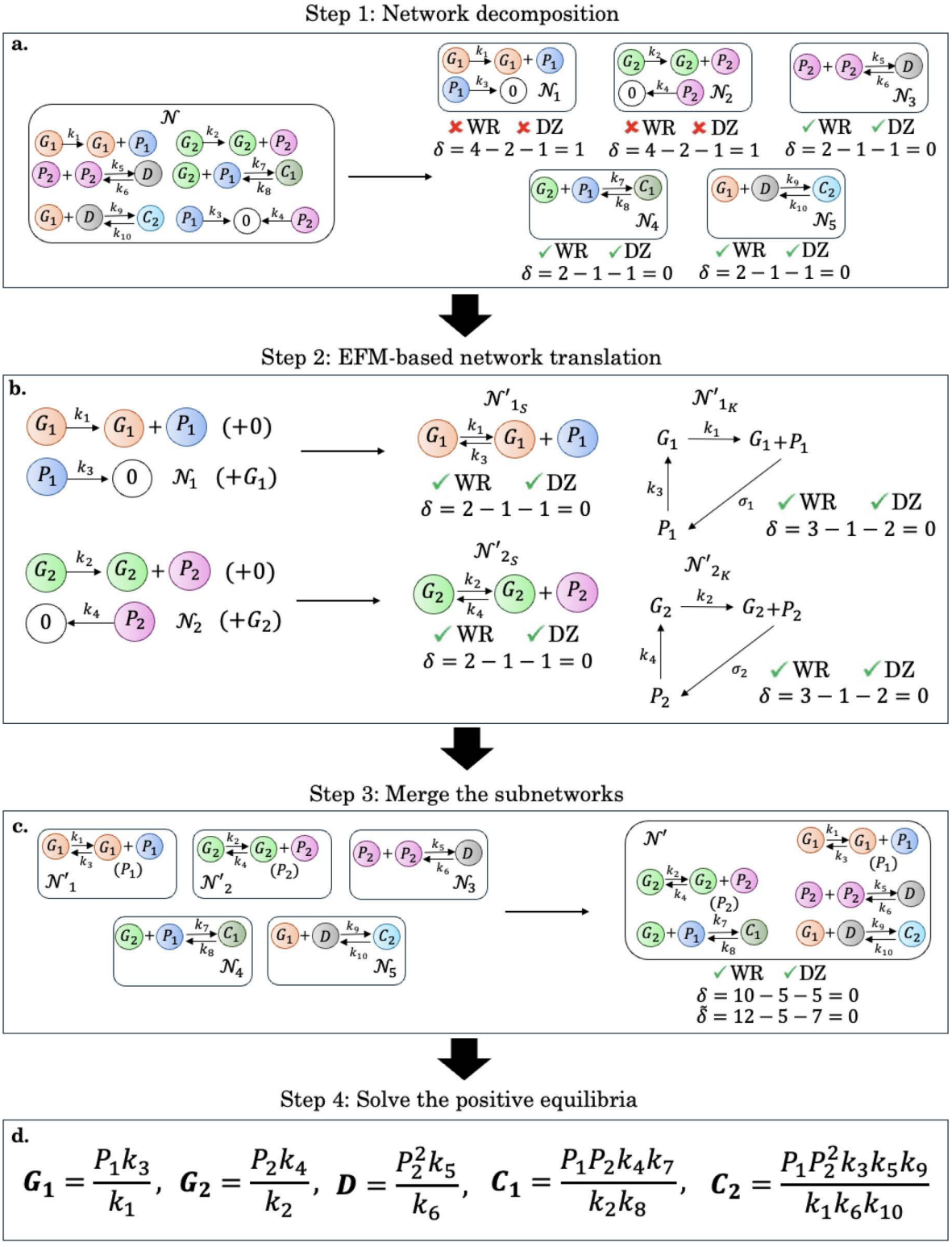

Fig 1. Derivation of the analytic equilibria using the enhanced framework. a The CRN $\mathcal{N}$ consisting of 10 reactions is decomposed into five independent subnetworks, each having two reactions. This decomposition has the property that the rank of the stoichiometric matrix of $\mathcal{N}$ is equal to the sum of the ranks of the stoichiometric matrices of the five subnetworks. **b** Of the five subnetworks, two ($\mathcal{N}_1$ and $\mathcal{N}_2$) are translated to become WR

and DZ. For $\mathcal{N}_1$, the complex G_1 serves as the translation complex for the third reaction while the first reaction remains unchanged. This produces a stoichiometric CRN ($\mathcal{N}'_{1s}$) and kinetic-order CRN ($\mathcal{N}'_{1\kappa}$) that are both WR and DZ. Hence, the translated network $\mathcal{N}'_1$ is WR and DZ, while retaining the same dynamics as $\mathcal{N}_1$. The same procedure is done for $\mathcal{N}_2$ by using G_2 as the translation complex for the fourth reaction and keeping the second reaction unchanged. This yields a translated network with the same dynamics as the original network but is now WR and DZ. The translation complexes for $\mathcal{N}_1$ and $\mathcal{N}_2$ are solved via Steps T1, T2, and T3 discussed in the previous subsection. **c** The five subnetworks are then merged into a single network. For $\mathcal{N}'_1$ and $\mathcal{N}'_2$, the complex in parentheses is the kinetic complex associated with that node. For nodes where no complex is shown in parentheses, it is assumed that the stoichiometric complex is the same as the kinetic complex. The kinetic complexes comprise the kinetic-order CRN, with additional edges, known as phantom edges, introduced to separate multiple complexes associated with a single node. There are two deficiencies associated to the merged network. The first one (δ) is for the deficiency of the stoichiometric CRN while the second one ($\tilde{\delta}$) is for the kinetic-order CRN. In the example, both deficiencies are equal to zero and hence the network is DZ. **d** Since the network is now WR and DZ, the equilibrium expressions are then analytically derived.

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more details on the basics of chemical reaction networks). Given this, an EFM is a flux vector that satisfies $Nv = 0$ together with the nonnegative flux constraint $v \geq 0$. The central mathematical constructs that describe the space of all flux vectors that satisfy $Nv = 0$ where $v \geq 0$ (i.e., admissible flux vectors) are polyhedral cones [6].

By defining $A = [I_r \;\; N \;\; -N]^T$, we can observe that the set

$$P = \{v \in \mathbb{R}^r_{\geq 0} \mid Av \geq 0\}$$

is a polyhedral cone that contains exactly the admissible flux vectors [6]. Moreover, it has been established in [6] that the extreme rays of this polyhedral cone correspond to the EFMs of the network. Alternatively, the set of EFMs is the minimal generating set of P.

The general method that computes this minimal generating set or the extreme rays of P is called the *Double Description Algorithm*. Several variations of the algorithm exist. In this paper, we use the *Canonical Basis Method*, which exploits the structure of the matrix A as defined above [6]. The outline of the method is presented in Algorithm 1.

Algorithm 1 Computing EFMs

Input: Stoichiometric matrix N
Output: A matrix R whose columns are the EFMs
1. **Initialize:** Set $k = r+2$, $A_{\text{old}} = I_r$, and $R_{\text{old}} = I_r$.
2. **Prepare rays in R_{old} for partitioning:** While $k < r+2m+2$, do:
   a. Select a row v of N not in A_{old}.
   b. Update $A_{\text{new}} = \begin{bmatrix} A_{\text{old}} \; ; \; v \; ; \; -v \end{bmatrix}$.
   c. Let $R_{\text{new}} = R_{\text{old}}$, and $\Lambda = \{1, 2, \ldots, q\}$ where q is the number of columns of R_{old}.
3. **Partition rays in R_{old}:** Let $\Lambda_+ = \{j \in \Lambda : v \cdot R_{\text{old}}(:, j) > 0\}$, $\Lambda_0 = \{j \in \Lambda : v \cdot R_{\text{old}}(:, j) = 0\}$, and $\Lambda_- = \{j \in \Lambda : v \cdot R_{\text{old}}(:, j) < 0\}$.
4. **Generate extreme rays:** For each $(j_+, j_-) \in \Lambda_+ \times \Lambda_-$, do:
   a. Compute $r_{j_+,j_-} = (v \cdot R_{\text{old}}(:, j_+)) R_{\text{old}}(:, j_-) - (v \cdot R_{\text{old}}(:, j_-)) R_{\text{old}}(:, j_+)$.
   b. Test for extremeness. If r_{j_+,j_-} is an extreme ray, append r_{j_+,j_-} to R_{new}.
5. **Update R_{old} and R_{new}:**
   a. Remove columns of R_{new} indexed by $\Lambda_+ \times \Lambda_-$.
   b. Update: $R_{\text{old}} = R_{\text{new}}$, $A_{\text{old}} = A_{\text{new}}$, and $k = k+2$.
6. **Repeat:** Go back to Step 2. Stop when no row of N remains unchosen.

Going back to the histidine kinase system consisting of four reactions, we can define its stoichiometric matrix N as follows

$$\begin{array}{c} \\ X \\ X_p \\ Y \\ Y_p \end{array} \begin{array}{c} \begin{array}{cccc} R_1 & R_2 & R_3 & R_4 \end{array} \\ \begin{bmatrix} -1 & 1 & -1 & 0 \\ 1 & -1 & 1 & 0 \\ 0 & -1 & 1 & 1 \\ 0 & 1 & -1 & -1 \end{bmatrix} \end{array}.$$

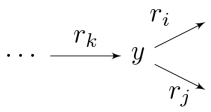
Applying Algorithm 1, the output R is given by

$$\begin{array}{cc} e_1 & e_2 \\ \begin{bmatrix} 0 & 1 \\ 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} \end{array}$$

which corresponds to the set of EFMs $\mathcal{E} = \{e_1, e_2\} = \{\{R_2, R_3\}, \{R_1, R_2, R_4\}\}$. See the Supplementary Information for the step-by-step procedure in the computation.

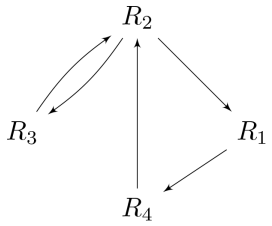
Step T2. Once the EFMs are computed, a reaction-to-reaction graph is then constructed that must be both CS-compatible and EM-compatible with the given CRN (see Theorem 3 in the Methods section).

A *reaction-to-reaction graph* is a directed graph in which the reactions of the CRN are treated as the vertices of the graph [5]. The CRN is said to be *CS-compatible* whenever reactions of the following form in the CRN



forces $(r_k, r_i) \in E^{\mathcal{R}}$ if and only if $(r_k, r_j) \in E^{\mathcal{R}}$, where $E^{\mathcal{R}}$ is the edge set of the reaction-to-reaction graph [5]. Moreover, both the CRN and the reaction-to-reaction graph are said to be *EM-compatible* if every EFM of the CRN corresponds to the vertices of a minimal directed cycle in the reaction-to-reaction graph, and vice versa [5].

In [5], a binary linear programming (BLP) problem was formulated to construct the desired reaction-to-reaction graph while enforcing CS compatibility and EM compatibility with the given CRN. In our histidine kinase system, the BLP model results in the reaction-to-reaction graph shown below. See the Supplementary Information for the detailed discussion.



Step T3. The final step of the translation procedure is to determine the translation complexes to obtain a WR and DZ network translation. Intuitively, *translation complexes* are complexes added to individual reactions in the translation process. This modifies the graphical structure of the CRN without changing its stoichiometric matrix [18]. This is crucial to preserve the dynamics of the system.

Theorem 3 provides the method for determining the translation complexes, which involves solving the linear system in Eq (9). A more efficient procedure, however, is proposed in [5] to determine the translation complexes instead of solving the linear system in Eq (9) directly.

The approach is similar to a graph traversal: once a value is fixed at a single node, the remaining values are computed incrementally using Eq (9) by traversing the directed edges of the reaction-to-reaction graph. The outline of the procedure is shown in Algorithm 2, which is based in [5]. This strategy exploits the structure of the reaction-to-reaction graph to propagate the values of the translation complexes step-by-step. This is more efficient than solving the full matrix system which may have redundancies that can lead to unnecessary computational overhead.

Algorithm 2 Determining the translation complexes

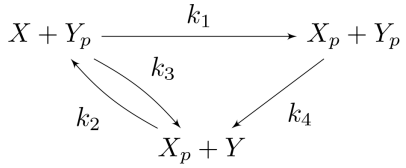
Input: Edges $E^{\mathcal{R}}$ of the reaction-to-reaction graph, matrix of source complexes Y_s and product complexes Y_p, linkage classes $\mathcal{L}$
Output: Translation complexes α

1. **Check consistency of the linear system.** Determine whether rank(A) = rank(B), where B is the augmented matrix $B = [A \mid b]$, and A and b come from Eq (9). Exit algorithm if rank(A) $\neq$ rank(B).
2. **Seed the process.** Set $\mathcal{P} = \mathcal{R}$. Select an arbitrary $r_i \in \mathcal{P}$ and set $\alpha_i = 0$. Move r_i to the "active" list $\mathcal{P}'$.
3. **Expand.** For every r_i in the "active" list, choose a neighbor $r_j \in \mathcal{P}$, and then calculate α_j based on Eq (9). Move r_j to the "processed" list $\mathcal{P}''$.
4. **Propagate.** Once we have checked all neighbors of the current "active" list $\mathcal{P}'$, set $\mathcal{P}' = \mathcal{P}''$. Set $\mathcal{P}'' = \emptyset$ and repeat Step 3.
5. **Check for completion.** If the "active" list is already empty but $\mathcal{P} \neq \emptyset$, go back to step 2. If $\mathcal{P} = \emptyset$, exit the algorithm.
6. **Update complexes.** Add the computed translation complexes α_i to reaction i.
7. **Ensure nonnegative complexes.** For each linkage class, shift complexes with negative entries to ensure that the stoichiometric coefficients of species per complex is nonnegative.

In our running example, the histidine kinase network consists of four reactions, and so four translation complexes must be determined. Applying Algorithm 2, the translation complex for each reaction is given by

$$\begin{aligned} X &\longrightarrow X_p && (+Y_p) \\ X_p + Y &\longrightarrow X + Y_p && (+\emptyset) \\ X + Y_p &\longrightarrow X_p + Y && (+\emptyset) \\ Y_p &\longrightarrow Y && (+X_p) \end{aligned}$$

which results in the following translated network which is guaranteed to be WR and DZ by Theorem 3. See the Supplementary Information for the step-by-step procedure in the computation.



It should be noted that a WR and DZ translation from the method of Theorem 3 is not unique. For instance, translating using the translation complexes $\alpha_1 = Y$, $\alpha_2 = \emptyset = \alpha_3$, and $\alpha_4 = X$ yields a different network but is still WR and DZ. This arises from Steps 2 and 3 of Algorithm 2, where different choices of r_i from $\mathcal{P}$ may lead to different outcomes.

In the next subsection, we demonstrate the enhanced computational framework for deriving analytic steady states using a two-protein gene transcription model. We use a different example to better illustrate the framework. The process involves decomposing the network into its independent subnetworks and subsequently merging translated versions of the subnetworks. The histidine kinase example does not admit a nontrivial independent decomposition, so it does not demonstrate these decomposition and merging steps.

Demonstration: An enhanced framework for deriving the analytic steady state of a two-protein gene transcription model

We now demonstrate the enhanced framework for deriving analytic equilibria by considering a two-protein transcription model, which is a mass-action system consisting of ten reactions that describe a gene transcription motif involving two

proteins P_1 and P_2, each produced by their respective genes G_1 and G_2 [7,21]. Table 1 shows the reactions of the network grouped according to their functional roles.

The first step of the procedure is to break down the network into its independent subnetworks as shown in Fig 1a. The rank of the entire network is given by

$$s = \operatorname{rank} \begin{array}{c} \\ G_1 \\ G_2 \\ P_1 \\ P_2 \\ D \\ C_1 \\ C_2 \end{array} \begin{array}{c} \begin{array}{cccccccccc} R_1 & R_2 & R_3 & R_4 & R_5 & R_6 & R_7 & R_8 & R_9 & R_{10} \end{array} \\ \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\ 1 & 0 & -1 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\ 0 & 1 & 0 & -1 & -2 & 2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & -1 & 1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{bmatrix} \end{array} = 5 \tag{1}$$

while the ranks of the subnetworks are as follows

$$s_1 = \operatorname{rank} \begin{array}{c} \begin{array}{cc} R_1 & R_3 \end{array} \\ \begin{bmatrix} 0 & 0 \\ 0 & 0 \\ 1 & -1 \\ 0 & 0 \\ 0 & 0 \\ 0 & 0 \\ 0 & 0 \end{bmatrix} \end{array} = 1 \quad s_2 = \operatorname{rank} \begin{array}{c} \begin{array}{cc} R_2 & R_4 \end{array} \\ \begin{bmatrix} 0 & 0 \\ 0 & 0 \\ 0 & 0 \\ 1 & -1 \\ 0 & 0 \\ 0 & 0 \\ 0 & 0 \end{bmatrix} \end{array} = 1 \quad s_3 = \operatorname{rank} \begin{array}{c} \begin{array}{cc} R_5 & R_6 \end{array} \\ \begin{bmatrix} 0 & 0 \\ 0 & 0 \\ 0 & 0 \\ -2 & 2 \\ 1 & -1 \\ 0 & 0 \\ 0 & 0 \end{bmatrix} \end{array} = 1$$

Table 1. The reactions of the two-protein gene transcription model and their descriptions.

Label	Reaction	Description
Gene transcription		
R_1	$G_1 \xrightarrow{k_1} G_1 + P_1$	basal production of protein P_1 through gene G_1
R_2	$G_2 \xrightarrow{k_2} G_2 + P_2$	basal production of protein P_2 through gene G_2
Protein clearance		
R_3	$P_1 \xrightarrow{k_3} 0$	clearance of protein P_1
R_4	$P_2 \xrightarrow{k_4} 0$	clearance of protein P_2
Dimerization		
R_5 and R_6	$2P_2 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} D$	dimerization of protein P_2
Regulatory complex formation		
R_7 and R_8	$G_2 + P_1 \underset{k_8}{\overset{k_7}{\rightleftharpoons}} C_1$	P_1 binds with G_2
R_9 and R_{10}	$G_1 + D \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} C_2$	P_2 dimer binds with G_1

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$$s_4 = \text{rank}\begin{array}{c} \begin{matrix} R_7 & R_8 \end{matrix} \\ \begin{bmatrix} 0 & 0 \\ -1 & 1 \\ -1 & 1 \\ 0 & 0 \\ 0 & 0 \\ 1 & -1 \\ 0 & 0 \end{bmatrix} \end{array} = 1 \qquad s_5 = \text{rank}\begin{array}{c} \begin{matrix} R_9 & R_{10} \end{matrix} \\ \begin{bmatrix} -1 & 1 \\ 0 & 0 \\ 0 & 0 \\ 0 & 0 \\ -1 & 1 \\ 0 & 0 \\ 1 & -1 \end{bmatrix} \end{array} = 1$$

Since $s = 5 = s_1 + s_2 + s_3 + s_4 + s_5$, the decomposition in Fig 1a is an independent network decomposition. Among the five subnetworks, three are already WR and DZ, whereas the first two require network translation to satisfy these conditions. Following the three-step translation scheme outlined in the previous subsection, we use G_1 as the translation complex for the third reaction, while leaving the first reaction unchanged. Refer to Fig 1b. As a result, the third reaction becomes $G_1 + P_1 \longrightarrow G_1$, which is the reverse of the first reaction. The translated subnetwork with reversible reactions defines the stoichiometric CRN, denoted by $\mathcal{N}'_{1_S}$, associated with $\mathcal{N}_1$. Clearly, it is both WR and DZ ($\delta' = n' - l' - s' = 2 - 1 - 1 = 0$).

Next, we construct the kinetic-order CRN, which is denoted by $\mathcal{N}'_{1_K}$, associated with $\mathcal{N}_1$. To do this, we preserve the reaction rates of the original network so that the translated network remains dynamically equivalent to the original system (see Methods section under Generalized chemical reaction networks for a more detailed discussion). In particular, we assign the reaction rate $k_3 P_1$ to the translated reaction $G_1 + P_1 \longrightarrow G_1$ because P_1 is the source complex of the corresponding reaction in the original network. In this manner, we preserve the reaction kinetics of the original reaction. Moreover, the reaction vector of the translated reaction is given by

$$(G_1) - (G_1 + P_1) = -P_1 = [0 \;\; 0 \;\; -1 \;\; 0 \;\; 0 \;\; 0 \;\; 0]^T,$$

which is precisely the third column in the stoichiometric matrix of the original network (see Eq (1)). These guarantee that the translated network generates the same dynamical system as the original network. Otherwise, the translated network would no longer be dynamically equivalent to the original network, and the subsequent steady state analysis would not correspond to the original biochemical system.

We then take all the edges of $\mathcal{N}'_{1_S}$ while assigning to each of the source nodes of the edges the corresponding source complexes from the original network. Since P_1 is the original source complex of the third reaction, two complexes become associated with a single node in $\mathcal{N}'_{1_K}$. We separate them using a *phantom edge*, defined as an edge equipped with a free parameter (σ_1) that serves as its "rate constant", in such a way that the resulting network is WR. For a phantom edge, we always get a zero stoichiometric vector, since both the head and tail of the edge correspond to the same complex in the stoichiometric CRN. This construction yields the kinetic-order CRN $\mathcal{N}'_{1_K}$, which is both WR and DZ ($\tilde{\delta} = \tilde{n} - \tilde{l} - \tilde{s} = 3 - 1 - 2 = 0$). The stoichiometric CRN $\mathcal{N}'_{1_S}$ and kinetic-order CRN $\mathcal{N}'_{1_K}$ constitute the *translated network* for $\mathcal{N}_1$. Since both $\mathcal{N}'_{1_S}$ and $\mathcal{N}'_{1_K}$ are WR and DZ, the translated network is WR and DZ as well.

Similarly, we apply the procedure to $\mathcal{N}_2$ using G_2 as the translation complex for the fourth reaction while keeping the second reaction unchanged. This yields a translated network that is both WR and DZ as shown in Fig 1b.

The next step is to merge the subnetworks into a single unified network instead of treating each subnetwork as an individual piece. This step is supported by Theorem S1 in the Supporting Information, stating that the preservation of weak reversibility and zero deficiency under merging of independent translated subnetworks possessing these properties is guaranteed. In the two-protein gene transcription example, Fig 1c illustrates how the subnetworks are merged. This is simply done by taking the union of the reaction sets of the translated subnetworks. Since the subnetworks do not share any common complexes, the merging process preserves the interconnections within each subnetwork, effectively treating

them as components of a single larger network. It can be easily verified that the resulting merged network is both WR and DZ. In particular, both the stoichiometric CRN and kinetic-order CRN of the merged network are WR and DZ. This is also guaranteed by Theorem S1 in the Supplementary Information.

Finally, we compute the equilibrium parametrization using the full network rather than treating each subnetwork individually. We refer the readers to [2,22] for a detailed discussion on equilibrium parametrization. Fig 1d shows the final equilibrium parametrization of the two-protein gene transcription model. It can be observed that P_1 and P_2 are free parameters in the parametrization. If we set particular values for the rate constants and free parameters P_1 and P_2, we get the steady state concentration values of the species G_1, G_2, D, C_1, and C_2.

Improvements in CRITERIA over COMPILES

In this subsection, we summarize the improvements introduced in CRITERIA over COMPILES. Specifically, we incorporate three key modifications to the COMPILES framework to address its issues and limitations. First, we adopt the method developed in [5] to translate networks into becoming WR and DZ using EFMs. This was discussed in detail in a previous subsection using a simple histidine kinase example. By replacing the original translation procedure in COMPILES, we resolve a major computational bottleneck making CRITERIA more practical for analyzing larger reaction networks.

Second, we reorder the steps of COMPILES. Instead of computing the equilibrium parametrization of each subnetwork individually, we first merge all subnetworks into a single network by taking the union of their reactions, and then compute the parametrization for the entire network. This modification is crucial for avoiding dependencies in the equilibrium expressions, as described in the Introduction section. To illustrate this issue more clearly, we consider again the MAPK example in the Introduction. The parametrization obtained by COMPILES for the species X_{pp} and $X_{pp}M$ is given by

$$\boldsymbol{X_{pp}} = \frac{X_p \boldsymbol{X_{pp}} \boldsymbol{M} k_8 k_{11} k_{12} \sigma_1 + X_p \boldsymbol{X_{pp}} \boldsymbol{M} k_9 k_{11} k_{12} \sigma_1}{X_p^* M k_7 k_{13} k_{16} \sigma_2 + X_p^* M k_7 k_{14} k_{16} \sigma_2}$$

$$\boldsymbol{X_{pp}M} = \frac{\boldsymbol{X_{pp}} X_p^* M k_7 (k_{13} + k_{14})}{X_p k_{12} (k_8 + k_9)}.$$

There is an interdependency issue in this example because the two parametrization equations depend on each other in a circular way, so that none of the involved species can be expressed solely in terms of the free parameters and rate constants without further algebraic manipulation. If we substitute the equation for $X_{pp}M$ to that of X_{pp}, we arrive at the equation

$$k_{16}\sigma_2 = k_{11}\sigma_1$$

which is a hidden algebraic condition on the parameters. However, after observing this relation, substituting the parametrization into the ODE system does not make all time derivatives vanish indicating that the output COMPILES obtained is incorrect. This is what we aim to address as far as the interdependency issue is concerned.

Finally, we extend the applicability of the parametrization procedure to handle cases in which the deficiency of the kinetic-order CRN is positive based on the theory established in [22]. In particular, the core algorithm in COMPILES does not consider the theoretical foundations for models with positive kinetic deficiency according to Case 2 of Theorem 2 (see the Methods section). This means that COMPILES neither processes nor outputs the additional algebraic conditions necessary for the final parametrization of networks with positive kinetic deficiency. Take, for example, the MAPK model considered above. Its kinetic deficiency is equal to one. By Case 2 of Theorem 2, one additional condition needs to be imposed in the final parametrization. Applying CRITERIA, we get

$$\sigma_2 = \frac{k_4 k_6 k_{12} k_{14} k_{16} \sigma_1 (k_2 + k_3)(k_8 + k_9)}{k_1 k_3 k_7 k_9 k_{11} (k_5 + k_6)(k_{13} + k_{14})}$$

as the additional condition which is not captured by COMPILES. By considering Case 2 of Theorem 2, we get a valid final parametrization for models whose kinetic deficiency is positive. In the next subsection, we compare the runtime performance of COMPILES and CRITERIA by applying them to a variety of models.

Runtime performance comparisons

We apply CRITERIA to 26 models and evaluate its performance by comparing its runtime with that of COMPILES. We also assess the performance gains of the new network translation scheme by comparing the EFM-based translation with the translation method used in COMPILES. Computational experiments were conducted on a MacBook Air equipped with an Apple M2 processor and 8 GB of RAM. All runtime measurements were obtained under identical hardware and software conditions.

To contextualize performance results, Table 2 summarizes the network properties of the benchmark models used for the comparisons. The models are listed in order of increasing number of reactions. It is worth noting here that all benchmark models are neither WR nor DZ.

We impose a maximum runtime of 3600 seconds; any execution that does not complete within this time limit is terminated. For our computational experiments, we perform five independent runs, and report the mean and standard deviation

Table 2. Network properties of benchmark models with r = number of reactions, m = number of species, n = number of complexes, s = rank of the network, l = number of linkage classes, δ = effective deficiency, and $\tilde{\delta}$ = kinetic deficiency.

Model	r	m	n	s	l	δ	$\tilde{\delta}$
Model A: Histidine-Kinase [21]	4	4	6	2	3	1	0
Model B: Miao's Influenza Virus Model [8]	5	3	7	3	2	2	0
Model C: IDHKP-IDH [9]	6	5	6	3	2	1	0
Model D: 1-Site PD Network [10]	6	6	6	3	2	1	0
Model E: Hybrid Histidine-Kinase [21]	6	6	10	4	4	2	0
Model F: PTM system [11]	9	8	9	5	3	1	0
Model G: 2-Protein Gene Transcription [7,21]	10	7	13	5	6	2	0
Model H: Reduced ERK Network [12]	10	10	12	7	2	3	0
Model I: 2-site PD Network [10]	12	9	10	6	2	2	0
Model J: Two-Substrate Phosphorylation [21]	12	10	12	6	4	2	0
Model K: Two-Layer Cascade of Modification Cycles [11]	12	10	12	6	4	2	0
Model L: EnvZ-OmpR [22]	14	9	13	7	4	2	1
Model M: Two-Component Osmoregulator in *E. coli* [13]	15	9	13	7	4	2	2
Model N: Hernandez's Influenza Virus Model [8]	16	7	16	7	3	6	4
Model O: Modified Two-Component Osmoregulator in *E. coli* [13]	16	9	15	7	5	3	2
Model P: CRISPRi Model 1 [25]	16	12	19	8	9	2	0
Model Q: MAPK [5]	16	11	12	8	2	2	1
Model R: Full ERK model [12]	18	12	14	9	2	3	1
Model S: 3-Site PD Network [10]	18	12	14	9	2	3	1
Model T: Zigzag Model of Plant-Pathogen Interactions [5]	21	13	23	9	6	8	0
Model U: CRISPRi Model 2 [25]	22	15	24	11	11	2	0
Model V: MAPK Signaling Cascade [11]	24	17	19	12	4	3	0
Model W: WNT Signaling Pathway [22]	31	19	28	14	10	4	0
Model X: Insulin Signaling Pathway [26]	35	20	35	15	13	7	1
Model Y: CRISPRi Model 3 [25]	38	19	36	17	15	4	6
Model Z: CRISPRi Model 4 [25]	42	17	38	15	13	10	10

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of the resulting runtimes. In the first experiment, we compare the runtime performance of the EFM-based network translation, which we call REWARDZ (tRanslation via Elementary flux modes towards WeAkly Reversible Deficiency Zero networks), with TOWARDZ (TranslatiOn toward WeAkly Reversible and Deficiency Zero networks), the translation method used in COMPILES [23]. Table 3 presents this comparison. It should be noted that network decomposition has not yet been integrated in both computational frameworks. We make the following observations:

- REWARDZ successfully produced a valid translation for nearly all models, with the exception of Hernandez's Influenza Virus Model and CRISPRi Model 3, whereas TOWARDZ succeeded in only five models. In particular, TOWARDZ failed to translate any model containing more than six reactions. It is important to note that the inability of TOWARDZ to generate the desired output within the prescribed maximum runtime is not caused by insufficient RAM. We monitored the memory usage of MATLAB throughout the computation for Model Z and observed that the peak real memory consumption is 209 MB which is relatively small compared with the 8 GB of available system memory of the machine.

Table 3. Comparison of running times (in seconds) between TOWARDZ and REWARDZ, and between COMPILES and CRITERIA. A maximum runtime of 3600 seconds is imposed for the computational experiments. Each entry represent the mean runtime ± standard deviation over five independent runs. Entries marked with "⋙ 3600" indicate that the method was unable to produce the desired output within the time limit. Entries labeled "No valid translation" signify that the method completed within the prescribed maximum runtime but did not identify a WR and DZ translation. Lastly, entries labeled "Error" encountered an error in the code.

Model	TOWARDZ	REWARDZ	COMPILES	CRITERIA
Model A	0.259 ± 0.009	0.498 ± 0.021	0.762 ± 0.028	0.991 ± 0.026
Model B	1.043 ± 0.011	0.541 ± 0.022	0.877 ± 0.024	0.993 ± 0.033
Model C	0.557 ± 0.003	0.683 ± 0.010	1.135 ± 0.020	1.200 ± 0.022
Model D	1.040 ± 0.006	0.731 ± 0.043	1.568 ± 0.017	1.192 ± 0.020
Model E	204.296 ± 0.968	0.749 ± 0.012	206.543 ± 2.665	1.390 ± 0.039
Model F	⋙ 3600	1.098 ± 0.028	⋙ 3600	1.836 ± 0.033
Model G	⋙ 3600	0.854 ± 0.024	1.039 ± 0.027	1.452 ± 0.032
Model H	⋙ 3600	1.199 ± 0.038	⋙ 3600	2.049 ± 0.006
Model I	⋙ 3600	1.216 ± 0.043	2.907 ± 0.017	2.068 ± 0.016
Model J	⋙ 3600	1.247 ± 0.025	2.738 ± 0.018	1.883 ± 0.044
Model K	⋙ 3600	1.222 ± 0.027	2.888 ± 0.023	1.897 ± 0.020
Model L	⋙ 3600	1.280 ± 0.011	⋙ 3600	2.560 ± 0.042
Model M	⋙ 3600	1.357 ± 0.047	⋙ 3600	2.716 ± 0.036
Model N	⋙ 3600	No valid translation	⋙ 3600	3.067 ± 0.093
Model O	⋙ 3600	1.471 ± 0.047	⋙ 3600	3.177 ± 0.024
Model P	⋙ 3600	1.071 ± 0.017	1.431 ± 0.035	2.009 ± 0.053
Model Q	⋙ 3600	1.158 ± 0.009	1223.452 ± 3.801	2.641 ± 0.057
Model R	⋙ 3600	1.397 ± 0.022	⋙ 3600	3.197 ± 0.053
Model S	⋙ 3600	1.483 ± 0.018	Error	2.961 ± 0.116
Model T	⋙ 3600	1.423 ± 0.010	15.845 ± 0.184	3.010 ± 0.176
Model U	⋙ 3600	1.335 ± 0.080	Error	2.498 ± 0.071
Model V	⋙ 3600	1.912 ± 0.030	⋙ 3600	3.773 ± 0.050
Model W	⋙ 3600	2.225 ± 0.058	⋙ 3600	3.803 ± 0.069
Model X	⋙ 3600	2.148 ± 0.038	3.675 ± 0.054	5.077 ± 0.068
Model Y	⋙ 3600	No valid translation	⋙ 3600	6.424 ± 0.069
Model Z	⋙ 3600	32.430 ± 0.266	⋙ 3600	12.946 ± 0.172

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This suggests that its failure to obtain a valid translation is more likely attributable to the computational complexity of the translation search process.

- Among the models for which REWARDZ successfully constructed a WR and DZ translation, the runtime was under 3 seconds in all cases except for the full CRISPRi model (CRISPRi Model 4). This model, which is the largest considered in the paper, contains 42 reactions and has deficiency equal to 10.
- There is no significant difference in runtime among the models for which both methods successfully produced a valid translation, with the exception of the Hybrid Histidine–Kinase Model. This model, which consists of six reactions and ten species and has a deficiency of two, exhibited a markedly better performance under REWARDZ.

The result in Table 3 demonstrates that REWARDZ offers a substantial improvement in terms of running time in generating a WR and DZ network translation, especially in larger models. It underscores the effectiveness and scalability of the enhanced computational framework in handling more complex biochemical systems.

We now proceed to compare the runtime performance of COMPILES and CRITERIA. Similar to the comparison above, we impose a maximum runtime of 3600 seconds. If steady states are not parametrized within the time limit, we exit the program. Moreover, we repeat the experiment five times, and the results are reported as the mean $\pm$ standard deviation across these runs. As shown in Table 3, COMPILES was unable to derive the analytic equilibria of several benchmark models. Of the 26 models, it failed to parametrize the equilibria of 11, whereas CRITERIA successfully obtained equilibrium parametrizations for all models. Notably, CRITERIA was able to solve the analytic equilibria of all models in less than 7 seconds except for the full CRISPRi model which required approximately 12 seconds. In addition, COMPILES encountered code errors in 2 models, namely the 3-site PD Network and CRISPRi Model 2. These results show the performance gains of CRITERIA over COMPILES.

It can be observed that COMPILES performed slightly better than CRITERIA in six models. This is because, in these models, network decomposition has broken down the network into WR and DZ subnetworks, eliminating the need for translation. To elucidate this, we consider the 2-Protein Gene Transcription Model, CRISPRi Model 1, and the Insulin Signaling Pathway. Fig 1 shows that the 2-Protein Gene Transcription Model consists of five independent subnetworks, three of which are already WR and DZ. The remaining two subnetworks that require translation each contain only two reactions. For the Insulin Signaling Pathway, five of the ten independent subnetworks are already WR and DZ. The remaining five subnetworks that require translation only have three reactions, four reactions, and two reactions in three subnetworks, respectively. Lastly, the CRISPRi Model 1 (smallest CRISPRi model considered in this paper) consists of eight independent subnetworks, none of which are WR or DZ, but each contains only two reactions.

Based on these results, COMPILES appears to handle smaller networks slightly more efficiently than CRITERIA. This is likely due to the fact that CRITERIA requires the solving of EFMs and a BLP, which can be computationally more demanding. For more details, see the Discussion section.

We verify the correctness of the output produced by CRITERIA by substituting the derived equilibrium expressions into the model's ODE system using MATLAB. To get the mass-action ODE system of the models, we use CHECKMATE (https://github.com/pvnlubenia/CHECKMATE), which is a MATLAB code used to generate the ODE of a CRN with mass-action kinetics. We then plug in the output produced by CRITERIA into the ODE system. Indeed, the time derivatives evaluate to zero, confirming that the solutions are indeed valid equilibria.

In the next subsection, we apply CRITERIA to investigate key dynamical properties of selected biochemical systems such as multistationarity and ACR.

Application: Using equilibrium parametrization to examine ACR and multistationarity

We now apply our method to the EnvZ-OmpR system, a two-component signaling pathway consisting of the sensor kinase EnvZ, denoted by X, and the response regulator OmpR, denoted by Y [19]. In the network below that corresponds

to Model L in Table 2, both X and Y exist in phosphorylated forms, denoted by X_p and Y_p, respectively. The sensor X undergoes autophosphorylation through ATP (species T) binding and hydrolysis. The phosphorylated sensor X_p catalyzes the transfer of the phosphoryl group to Y. Furthermore, X dephosphorylates Y_p using either ATP or ADP (species D) as a cofactor [19]. In the model, the effects of both ATP and ADP are simultaneously considered. Experimental observations indicate that the system exhibits concentration robustness, which has been analytically verified by demonstrating that the equilibrium concentration of Y_p depends solely on the rate constants. [14,19,22].

The reaction network of the system consisting of 14 reactions is shown below.

$$XD \underset{k_2}{\overset{k_1}{\rightleftarrows}} X \underset{k_4}{\overset{k_3}{\rightleftarrows}} XT \xrightarrow{k_5} X_p$$

$$X_p + Y \underset{k_7}{\overset{k_6}{\rightleftarrows}} X_pY \xrightarrow{k_8} X + Y_p$$

$$XD + Y_p \underset{k_{10}}{\overset{k_9}{\rightleftarrows}} XDY_p \xrightarrow{k_{11}} XD + Y$$

$$XT + Y_p \underset{k_{13}}{\overset{k_{12}}{\rightleftarrows}} XTY_p \xrightarrow{k_{14}} XT + Y$$

This can be decomposed into two independent subnetworks. The first subnetwork is given by

$$XD \underset{k_2}{\overset{k_1}{\rightleftarrows}} X$$

which is already WR and DZ. The second subnetwork, however, requires translation. It is shown below together with the translation complexes to achieve WR and DZ.

$$X \underset{k_4}{\overset{k_3}{\rightleftarrows}} XT \xrightarrow{k_5} X_p \qquad (+XD + XT + Y_p)$$

$$X_p + Y \underset{k_7}{\overset{k_6}{\rightleftarrows}} X_pY \xrightarrow{k_8} X + Y_p \qquad (+XD + XT)$$

$$XD + Y_p \underset{k_{10}}{\overset{k_9}{\rightleftarrows}} XDY_p \xrightarrow{k_{11}} XD + Y \qquad (+XT + X_p)$$

$$XT + Y_p \underset{k_{13}}{\overset{k_{12}}{\rightleftarrows}} XTY_p \xrightarrow{k_{14}} XT + Y \qquad (+XD + X_p)$$

By applying CRITERIA, we obtain the following equilibrium parametrization

$$X = \frac{X_p Y k_8 (k_4 + k_5)}{k_3 k_5}$$

$$XD = \frac{X_p Y k_2 k_8 (k_4 + k_5)}{k_1 k_3 k_5}$$

$$XDY_p = \frac{X_p Y k_8 k_9 (k_{13} + k_{14})}{k_9 k_{11} k_{13} + k_9 k_{11} k_{14} + \frac{k_1 k_3 k_{10} k_{12} k_{14}}{k_2 (k_4 + k_5)} + \frac{k_1 k_3 k_{11} k_{12} k_{14}}{k_2 (k_4 + k_5)}}$$

$$XT = \frac{X_p Y k_8}{k_5}$$

$$XTY_p = \frac{X_p Y k_1 k_3 k_8 k_{12} (k_{10} + k_{11})}{k_2 (k_4 + k_5) \left(k_9 k_{11} k_{13} + k_9 k_{11} k_{14} + \frac{k_1 k_3 k_{10} k_{12} k_{14}}{k_2 (k_4 + k_5)} + \frac{k_1 k_3 k_{11} k_{12} k_{14}}{k_2 (k_4 + k_5)} \right)}$$

$$X_p = \frac{X_p Y (k_7 + k_8)}{Y k_6}$$

$$Y_p = \frac{k_1 k_3 k_5 (k_{10} + k_{11})(k_{13} + k_{14})}{k_2 (k_4 + k_5) \left(k_9 k_{11} k_{13} + k_9 k_{11} k_{14} + \frac{k_1 k_3 k_{10} k_{12} k_{14}}{k_2 (k_4 + k_5)} + \frac{k_1 k_3 k_{11} k_{12} k_{14}}{k_2 (k_4 + k_5)} \right)}$$

with free parameters $X_p Y$ and Y, and set of conservation laws

$$\begin{aligned} \frac{dX}{dt} + \frac{dXD}{dt} + \frac{dXDY_p}{dt} + \frac{dXT}{dt} + \frac{dXTY_p}{dt} + \frac{dX_p}{dt} + \frac{dX_pY}{dt} &= 0 \\ -\frac{dX}{dt} - \frac{dXD}{dt} - \frac{dXT}{dt} - \frac{dX_p}{dt} + \frac{dY}{dt} + \frac{dY_p}{dt} &= 0 \end{aligned}$$

Observe that the equilibrium concentration of Y_p depends only on the rate constants confirming the result in [22]. Now, integrating the conservation equations with respect to t, we get

$$X + XD + XDY_p + XT + XTY_p + X_p + X_pY = T_1 \tag{2}$$

$$-X - XD - XT - X_p + Y + Y_p = T_2 \tag{3}$$

where T_1 and T_2 are nonnegative constants dependent on the initial conditions of the species involved in their respective equation. It is important to note that the conservation laws reported by CRITERIA are obtained by computing a basis for the left null space of the stoichiometric matrix of the reaction network [24,29,30]. Consequently, the reported conservation laws are not guaranteed to have positive coefficients as can be observed by the second conservation law of the EnvZ–OmpR model. This, however, does not affect the final equilibrium parametrization since conservation laws are not used in its derivation. In other words, the derivation of the equilibrium parametrization and the computation of conservation laws are independent procedures.

Therefore, the choice of a basis for the left null space of the stoichiometric matrix has no effect on the equilibrium parametrization or its positivity properties. In particular, replacing a reported conservation law with another valid conservation law with negative coefficients would not alter any of the steady state expressions generated by CRITERIA. Such valid conservation laws can always be obtained as linear combinations of the reported basis vectors. A detailed discussion on the computation of conservation laws is provided in the Supplementary Information.

To facilitate our computation, we express the rate constants in the equilibrium parametrization in terms of alphas so that $X = X_p Y\alpha_1$, $XD = X_p Y\alpha_2$, $XDY_p = X_p Y\alpha_3$, $XT = X_p Y\alpha_4$, $XTY_p = X_p Y\alpha_5$, $X_p = \frac{X_p Y\alpha_6}{Y}$, and $Y_p = \alpha_7$, where $\alpha_i > 0$ for $i = 1,2,3,4,5,6,7$. Plugging these expressions into Eq (2) and Eq (3), we get

$$X_p Y\alpha_1 + X_p Y\alpha_2 + X_p Y\alpha_3 + X_p Y\alpha_4 + X_p Y\alpha_5 + \frac{X_p Y\alpha_6}{Y} + X_p Y = \quad T_1 \tag{4}$$

$$-X_p Y\alpha_1 - X_p Y\alpha_2 - X_p Y\alpha_3 - \frac{X_p Y\alpha_6}{Y} + Y + \alpha_7 = \quad T_2 \tag{5}$$

From Eq (4), we have $Y = \frac{X_p Y\alpha_6}{T_1 - X_p Y\gamma_1}$, where $\gamma_1 = 1 + \alpha_1 + \alpha_2 + \alpha_3 + \alpha_4 + \alpha_5$. Similarly, we can write Eq (5) as

$$-Y \cdot X_p Y\gamma_2 - X_p Y\alpha_6 + Y^2 + Y\alpha_7 = Y \cdot T_2$$

where $\gamma_2 = \alpha_1 + \alpha_2 + \alpha_3$ so that

$$-\left(\frac{X_p Y\alpha_6}{T_1 - X_p Y\gamma_1}\right) X_p Y\gamma_2 - X_p Y\alpha_6 + \left(\frac{X_p Y\alpha_6}{T_1 - X_p Y\gamma_1}\right)^2 + \left(\frac{X_p Y\alpha_6}{T_1 - X_p Y\gamma_1}\right)\alpha_7 = \left(\frac{X_p Y\alpha_6}{T_1 - X_p Y\gamma_1}\right) T_2 \tag{6}$$

$$\begin{aligned} \Longrightarrow \left(\gamma_1\alpha_6\gamma_2 - \gamma_1^2\alpha_6\right)(X_p Y)^3 + \left(2T_1\gamma_1\alpha_6 - T_1\alpha_6\gamma_2 + \alpha_6^2 - \gamma_1\alpha_6\alpha_7 + \gamma_1\alpha_6 T_2\right)(X_p Y)^2 + \\ (T_1\alpha_6\alpha_7 - T_1^2\alpha_6 - T_1\alpha_6 T_2)X_p Y = 0 \end{aligned} \tag{7}$$

The left hand side of the equation is a cubic polynomial in $X_p Y$ with two sign changes indicating that the equation can either have two or zero positive real solutions by Descartes' Rule of Signs. Refer to the Supplementary Information for the proof. This means that $X_p Y$ can admit either two positive equilibria or none at all, implying that the system has the capacity for multistationarity. In other words, the system can have two positive equilibria for a particular choice of rate constants and initial conditions.

Alternatively, observe that the cubic expression in Eq (7) has a common factor $X_p Y$. We can then write the cubic polynomial as $f(X_p Y) = X_p Y \cdot g(X_p Y)$, with g being quadratic. Setting $f(X_p Y) = 0$, we have $g(X_p Y) = A(X_p Y)^2 + BX_p Y + C = 0$, where

$$\begin{aligned} A &= \gamma_1\alpha_6\gamma_2 - \gamma_1^2\alpha_6 \\ B &= 2T_1\gamma_1\alpha_6 - T_1\alpha_6\gamma_2 + \alpha_6^2 - \gamma_1\alpha_6\alpha_7 + \gamma_1\alpha_6 T_2 \\ C &= T_1\alpha_6\alpha_7 - T_1^2\alpha_6 - T_1\alpha_6 T_2. \end{aligned}$$

We discard the case when $X_p Y = 0$ since we are interested in looking at the positive equilibrium. When the discriminant $B^2 - 4AC$ of g is positive, an explicit parameter condition for the existence of two distinct equilibria is obtained. Suppose $B^2 - 4AC > 0$. From the Supplementary Information, it has been shown that $A < 0$, $B > 0$, and $C < 0$. We can infer from the Vieta's formulas that the product of the roots of g is $\frac{C}{A} > 0$ so that either both roots are positive or both of them are negative. By the quadratic formula, we have

$$x_1 = \frac{-B}{2A} + \frac{\sqrt{B^2 - 4AC}}{2A} \qquad x_2 = \frac{-B}{2A} - \frac{\sqrt{B^2 - 4AC}}{2A}$$

Note that $\frac{-B}{2A} > 0$ and $\frac{\sqrt{B^2-4AC}}{2A} < 0$. So, we have $x_2 > 0$ implying that $x_1 > 0$. Under the $B^2 - 4AC > 0$ assumption, these two positive roots correspond to the two distinct and positive equilibria of the system. This behavior is supported by Fig 2 which shows representative cases for specific choices of rate constants and initial conditions. In particular, the first function exhibits two positive roots, whereas the second admits none.

In the context of the EnvZ-OmpR system, the possibility for the existence of two positive equilibria or none at all provides a mechanistic explanation for how the EnvZ–OmpR pathway can implement threshold-based osmotic sensing. One equilibrium may represent a basal regulation, while the second may correspond to a fully activated stress-response state. This provides a rigorous mathematical foundation for its role as a master regulator of osmotic stress response. This result demonstrates the predictive power of CRITERIA over numerical simulations. While the latter can show specific cases of multistationarity, the analytical result provides a guarantee that no more than two positive steady states can exist regardless of the chosen rate constants. CRITERIA reveals how the network's structure, rather than just specific parameter values, dictates these functions.

For our next application, we consider the full CRISPRi toggle switch model which consists of 17 species and 42 reactions [25]. This is Model Z of Table 2. In this system, a catalytically inactive protein (dCas9) forms complexes with two single-guide RNAs (sg1 and sg2). These complexes subsequently bind both to their target genes (G_1 and G_2, respectively) and also to the opposing genes [2,25]. Binding to the intended target genes is referred to as specific binding, whereas

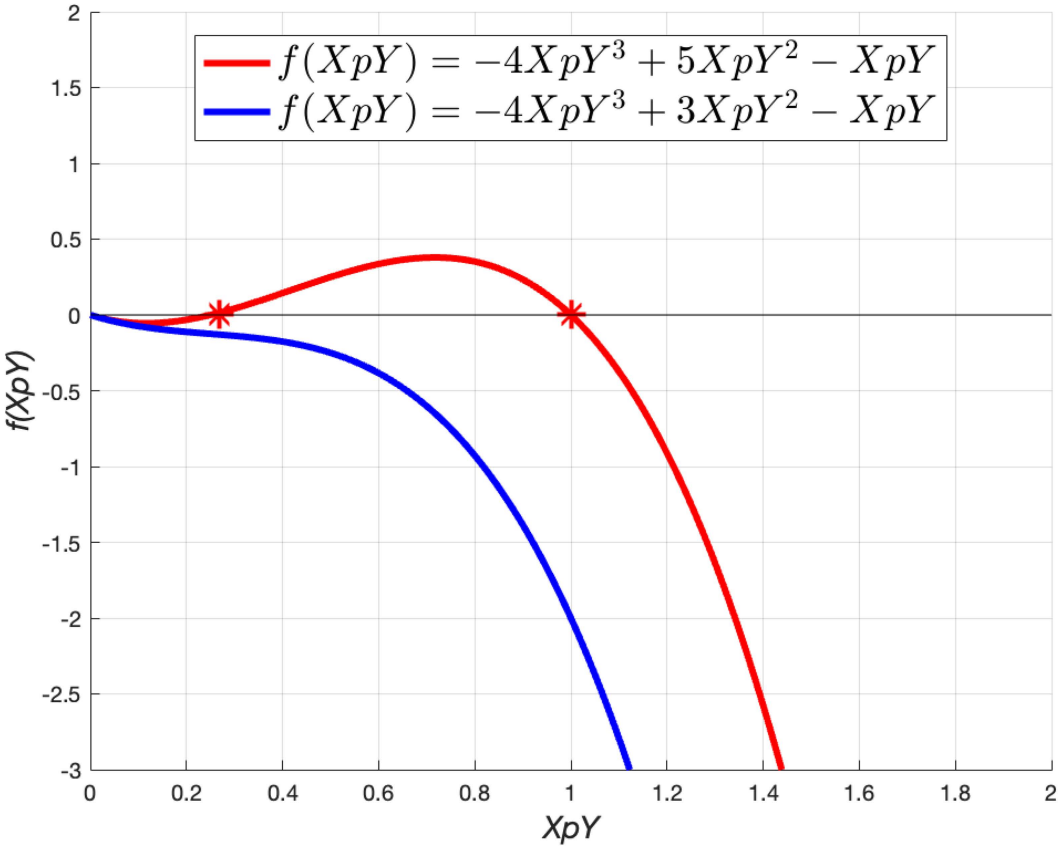

Fig 2. Graphs of two functions of the form $f(X_pY) = -a_3X_pY^3 + a_2X_pY^2 - a_1X_pY$ with positive constants a_i. The *x*-axis represents the value of X_pY at steady state. The first function, shown in red, has two positive zeroes while the second function, shown in blue, has no positive zero.

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binding to the opposing genes is called unspecific binding. The analysis in [25] shows that the interplay between specific and unspecific binding of dCas9/sgRNA complexes to the genes explains bistability in the CRISPRi toggle switch model.

Here, we show that, in addition to the system's capacity for multistationarity, the model also exhibits ACR in the dCas9 species. To establish this, we parametrize the equilibria of the system by applying CRITERIA. Fig 3 illustrates the decomposition, translation, and subsequent merging of the reaction network prior to deriving the equilibrium expressions. The species of interest in this analysis is $X13$, which represents the dCas9 protein. As shown in Fig 3b, the network has five independent subnetworks, two of which are neither WR nor DZ. Using the EFM-based network translation outlined above, we apply the translation complexes indicated on the right in each of the reactions in Fig 3b producing a generalized reaction network that is both WR and DZ.

A generalized reaction network consists of two components: the *stoichiometric CRN*, which contains the complexes obtained after the translation complexes are added to the reactions, and the *kinetic-order CRN*, which retains the original source complexes of the reaction network, thereby preserving the dynamics of the system. The reactions in red in the kinetic-order CRN are called *phantom edges*, which connect identical complexes in the stoichiometric CRN. If both stoichiometric CRN and kinetic-order CRN are WR and DZ, then the translated network is WR and DZ. See the Methods section for more information.

Using CRITERIA, the derived equilibrium expression for dCas9 is given by

$$\mathrm{X13} = \frac{k_{28}}{k_{27}},$$

where k_{28} and k_{27} represent the rate constants for the production (R_{28}) and degradation (R_{27}) of dCas9, respectively. This tells us that the equilibrium concentration of the dCas9 species is dependent only on these specific rate constants. So, the equilibrium concentration of dCas9 remains robustly constant for any initial conditions under fixed parameters. This remarkable property is known as *absolute concentration robustness* (ACR) [19]. This finding has implications for the design of robust synthetic systems:

- **Buffering Against Network Fluctuations**: ACR suggests an intrinsic buffering mechanism in which the equilibrium concentration of dCas9 remains invariant despite fluctuations in other network components, such as target gene concentrations or guide RNA levels.
- **Predictable Tuning**: In synthetic biology, achieving predictable and robust behavior is a primary concern. Our discovery shows that the long-term concentration of dCas9 is robustly constant over the concentrations of all other species.
- **Simplified Design Parameters**: Fine-tuning the long-term concentration of dCas9 in the circuit can only be achieved by altering its production rate k_{28} or degradation rate k_{27}. This decoupling from other parameters allows for modular circuit design where dCas9 levels can be set independently of the complexity of the broader network.

By maintaining a stable pool of the dCas9 engine, the CRISPRi circuit ensures that the regulation machinery remains consistent in varying cellular environments, effectively anchoring the stability of the toggle switch.

Computational package, CRITERIA

We developed CRITERIA (https://github.com/evvillejo/CRITERIA), a user-friendly, open-source, and publicly available computational package that automates the computation of equilibria of biochemical reaction networks. The framework enhances and extends COMPILES by incorporating several methodological improvements that overcome previously identified limitations (see Fig 4 and its accompanying discussion).

Taking the set of reactions of a CRN as input, CRITERIA outputs the following: an equilibrium parametrization, kinetic deficiency of the GCRN along with the additional equations required by Case 2 of Theorem 2, and the conservation laws

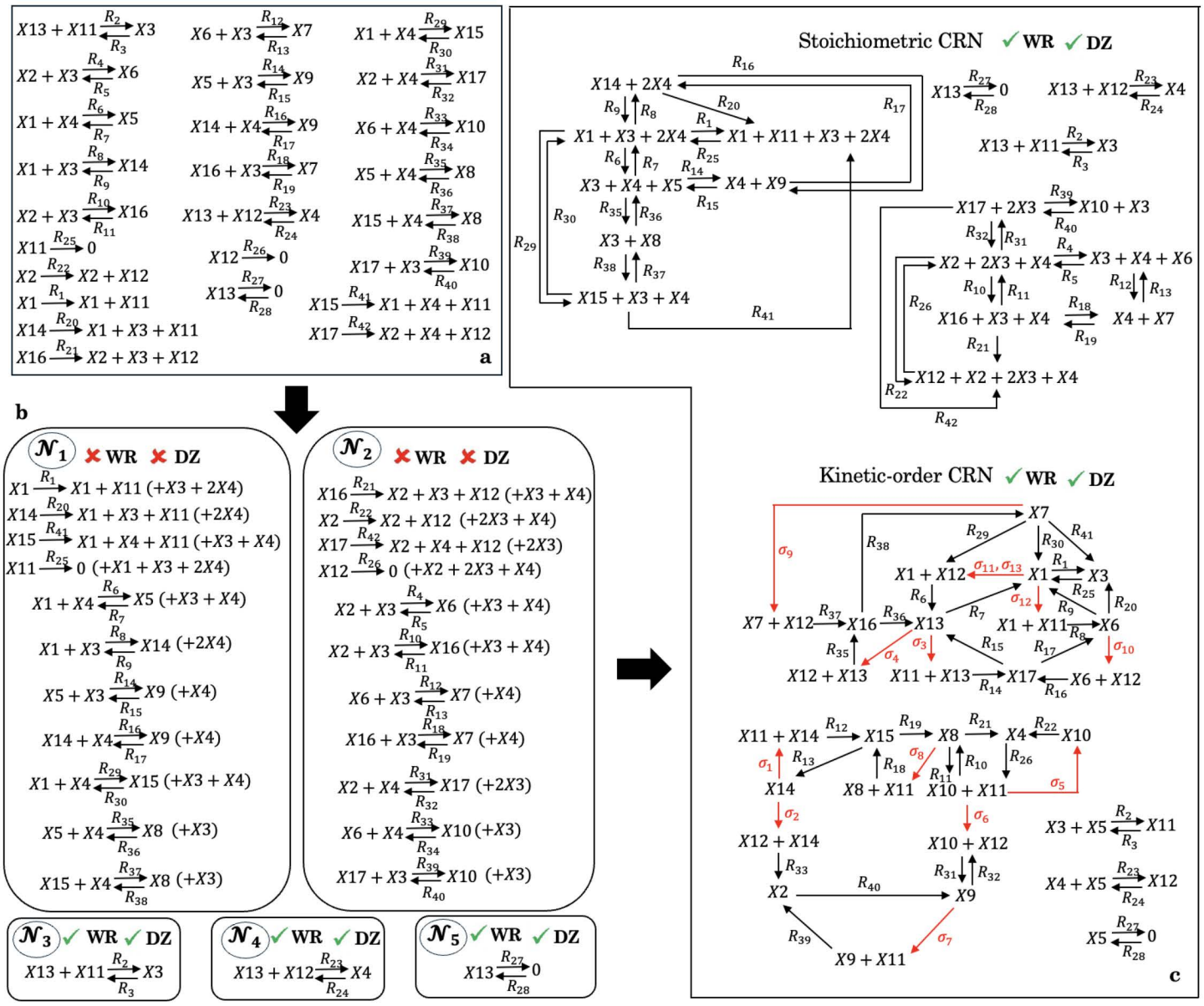

Fig 3. Network decomposition, translation, and merging of the full CRISPRi model for equilibrium parametrization. a The reaction network consists of 17 species and 42 reactions. The species of interest in our analysis is $X13$, which represents the catalytically inactive dCas9 protein. The full correspondence between model species and network variables is provided in the Supplementary Information. **b** The network has five independent subnetworks, two of which are neither WR nor DZ. The other three subnetworks consist only of two reactions which are reversible. The complexes to the right of each reaction are the translation complexes after applying the EFM-based translation method described above. **c** Adding the translation complexes to each reaction yields the following stoichiometric CRN and kinetic-order CRN, which together constitute the generalized reaction network for the full CRISPRi model. The stoichiometric CRN is the resulting network after adding the translation complexes to each of the reactions in the original model. On the other hand, the kinetic-order CRN, which determines the kinetics of the system, consists of the original source complex of the reaction network before translation, thereby ensuring that the dynamics is preserved. The reactions shown in red in the kinetic-order CRN are referred to as phantom edges. They connect identical complexes in the stoichiometric CRN. In the example, both the stoichiometric and kinetic-order CRNs are WR and DZ. Hence, the translated network is also WR and DZ. This now enables the parametrization of the equilibria of the model.

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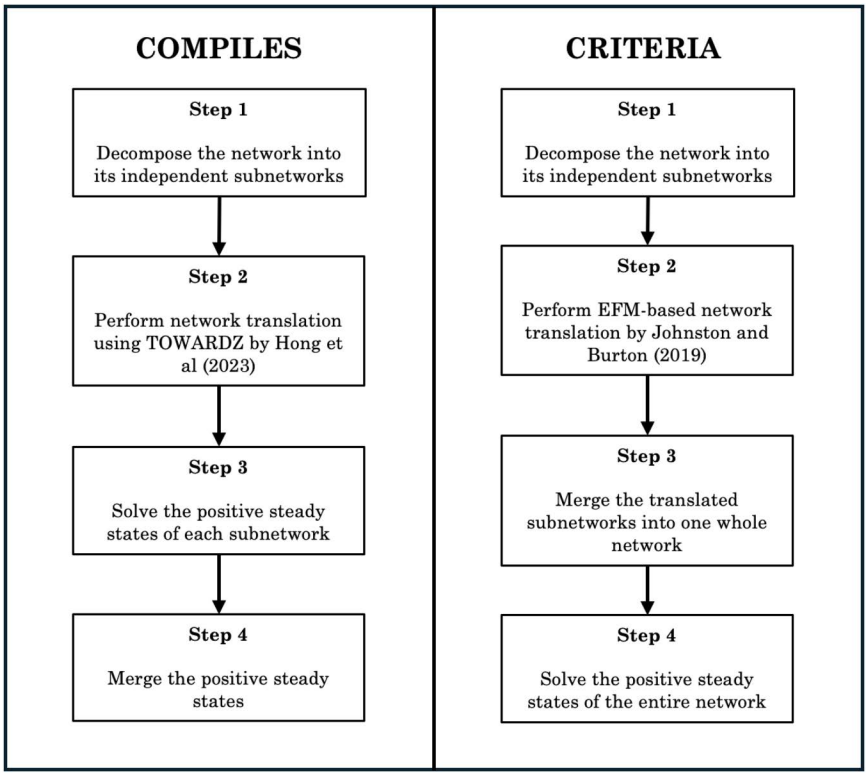

Fig 4. Side-by-side comparison of the COMPILES and CRITERIA frameworks for deriving analytic equilibria. For both frameworks, the method starts by decomposing the network into its independent subnetworks, thereby simplifying the analysis by focusing on smaller individual pieces rather than looking at the network as a whole. The next step is to perform network translation for each subnetwork that is not WR and DZ. For COMPILES, translation is performed using the TOWARDZ approach introduced in [23]. On the other hand, CRITERIA uses an EFM-based approach [5]. Both frameworks differ in the last two steps. Once all subnetworks satisfy WR and DZ, COMPILES proceeds by determining the positive equilibria of each subnetwork through the parametrization method in [22]. Finally, the equilibria of the original network are obtained by merging the equilibria of the subnetworks. However, for CRITERIA, it first performs merging of the translated subnetworks into a single network by taking the union of the reactions in the subnetworks before proceeding to compute the positive equilibria of the entire network, which constitutes the last step of the enhanced procedure.

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of the system. As with COMPILES, CRITERIA does its best to output the most simplified analytic solution in terms of rate constants and free parameters.

Discussion

In this paper, we have developed an enhanced framework together with a corresponding computational package for computing the positive equilibria of biochemical reaction networks. This approach substantially extends and improves the method introduced in [2], called COMPILES, for several key reasons (see Fig 4 for the side-by-side comparison of the schematic diagrams of both frameworks):

- Its network translation step constitutes a major computational bottleneck in the procedure. In particular, TOWARDZ, the package used to perform network translation in COMPILES, takes too long to output a WR and DZ translation.
- It has a “merging” issue that leads to interdependencies in the resulting equilibrium parametrization.
- COMPILES is limited only to reaction networks with zero kinetic deficiency.

Our approach addresses the issues and limitations enumerated above by adopting the EFM-based translation approach in [5], reordering the steps in the overall procedure of COMPILES (i.e., we first merge the translated subnetworks into one whole network before solving the equilibria), and incorporating additional conditions required to parametrize the equilibria of networks with positive kinetic deficiency (Theorem 15 of [22]). These modifications form the foundation of the computational package we developed, termed CRITERIA, which implements the improved process.

We were able to show that CRITERIA indeed outperforms COMPILES, particularly for large reaction networks, in terms of computational runtime (see Table 3). In fact, COMPILES was unable to parametrize the equilibria of 11 of the 26 models considered in this study. Moreover, 10 of the 26 models have positive kinetic deficiency, highlighting a class of networks that COMPILES cannot accommodate but are handled by CRITERIA.

It has been observed that COMPILES may handle smaller networks slightly more efficiently than CRITERIA, as the latter requires the solving of EFMs and a BLP, both of which are not trivial tasks. This advantage arises when independent decomposition directly yields WR and DZ subnetworks, thereby eliminating the need for translation. However, such favorable decompositions cannot be expected in every biochemical system, as they depend strongly on the network's structure and interconnections. Indeed, the runtime differences observed for the 2-Protein Gene Transcription Model, Insulin Signaling Pathway, and CRISPRi Model 1 contrast sharply with those for the Hybrid Histidine–Kinase Model, MAPK Model, and Zigzag Model, where CRITERIA significantly outperformed COMPILES.

The improvements in the computational runtime are largely attributable to the integration of the EFM-based network translation, which removes a key bottleneck in COMPILES. Compared with the earlier approach, this translation strategy is more structured and systematic, leading to enhanced numerical stability and improved scalability. Furthermore, the observations above suggest that the improved ordering of steps in CRITERIA alleviates the computational burden arising from handling subnetworks separately, thereby improving the parametrization stage of the procedure. As a result, the interdependencies that previously appeared in the derived equilibrium expressions are effectively resolved. Taken together, the results indicate that CRITERIA is not merely a computational enhancement but also a conceptual extension of the original framework. It broadens the range of networks for which analytic equilibria can be derived while maintaining practical efficiency, making it a promising tool for the systematic analysis of increasingly large and structurally complex biochemical reaction networks.

Finally, we demonstrate the utility of having a closed form equilibrium expression for analyzing key dynamical properties of biochemical systems. Using CRITERIA, we were able to easily obtain the analytic equilibria of the EnvZ-OmpR model, which COMPILES was not able to do within the prescribed maximum runtime. Using the system's conservation laws, we showed that the model has the capacity to admit multiple positive steady states. In addition to exhibiting ACR, as previously established in [19,22], our analysis provides further insight that the system admits two positive equilibria or none at all. As a second application, we easily obtained an equilibrium parametrization of the full CRISPRi toggle switch model, which COMPILES also failed to compute within the prescribed maximum runtime. Our analysis reveals that the dCas9 species exhibits ACR, indicating that its concentration is invariant across all positive equilibria and independent of the initial conditions. This robustness property suggests a potential buffering mechanism in CRISPRi-based regulatory circuits, by which the availability of dCas9 is maintained despite fluctuations elsewhere in the network. From a design perspective, this feature may be advantageous in synthetic biology settings, where predictable regulator levels are essential for reliable circuit performance.

These applications demonstrate how explicit equilibrium parametrizations enable a more general analysis of biochemical systems beyond simulation-based methods. In particular, closed-form equilibrium expressions make analysis of models possible without committing to specific parameter values, thereby separating intrinsic dynamical features from numerical artifacts. By enabling analytic equilibrium parametrizations for broader classes of biochemical reaction networks, CRITERIA opens the door to parameter-free analyses of complex models, allowing one to characterize multistationarity, robustness, and other qualitative dynamical behavior directly from network structure. This capability not

only reduces dependence on uncertain or experimentally inaccessible parameter estimates but also enables systematic structural comparisons between models and reveals general mechanistic principles underlying their dynamical behavior. Looking ahead, this study presents several avenues for future work.

1. The translation method is currently applicable only to reaction networks whose set of EFMs are unitary (see Remark of Theorem 3 in the Methods section). Future work will focus on adapting the translation procedure to account for models whose EFMs are not unitary. A promising avenue is to investigate the effect of network decomposition on the EFMs of a reaction network. In particular, it would be of interest to characterize the conditions under which network decomposition transforms a non-unitary set of EFMs into a unitary one.
2. A promising direction for future work is to assess multistationarity using Sturm's Theorem, which is a result in algebraic geometry [7]. This approach follows a systematic sequence of steps that ultimately yields a polynomial in the species concentrations, with coefficients depending on the system's parameters. By analyzing the sign structure of this polynomial, one can infer the existence or absence of multiple equilibria as a function of both parameters and variables. A key prerequisite for this method is an equilibrium parametrization, which our current framework already provides. As a next step, we plan to automate this method by integrating it with the equilibrium parametrization generated by our approach.
3. Another important direction for further study is the automation of boundary equilibrium analysis, where the equilibrium value of one or more species is zero. This approach would extend the framework to include biologically relevant cases such as extinction states or other switch-like behaviors, offering a more comprehensive understanding of the dynamics of a system.
4. Future work may also focus on developing a user-friendly interface or application that implements the enhanced computational framework proposed in this study, and hence extends its accessibility beyond the current MATLAB environment. In addition, this could be expanded to incorporate other analyses and computational methods in CRN theory that are not yet available in existing applications. This would provide a more comprehensive platform that researchers in the field of applied mathematics, biochemistry, and systems biology can readily use.

More broadly, building on the current framework could support large-scale, structure-driven analyses of complex biochemical networks, unlocking new opportunities to translate theoretical insights from reaction network theory into practical tools for systems and synthetic biology.

Methods

Chemical reaction networks

A *chemical reaction network* (CRN) is defined by three sets [24]. The first set is the set of species $\mathcal{S} = \{X_1, ..., X_m\}$, which contains the fundamental units of the network called *species*. The second set is the set of complexes $\mathcal{C} = \{y_1, ..., y_n\}$, which contains entities called *complexes* that are linear combinations of species. That is,

$$y_i = \sum_{j=1}^{m} y_{ij} X_j, \quad i = 1, ..., n.$$

The coefficients $y_{ij} \in \mathbb{Z}_{\geq 0}$ are called stoichiometric coefficients. We use y_i as both the complex itself and the corresponding complex vector $y_i = (y_{i1}, ..., y_{im}) \in \mathbb{Z}^m_{\geq 0}$. And lastly, the third set is the set of reactions $\mathcal{R} = \{r_1, ..., r_r\} \subset \mathcal{C} \times \mathcal{C}$.

The structure of a CRN can be seen as a directed graph where the edges are the reactions, and the vertices are the complexes. So, we can represent reactions as ordered pairs of complexes (i.e., $r_k = (y_i, y_j)$) or as directed edges (i.e., $r_k = y_i \to y_j$). Here, we call y_i the *reactant* or *source complex* and y_j the *product complex*. A weight $k_i \in \mathbb{R}_{\geq 0}$ is

associated to each reaction called the *reaction rate coefficient*. For each reaction $r_k = y_i \to y_j$, we can associate a *reaction vector* $y_j - y_i \in \mathbb{Z}^m$ which serves as the columns of the *stoichiometric matrix N* of the CRN.

Example 1. *Consider the following toy CRN with five reactions:*

$$R_1 : A + B \xrightarrow{k_1} C + B \qquad R_2, R_3 : A \underset{k_3}{\overset{k_2}{\rightleftharpoons}} B \qquad R_4 : C \xrightarrow{k_4} 0 \qquad R_5 : 0 \xrightarrow{k_5} A$$

In this CRN, R_1 represents the conversion of A to C in the presence of B, with B effectively acting as a catalyst for the reaction. The reversible reactions R_2 and R_3 can be interpreted as conversion of A to B, and vice versa. Reaction R_4 models the degradation or removal of C from the system, while R_5 represents the inflow or external production of A. The network consists of three species, six complexes, and five reactions. In particular,

$$\mathcal{S} = \{A, B, C\},$$
$$\mathcal{C} = \{A + B, C + B, C, A, B, 0\},$$
$$\mathcal{R} = \{A + B \to C + B, A \to B, B \to A, C \to 0, 0 \to C\}.$$

The zero complex is not considered as a species; rather, it is a formal placeholder that represents the external environment of the system, where it acts as a "sink" or "source" of species and complexes. In $R_1 : A + B \to C + B$, A+B acts as the source complex and C+B acts as the product complex. Furthermore, the following are the reaction vectors of the CRN:

$$R_1 : (C + B) - (A + B) = C - A = [-1 \;\; 0 \;\; 1]^T \qquad R_4 : (0) - (C) = -C = [0 \;\; 0 \;\; -1]^T$$
$$R_2 : (B) - (A) = B - A = [-1 \;\; 1 \;\; 0]^T \qquad R_5 : (A) - (0) = A = [1 \;\; 0 \;\; 0]^T$$
$$R_3 : (A) - (B) = A - B = [1 \;\; -1 \;\; 0]^T$$

These reaction vectors comprise the columns of the stoichiometric matrix, which can be written as

$$N = \begin{array}{c} \begin{matrix} R_1 & R_2 & R_3 & R_4 & R_5 \end{matrix} \\ \begin{bmatrix} -1 & -1 & 1 & 0 & 1 \\ 0 & 1 & -1 & 0 & 0 \\ 1 & 0 & 0 & -1 & 0 \end{bmatrix} \end{array} \begin{matrix} A \\ B \\ C \end{matrix}$$

whose rank is given by s = 3.

Since a CRN can be seen as a directed graph, we can draw parallels between concepts in graph theory and CRN theory. In graph theory, we say that two vertices are connected if there is a directed path between them. Furthermore, two vertices are strongly connected if there is a directed path to and from each other. Given these definitions, we call the connected components of the network the *linkage classes* of the CRN and the strongly connected components the *strong linkage classes* of the CRN. In other words, a linkage class is a set of complexes that are connected to one another but not to any other complex not in the set, whereas a strong linkage class consists of complexes that are strongly connected to each other. If the number of linkage classes is equal to the number of strong linkage classes, then the CRN is said to be *weakly reversible*. Alternatively, a CRN is said to be *weakly reversible* if every reaction is part of a directed cycle.

Example 2. *In Example 1, there are two linkage classes, namely {A+B,C+B} and {A,B,C,0}. On the other hand, there are five strong linkage classes which are the following: {A,B}, {C+B}, {A+B}, {C}, {0}. Note that a complex, if not strongly*

connected to other complex/es, is a trivial strong linkage class. Since the number of linkage classes does not coincide with the number of strong linkage classes, the CRN is not weakly reversible. Alternatively, this can be seen from the fact that reaction R_1 is not contained in any directed cycle, and hence the network fails to be weakly reversible.

One of the central concepts in CRN theory is called the *deficiency* of the CRN which is denoted by δ. It is a nonnegative integer with formula $\delta = n - l - s$, where n is the number of complexes, l is the number of linkage classes of the CRN, and s is the rank of the stoichiometric matrix. It can be interpreted as a measure of the linear dependence among the reactions of the reaction network. The higher the deficiency, the *l*ower the extent of linear independence [20].

Example 3. *In Example 1, we have $\delta = 6 - 2 - 3 = 1$. So, it is a deficiency one network.*

A CRN is endowed by kinetics to describe the evolution of the concentration of the species over time. One of the widely-used kinetics is *mass-action* which states that the rate at which the reaction proceeds is proportional to the product of the concentration of the species in its reactant complex.

Example 4. *In our running example, suppose a, b, and c denote the concentrations of species A, B, and C, respectively. Then, the rate function of each reaction under the assumption of mass-action is given by*

$$R_1 : k_1 ab \quad R_2 : k_2 a \quad R_3 : k_3 b \quad R_4 : k_4 c \quad R_5 : k_5.$$

Together with N, the system of ordinary differential equations for the model is given by

$$\begin{bmatrix} \frac{da}{dt} \\ \frac{db}{dt} \\ \frac{dc}{dt} \end{bmatrix} = k_1 ab \begin{bmatrix} -1 \\ 0 \\ 1 \end{bmatrix} + k_2 a \begin{bmatrix} -1 \\ 1 \\ 0 \end{bmatrix} + k_3 b \begin{bmatrix} 1 \\ -1 \\ 0 \end{bmatrix} + k_4 c \begin{bmatrix} 0 \\ 0 \\ -1 \end{bmatrix} + k_5 \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix},$$

where $\frac{da}{dt}$, $\frac{db}{dt}$, and $\frac{dc}{dt}$ are the time derivatives of the concentration functions of the species A, B, and C, respectively. The system of ODEs can be written as

$$\begin{aligned} \frac{da}{dt} &= -k_1 ab - k_2 a + k_3 b + k_5 \\ \frac{db}{dt} &= k_2 a - k_3 b \\ \frac{dc}{dt} &= k_1 ab - k_4 c \end{aligned} \cdot$$

The long-term behavior of a system is described by the *equilibrium* or *steady states*, which can be solved by setting the time derivatives to zero and then solving for the concentration of the species.

Example 5. *The positive equilibrium of the network in Example 1 is given by*

$$(a, b, c) = \left(\sqrt{\frac{k_3 k_5}{k_1 k_2}}, \sqrt{\frac{k_2 k_5}{k_1 k_3}}, \frac{k_5}{k_4} \right),$$

where $k_1, k_2, k_3, k_4, k_5 > 0$.

Network decomposition

A *decomposition* of a CRN is induced by a partition of its reaction set $\mathcal{R}$ [2]. This partition then gives rise to a decomposition of the reaction network into subnetworks. One special type of network decomposition is called *independent decomposition*, where the rank of the stoichiometric matrix of the whole network is equal to the sum of the ranks of the stoichiometric matrices of its subnetworks [24].

Example 6. *In Example 1, the following partition of the reaction set $\mathcal{R}$ into*

$$R_1 : \{R_1, R_4, R_5\} \quad \text{and} \quad R_2 : \{R_2, R_3\}$$

induces a decomposition of the CRN into subnetworks $\mathcal{N}_1$ and $\mathcal{N}_2$ with stoichiometric matrices

$$\begin{array}{c} \\ A \\ B \\ C \end{array}\!\!\begin{array}{c} \begin{array}{ccc} R_1 & R_4 & R_5 \end{array} \\ \begin{bmatrix} -1 & 0 & 1 \\ 0 & 0 & 0 \\ 1 & -1 & 0 \end{bmatrix} \end{array} \quad \textit{and} \quad \begin{array}{c} \\ A \\ B \\ C \end{array}\!\!\begin{array}{c} \begin{array}{cc} R_2 & R_3 \end{array} \\ \begin{bmatrix} -1 & 1 \\ 1 & -1 \\ 0 & 0 \end{bmatrix} \end{array},$$

respectively. It can easily be verified that $s_1 = 2$ and $s_2 = 1$. Since we have equality $s = 3 = 2 + 1 = s_1 + s_2$, the decomposition is independent.

A method was developed in [27] to obtain an independent decomposition of a reaction network that employs a coordinate graph to find the desired decomposition. It was then proven that the obtained independent decomposition in [27] is already the finest (i.e., the independent decomposition with the maximum number of subnetworks) [28].

A key feature of independent decomposition is that it allows us to compute the positive steady states of the entire network by first deriving the positive steady states of each independent subnetwork and then intersecting them. This is formalized in the following theorem which is taken from [24].

Theorem 1. *Let $\mathcal{N}$ be a reaction network with kinetics $\mathcal{K}$ decomposed into subnetworks $\mathcal{N}_1, \mathcal{N}_2, ..., \mathcal{N}_\alpha$ and $\mathcal{K}_i$ be the restriction of $\mathcal{K}$ to reactions in $\mathcal{N}_i$. Then*

$$E_1 \cap E_2 \cap \cdots \cap E_\alpha \subseteq E$$

where E is the set of positive steady states of the whole network while E_i is the set of positive steady states of subnetwork $\mathcal{N}_i$. Furthermore, if the network decomposition is independent, then equality holds, i.e.,

$$E_1 \cap E_2 \cap \cdots \cap E_\alpha = E.$$

Network translation

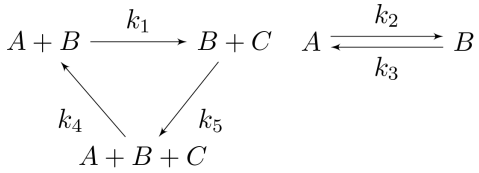
The parametrization method in [22] requires a CRN to be weakly reversible and deficiency zero. When these conditions are not satisfied, the reaction network must be transformed to obtain a network with the desired structural properties. This is done by the process of *network translation*, first introduced in [18], which modifies the graphical properties of the CRN but preserves the reaction vectors, and allows the original source complex to determine the kinetics so that the dynamics of the system is preserved.

Network translation can be visualized by the operation of adding or subtracting linear combinations of species, known as *translation complexes*, from individual reactions [5]. Formally, we let $\alpha_k \in \mathbb{Z}^m_{\geq 0}$ to be a translation complex for reaction k. We represent the operation of translating the reaction $r_k = y_i \to y_j \in \mathcal{R}$ by the translation complex α_k as

$$y_i \to y_j \qquad (+\alpha_k) \tag{8}$$

for $k = 1, ..., r$. Here, we add the translation complex to both reactant and product complexes to produce the translated reaction.

Example 7. *Going back to Example 1, translating R_4 by $\alpha_4 = A + B$ and R_5 by $\alpha_5 = B + C$ gives us the following translated network:*



Clearly, the translated network is weakly reversible. Furthermore, it can be verified that its deficiency is equal to zero, which is not the case for the original network.

The translation scheme in (8) produces a new set of complexes, containing $y_i + \alpha_k$ and $y_j + \alpha_k$, which are called *stoichiometric complexes* in the translated network. Since the original source complex determines the kinetics of the translated network, another set of complexes is produced called the *kinetic complexes*. Therefore, the translated network can be thought of in terms of a generalized chemical reaction network (GCRN).

Generalized chemical reaction networks

In this subsection, we formally define the concept of a GCRN, which was pioneered by Müller and Regensburger [17], and show how this is useful in deriving the analytic equilibria of a reaction network.

Let $G=(V,E)$ be a directed graph with vertex set V and edge set $E \subset V \times V$. Furthermore, denote $V_s = \{i | i \to j \in E\}$ to be the set of all source vertices in G. A GCRN is a directed graph $G=(V,E)$ with two maps

- $y : V \to \mathbb{R}^m_{\geq 0}$ that assigns to each vertex a stoichiometric complex; and
- $\tilde{y} : V_s \to \mathbb{R}^m_{\geq 0}$ that assigns to each vertex a kinetic complex.

Given a CRN with an associated graph G, we can construct a GCRN with graph G' via network translation such that the resulting system of ODE matches that of the original CRN. We can do this by allowing the kinetic complex to determine the reaction rate while having the stoichiometric complex determine the reaction vector. Doing this for all reactions, we are assured to get the same ODE system since the original reaction rates and stoichiometric matrix are preserved. In such case, the CRN and the GCRN are said to be *dynamically equivalent*. The two maps y and $\tilde{y}$ give rise to two associated CRNs for a GCRN: the *stoichiometric CRN* (G', y) and the *kinetic-order CRN* $(G', \tilde{y})$. Consequently, we can define deficiencies for both the stoichiometric CRN and kinetic-order CRN, which are called *effective deficiency* δ and *kinetic deficiency* $\tilde{\delta}$, respectively. Both are calculated with the same formula $n - l - s$ but using the CRN associated with them.

There are two kinds of edges in a GCRN: phantom edge and effective edge. A *phantom edge* connects identical stoichiometric complexes in the GCRN. Otherwise, it is an *effective edge*. A phantom edge does not contribute to the system of ODEs since we would get 0 as its corresponding reaction vector. Thus, we assign a dummy reaction rate constant σ, which is considered as a free parameter, for phantom edges. Lastly, we denote the sets of phantom edges and effective edges of the GCRN by E^0 and E^*, respectively.

Given these, the main parametrization method is given in the theorem below [2,22].

Theorem 2. *Consider a weakly reversible translated network, which is a GCRN. Let $\mathcal{F}$ be any spanning forest containing all the nodes of the kinetic-order CRN. For each edge of $\mathcal{F}$, we define the kinetic difference as the vector produced by subtracting the head kinetic complex by the tail kinetic complex. Furthermore, M is the matrix containing all the kinetic differences as rows where the entries per row are arranged according to the order of species. Let H be a generalized inverse of M (i.e., MHM = M). Finally, define B and C such that im(B) = ker(M) and ker(B) = {0}, and im(C) = ker$\left(M^T\right)$ and ker(C) = {0}.*

- *(Case 1) If the kinetic deficiency is zero, the set of parametrized complex-balanced equilibria is given by*

$$\bar{Z} = \left\{ \kappa \left(k^*, \sigma\right)^{H^T} \circ \tau^{B^T} | \sigma \in \mathbb{R}^{E^0}_{\geq 0}, \ \ \tau \in \mathbb{R}^{m-\tilde{s}}_{>0} \right\} \neq \emptyset$$

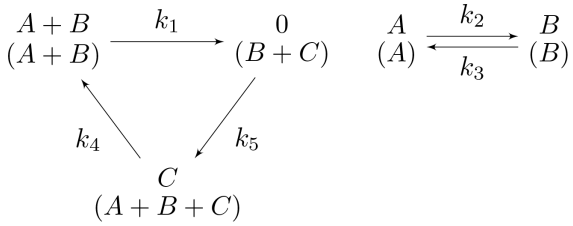
- *(Case 2) If the kinetic deficiency is positive, define the $\tilde{\delta}$ equations*

$$\kappa \left(k^*, k^0\right)^C = 1^{\tilde{\delta} \times 1}$$

and add them as additional conditions to the parametrization in Case 1.

In this formalization, the product $\kappa \left(k^*, \sigma\right)^{H^T} \circ \tau^{B^T}$ is the Hadamard product (the number of components of κ is the number of edges in a spanning forest, which can be effective of phantom) with the component of κ associated with the edge $i \to i'$ as $\kappa_{i \to i'} = \frac{K_{i'}}{K_i}$ and tree constant K_i as the sum (over all spanning trees of the kinetic-order CRN towards node i) of the products of the rate constants associated with the edges of each spanning tree. In addition, if the effective deficiency is zero, then the set of positive steady states of the original network is precisely $\bar{Z}$.

Example 8. *In Example 1 and its translation scheme in Example 7, we define the GCRN as follows*



Here, the complexes in parentheses are the stoichiometric complexes, while the complexes above them are the kinetic complexes. Determining the reaction rates of the GCRN, we have

$$R_1 : k_1 ab \quad R_2 : k_2 a \quad R_3 : k_3 b \quad R_4 : k_4 c \quad R_5 : k_5.$$

Moreover, the reaction vectors of the GCRN are the following:

$$R_1 : (B + C) - (A + B) = C - A = [-1 \ \ 0 \ \ 1]^T$$
$$R_2 : (B) - (A) = B - A = [-1 \ \ 1 \ \ 0]^T$$
$$R_3 : (A) - (B) = A - B = [1 \ \ -1 \ \ 0]^T$$
$$R_4 : (A + B) - (A + B + C) = -C = [0 \ \ 0 \ \ -1]^T$$
$$R_5 : (A + B + C) - (B + C) = A = [1 \ \ 0 \ \ 0]^T$$

It can be observed that the reaction vectors we have computed coincide with those obtained in Example 1, while the reaction rates agree exactly with those derived in Example 4. Hence, the original CRN and the GCRN in this example are dynamically equivalent. Finally, it can be verified that the GCRN has zero effective deficiency ($\delta = 0$) and zero kinetic deficiency ($\tilde{\delta} = 0$), so Case 1 of Theorem 2 applies.

Elementary flux modes

Johnston and Burton [5] developed a computational method for performing structural translation through an elementary flux mode-based approach. An *elementary flux mode* (EFM) represents a minimal, non-decomposable set of reactions that operate at steady state. In other words, EFMs are flux-balanced pathways that cannot be simplified in the sense that it is not possible to remove a subset of active reactions from an EFM and still be able to build a flux-balanced path using only the remaining active reactions [6].

Formally, a vector $e_i \in \mathbb{R}^r_{\geq 0}$ is an EFM of the CRN if $e_i \in \ker(N)$ and e_i is not a convex combination of any other $e_j, e_k \in \ker(N) \cap \mathbb{R}^r_{\geq 0}$. The set $\ker(N) \cap \mathbb{R}^r_{\geq 0}$ is the set of all admissible/feasible flux vectors, sometimes referred to as the *elementary flux cone* $\text{cone}(\mathcal{E})$, where $\mathcal{E}$ is the set of all EFMs of a CRN. We say that $\mathcal{E}$ is *unitary* if every entry in every $e_i \in \mathcal{E}$ is a one or a zero. Furthermore, $\mathcal{E}$ *covers* the reaction set $\mathcal{R}$ is $\text{cone}(\mathcal{E}) \cap \mathbb{R}^r_{>0} \neq \emptyset$ [5].

If the set of EFMs of a CRN is unitary and covers $\mathcal{R}$, then each EFM e_i is completely determined by its support, $\text{supp}(e_i)$, which identifies the subset of reactions active in that mode. As a result, it is sufficient to represent an EFM by the positions of its nonzero entries, allowing us to use the binary vector notation (i.e., $e_i = (1, 0, 1, 1, \ldots)$) and the corresponding reaction set notation (i.e., $e_i = \{R_1, R_3, R_4, \ldots\}$) interchangeably.

The EFMs can be interpreted as the sets of reactions that, if taken in any order, would result in no net gain or loss of any species. In other words, each is a flux path through the reaction network with the property that the net total flux into and out of all nodes/vertices balances. Furthermore, EFMs are nondecomposable flux vectors from which all possible flux vectors of the network can be completely described [6]. That is, the EFMs are the extremal generators of $\text{cone}(\mathcal{E})$.

Example 9. *Note that the stoichiometric matrices of the network in Example 1 and its weakly reversible, deficiency zero translation in Example 7 coincide since both networks have the same set of reaction vectors. Since EFMs are, by definition, elements of the null space of the stoichiometric matrix, the original and translated networks have the same set of EFMs. In particular, they have two EFMs, which are given by $e_1 = \{R_1, R_4, R_5\}$ and $e_2 = \{R_2, R_3\}$.*

Reaction-to-reaction graph

A *reaction-to-reaction graph* is a directed graph whose vertices correspond to the reactions of the CRN [5]. Directed cycles in the reaction-to-reaction graph are formed using the EFMs of the reaction network. Formally, a directed graph $G^{\mathcal{R}} = (V^{\mathcal{R}}, E^{\mathcal{R}})$ is a reaction-to-reaction graph of a CRN if $V^{\mathcal{R}} = \mathcal{R}$ and $E^{\mathcal{R}} \subset \mathcal{R} \times \mathcal{R}$.

It would be convenient to use the mappings $s, p : \text{supp}(\mathcal{R}) \mapsto \text{supp}(\mathcal{C})$ in the subsequent definitions, where s and p map the source and product of each reaction to its corresponding complex, respectively. That is, we may use the notation $r_k = y_{s(k)} \to y_{p(k)}$ instead of $r_k = y_i \to y_j$. Now, we say that the CRN and the reaction-to-reaction graph are:

- *product-to-source compatible* (PS-compatible) if, for any $r_i = y_{s(i)} \to y_{p(i)}$ *and* $r_j = y_{s(j)} \to y_{p(j)}$, $(r_i, r_j) \in E^{\mathcal{R}}$ *if and only if* $y_{p(i)} = y_{s(j)}$.
- *common source compatible* (CS-compatible) if $y_{s(i)} = y_{s(j)}$ *and* $(r_k, r_i) \in E^{\mathcal{R}}$ *implies* $(r_k, r_j) \in E^{\mathcal{R}}$
- *elementary flux mode compatible* (EM-compatible) if every EFM of the CRN corresponds to the vertices of a minimal directed cycle in the reaction-to-reaction graph, and vice versa

The theorem below relates the properties of PS-, CS-, and EM-compatibility to a weakly reversible, deficiency zero translation of a network. This is taken from [5].

Theorem 3. *Consider a CRN* $(\mathcal{S},\mathcal{C},\mathcal{R})$ *with a set of elementary flux modes* $\mathcal{E} = \{e_1, e_2, \ldots, e_p\}$ *which is unitary and covers* $\mathcal{R}$. *If there is a reaction-to-reaction graph* $G^{\mathcal{R}} = (V^{\mathcal{R}}, E^{\mathcal{R}})$ *which is CS- and EM-compatible to* $(\mathcal{S},\mathcal{C},\mathcal{R})$, *then there is a CRN* $(\mathcal{S},\mathcal{C}',\mathcal{R}')$ *which is PS- CS, and EM-compatible with* $G^{\mathcal{R}}$. *Furthermore,* $(\mathcal{S},\mathcal{C}',\mathcal{R}')$ *is a weakly reversible, zero deficiency structural translation of* $(\mathcal{S},\mathcal{C},\mathcal{R})$. *In particular, the translation complexes* $\alpha_i \in \mathbb{R}^m_{\geq 0}, i = 1, \ldots, r$, *required to produce such a translation satisfy the following linear system, which is necessarily consistent:*

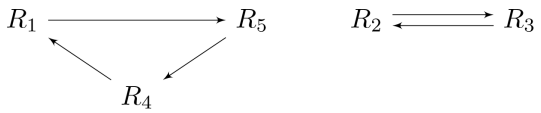
$$\alpha_i - \alpha_j = y_{s(j)} - y_{p(i)}, \qquad (r_i, r_j) \in E^{\mathcal{R}}. \tag{9}$$

Remarks

1. *It is important to note that Theorem 3 is only applicable when the set of EFMs of the reaction network is unitary. Consequently, CRITERIA cannot obtain an equilibrium parametrization of a CRN when its set of EFMs is non-unitary. The limitation of the method in [5] is inherited in CRITERIA.*
2. *As shown in Table 3, REWARDZ without network decomposition was unable to generate a valid translation for Model N because its set of EFMs is non-unitary. However, this limitation was overcome by first performing network decomposition, after which REWARDZ successfully obtained a valid translation.*

The goal then is to construct a reaction-to-reaction graph which are CS- and EM-compatible with a given CRN and then enforce PS-compatibility to construct a weakly reversible, deficiency zero translation via solving a linear system.

Example 10. *Consider the reaction-to-reaction graph below.*



Indeed, the EFMs computed in Example 9 given by $e_1 = \{R_1, R_4, R_5\}$ *and* $e_2 = \{R_2, R_3\}$ *correspond to minimal cycles in the reaction-to-reaction graph. Moreover, since there are no reactions with common source complex in the network in Example 1, then the CRN and the reaction-to-reaction graph are automatically CS-compatible. However, the reaction-to-reaction graph is not PS-compatible to the CRN. For instance, consider* $(R_5, R_4) \in E^{\mathcal{R}}$. We have $y_{p(5)} = A \neq C = y_{s(4)}$. *Nevertheless, the reaction-to-reaction graph is EM- and CS-compatible with the network in Example 1. Moreover, the set of EFMs* $\mathcal{E} = \{e_1, e_2\}$ *is unitary and covers* $\mathcal{R}$. *By Theorem, 3, there exists a weakly reversible, deficiency zero translation of the network. Indeed, such a translation was constructed in Example 7.*

Supporting information

S1 Text. Supplementary Methods, Table, and Figures.
(PDF)

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References

1. Allen L. An introduction to mathematical biology. Pearson/Prentice Hall; 2007.
2. Hernandez BS, Lubenia PVN, Johnston MD, Kim JK. A framework for deriving analytic steady states of biochemical reaction networks. PLoS Comput Biol. 2023;19(4):e1011039. https://doi.org/10.1371/journal.pcbi.1011039 PMID: 37053305
3. Pérez Millán M, Dickenstein A, Shiu A, Conradi C. Chemical reaction systems with toric steady states. Bull Math Biol. 2012;74(5):1027–65. https://doi.org/10.1007/s11538-011-9685-x PMID: 21989565
4. Thomson M, Gunawardena J. The rational parameterization theorem for multisite post-translational modification systems. J Theor Biol. 2009;261(4):626–36. https://doi.org/10.1016/j.jtbi.2009.09.003 PMID: 19765594
5. Johnston MD, Burton E. Computing Weakly Reversible Deficiency Zero Network Translations Using Elementary Flux Modes. Bull Math Biol. 2019;81(5):1613–44. https://doi.org/10.1007/s11538-019-00579-z PMID: 30790189
6. Klapper I, Szyld DB, Zhao K. Metabolic networks, elementary flux modes, and polyhedral cones. Society for Industrial and Applied Mathematics; 2021.
7. Siegal-Gaskins D, Franco E, Zhou T, Murray RM. An analytical approach to bistable biological circuit discrimination using real algebraic geometry. J R Soc Interface. 2015;12(108):20150288. https://doi.org/10.1098/rsif.2015.0288 PMID: 26109633
8. Peter S, Hölzer M, Lamkiewicz K, di Fenizio PS, Al Hwaeer H, Marz M, et al. Structure and Hierarchy of Influenza Virus Models Revealed by Reaction Network Analysis. Viruses. 2019;11(5):449. https://doi.org/10.3390/v11050449 PMID: 31100972
9. Shinar G, Rabinowitz J, Alon U. Robustness in glyoxylate bypass regulation. PLOS Comp Biol. 2009;5(3):e1000297.
10. Villareal KAM, Hernandez BS, Lubenia PVN. Derivation of Steady State Parametrizations of Chemical Reaction Networks with n Independent and Identical Subnetworks. MATCH Commun Math Comput Chem. 2023;91(2):337–65. https://doi.org/10.46793/match.91-2.337v
11. Feliu E, Wiuf C. Variable elimination in post-translational modification reaction networks with mass-action kinetics. J Math Biol. 2013;66(1–2):281–310. https://doi.org/10.1007/s00285-012-0510-4 PMID: 22311196
12. Obatake N, Shiu A, Tang X, Torres A. Oscillations and bistability in a model of ERK regulation. J Math Biol. 2019;79(4):1515–49. https://doi.org/10.1007/s00285-019-01402-y PMID: 31346693
13. Karp R, Perez Millan M, Dasgupta T, Dickenstein A, Gunawardena J. Complex-linear invariants of biochemical networks. J Theor Biol. 2012;311:130–8.
14. Batchelor E, Goulian M. Robustness and the cycle of phosphorylation and dephosphorylation in a two-component regulatory system. Proc Natl Acad Sci U S A. 2003;100(2):691–6. https://doi.org/10.1073/pnas.0234782100 PMID: 12522261
15. Price ND, Shmulevich I. Biochemical and statistical network models for systems biology. Curr Opin Biotechnol. 2007;18(4):365–70. https://doi.org/10.1016/j.copbio.2007.07.009 PMID: 17681779
16. Horn F, Jackson R. General mass action kinetics. Arch Ration Mech Anal. 1972;47:81–116.
17. Müller S, Regensburger G. Generalized Mass Action Systems: Complex Balancing Equilibria and Sign Vectors of the Stoichiometric and Kinetic-Order Subspaces. SIAM J Appl Math. 2012;72(6):1926–47. https://doi.org/10.1137/110847056
18. Johnston MD. Translated chemical reaction networks. Bull Math Biol. 2014;76(5):1081–116. https://doi.org/10.1007/s11538-014-9947-5 PMID: 24610094
19. Shinar G, Feinberg M. Structural sources of robustness in biochemical reaction networks. Science. 2010;327(5971):1389–91. https://doi.org/10.1126/science.1183372 PMID: 20223989
20. Shinar G, Feinberg M. Design principles for robust biochemical reaction networks: what works, what cannot work, and what might almost work. Math Biosci. 2011;231(1):39–48. https://doi.org/10.1016/j.mbs.2011.02.012 PMID: 21377478
21. Conradi C, Feliu E, Mincheva M, Wiuf C. Identifying parameter regions for multistationarity. PLoS Comput Biol. 2017;13(10):e1005751. https://doi.org/10.1371/journal.pcbi.1005751 PMID: 28972969
22. Johnston MD, Müller S, Pantea C. A Deficiency-Based Approach to Parametrizing Positive Equilibria of Biochemical Reaction Systems. Bull Math Biol. 2019;81(4):1143–72. https://doi.org/10.1007/s11538-018-00562-0 PMID: 30599071
23. Hong H, Hernandez BS, Kim J, Kim JK. Computational Translation Framework Identifies Biochemical Reaction Networks with Special Topologies and Their Long-Term Dynamics. SIAM J Appl Math. 2023;83(3):1025–48. https://doi.org/10.1137/22m150469x
24. Feinberg M. Foundations of Chemical Reaction Network Theory. Springer International Publishing; 2019.
25. Santos-Moreno J, Tasiudi E, Stelling J, Schaerli Y. Multistable and dynamic CRISPRi-based synthetic circuits. Nat Commun. 2020;11(1):2746. https://doi.org/10.1038/s41467-020-16574-1 PMID: 32488086

26. Lubenia PVN, Mendoza ER, Lao AR. Reaction Network Analysis of Metabolic Insulin Signaling. Bull Math Biol. 2022;84(11):129. https://doi.org/10.1007/s11538-022-01087-3 PMID: 36168001
27. Hernandez BS, De la Cruz RJL. Independent Decompositions of Chemical Reaction Networks. Bull Math Biol. 2021;83(7):76. https://doi.org/10.1007/s11538-021-00906-3 PMID: 34008093
28. Hernandez B, Amistas D, De la Cruz RJ, Fontanil L, de los Reyes A, Mendoza E. Independent, Incidence Independent and Weakly Reversible Decompositions of Chemical Reaction Networks. MATCH Commun Math Comput Chem. 2021;87(2):367–96. https://doi.org/10.46793/match.87-2.367h
29. Feliu E, Wiuf C. Variable Elimination in Chemical Reaction Networks with Mass-Action Kinetics. SIAM J Appl Math. 2012;72(4):959–81. https://doi.org/10.1137/110847305
30. Mahdi A, Ferragut A, Valls C, Wiuf C. Conservation Laws in Biochemical Reaction Networks. SIAM J Appl Math. 2017;16(4):2213–32. https://doi.org/10.1137/17m1138418
31. Gatermann K. Counting stable solutions of sparse polynomial systems in chemistry. In: Green EL, Hosten S, Laubenbacher RC, Powers VA, editors. Symbolic Computation: Solving Equations in Algebra, Geometry and Engineering. Contemporary Mathematics, vol. 286. 2001. p. 53–69.
32. Gatermann K, Wolfrum M. Bernstein's second theorem and Viro's method for sparse polynomial systems in chemistry. Adv Appl Math. 2005;34(2):252–94. https://doi.org/10.1016/j.aam.2004.04.003
33. Gatermann K, Huber B. A Family of Sparse Polynomial Systems Arising in Chemical Reaction Systems. J Symbolic Computat. 2002;33(3):275–305. https://doi.org/10.1006/jsco.2001.0512
34. Thomson M, Gunawardena J. Unlimited multistability in multisite phosphorylation systems. Nature. 2009;460(7252):274–7. https://doi.org/10.1038/nature08102 PMID: 19536158