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A computational method to preclude multistationarity in networks of interacting species

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ABSTRACT

Motivation: Modeling and analysis of complex systems are important aspects of understanding systemic behavior. In the lack of detailed knowledge about a system, we often choose modeling equations out of convenience and search the (high-dimensional) parameter space randomly to learn about model properties. Qualitative modeling sidesteps the issue of choosing specific modeling equations and frees the inference from specific properties of the equations. We consider classes of ordinary differential equation (ODE) models arising from interactions of species/entities, such as (bio)chemical reaction networks or ecosystems. A class is defined by imposing mild assumptions on the interaction rates. In this framework, we investigate whether there can be multiple positive steady states in some ODE models in a given class.

Results: We have developed and implemented a method to decide whether any ODE model in a given class cannot have multiple steady states. The method runs efficiently on models of moderate size. We tested the method on a large set of models for gene silencing by sRNA interference and on two publicly available databases of biological models, KEGG and Biomodels. We recommend that this method is used as (i) a pre-screening step for selecting an appropriate model and (ii) for investigating the robustness of non-existence of multiple steady state for a given ODE model with respect to variation in interaction rates.

Availability and Implementation: Scripts and examples in Maple are available in the Supplementary Information.

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Supplementary information: Supplementary data are available at *Bioinformatics* online.

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1 INTRODUCTION

Systems of interacting species are ubiquitous in many areas of science, from biology and ecology to epidemiology and sociology (Anderson and May, 1991; May, 1974; Murray, 2002). The dynamics of a system are typically specified by a system of ordinary differential equations (ODEs), potentially depending on many (unknown) parameters. The variables of the system are the concentrations (or abundances) of species, such as chemical or molecular species in systems biology, animal species in ecology or infected and susceptible individuals in epidemiology.

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Specifying the modeling equations is rarely trivial. For example, in systems biology, the species concentrations change according to the molecular reactions that take place, but the precise reaction mechanisms and rates are typically unknown. For example, choosing between mass-action, power-law or Hill-type kinetics might be a matter of tradition or convenience rather than biological knowledge. Mass-action kinetics has a simpler functional form than the other two kinetics and only one parameter. These, on the other hand, are more adaptable to systems in biologically constrained environments, such as cellular systems. The choice of kinetics might thus affect the biological validity of a conclusion derived from the ODEs. This remains true even if we are comfortable with a particular system of ODEs: the parameter space is generally high-dimensional and it is standard to explore it numerically or by choosing parameters randomly. However, this is difficult to do efficiently when the number of parameters is large. Consequently, we can only investigate a small part of the parameter space.

These concerns have lead to an increased interest in *qualitative properties* (Atay and Jost, 2011; Silk *et al.*, 2011). In the context of this article, qualitative properties refer to properties of dynamical systems with a common underlying structure. We focus on a particular qualitative property, namely the *capacity for multiple steady states*, or multistationarity. The common structure is defined by an *interaction network* (defined in Section 2.1). Multistationarity underlies the emergence of hysteresis and switch-like behavior, that is, the transformation of a gradual input into a steep change in the response. It plays an important role in understanding systemic behavior (Markevich *et al.*, 2004; May, 1974; Murray, 2002).

We have developed and implemented a computationally simple and efficient criterion to determine if a class of dynamical systems, compatible with the same underlying interaction network, cannot have multiple steady states. The reaction (interaction) rates are constrained by how they vary with the species concentrations. The criterion can be refined to preclude multistationarity for particular classes of kinetics such as *mass-action kinetics* and, more generally, *power-law kinetics*. Both of these are used widely outside systems biology and biochemistry, but without the biochemical labeling, e.g. Wiuf and Feliu (2013). The method is well suited to screen large sets of networks for the possible emergence of switch behavior. The applicability of our approach is demonstrated by studying a series of small motifs in gene silencing by RNA interference and by analysis of 408 models from the KEGG (Kanehisa and Goto, 2000) and Biomodels databases (Li *et al.*, 2010). In the

discussion we relate our method to other methods to preclude multistationarity.

2 METHODS

2.1 Interaction networks

We define a qualitative model as an interaction network together with a class of associated ODE systems. Interaction networks are qualitative representations of how species interact and influence reaction rates. An interaction network is a bipartite signed graph consisting of a set of *species* nodes $\{S_1, \ldots, S_n\}$ and a set of *reaction* nodes $\{r_1, \ldots, r_m\}$. Reactions are biochemical reactions between species nodes:

$$r_u : \sum_{i=1}^{n} \alpha_{i,u} S_i \to \sum_{i=1}^{n} \beta_{i,u} S_i, \tag{1}$$

where $\alpha_{i,u}, \beta_{i,u}$ are positive integers or zero. Edges are undirected and between species and reactions. A positive (resp. negative) edge indicates that the species has positive (resp. negative) influence on the rate of the reaction, that is, the rate increases (resp. decreases) with the concentration of the species. The absence of an edge implies that the reaction takes place independently of the presence of the species.

As an example, consider the simple reversible Michaelis–Menten mechanism for activation of a substrate S:

$$S + E \rightleftarrows X \rightleftarrows S^* + E, \tag{2}$$

where E is an enzyme, S^* the activated substrate and X an intermediate complex. In mass-action kinetics, the reactant species, and only those, influence positively the rate of a reaction. With this assumption, the interaction network corresponding to the Michaelis–Menten mechanism is given in Figure 1.

2.2 Compatible dynamical systems

Let c_i be the concentration of species S_i and $c = (c_1, \ldots, c_n)$. To each reaction r_u, we associate a *rate function* $K_u(c)$ defined on a set Ω_u that includes $\mathbb{R}^n_+$ (all points with $c_i > 0$). A rate function K_u is *compatible with the influences* if it fulfills a monotonicity requirement:

- K_u is increasing in c_i if there is a positive edge (S_i, r_u).
- K_u is decreasing in c_i if there is a negative edge (S_i, r_u).
- K_u is constant in c_i if there is no edge between S_i and r_u.

If there is a positive edge between S_i and r_u, we additionally require that $K_u(c) = 0$ whenever $c_i = 0$, that is, the reaction only takes place in the presence of the species with positive influence.

Let K_u, $u = 1, \ldots, m$, be rate functions compatible with the influences. A dynamical system compatible with the interaction network is given by

$$\dot{c}_i = \sum_{u=1}^{m} (\beta_{i,u} - \alpha_{i,u}) K_u(c_1, \ldots, c_n), \tag{3}$$

for $i = 1, \ldots, n$. The rate of change in c_i is a weighted sum of the rate functions involving species S_i. The weight $\beta_{i,u} - \alpha_{i,u}$, potentially zero, of reaction r_u is the net production of S_i in that reaction.

For example, consider the Michalis–Menten mechanism in Figure 1 with the species ordered as S, S^*, E, X, and the reactions ordered as $S + E \to X$, $X \to S + E$, $X \to S^* + E$, $S^* + E \to X$. The concentration of a species Y is denoted by the same letter in lowercase, y. Then any model, qualitatively identical to a mass-action model, has the form

$$\dot{s} = K_2(c) - K_1(c), \quad \dot{e} = K_2(c) + K_3(c) - K_1(c) - K_4(c),$$

$$\dot{s}^* = K_3(c) - K_4(c), \quad \dot{x} = K_1(c) + K_4(c) - K_2(c) - K_3(c),$$

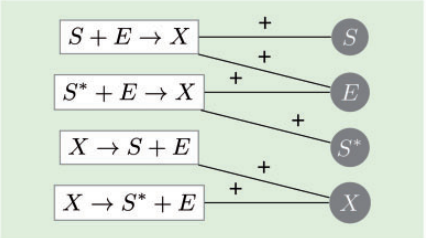

Fig. 1. The Michaelis–Menten mechanism in the form of an interaction network. The model consists of a network of reactions together with a qualitative specification of influences. Reactions are shown as squared nodes and species as round nodes. + indicates a positive edge

where $c = (s, s^*, e, x)$ and K_2, K_3 are increasing in x, K_1 is increasing in s and e and K_4 is increasing in s^* and e.

2.3 Conservation laws

Linear combinations of species concentrations may be preserved over time. For example in (2), the sums $E_{\text{tot}} = e + x$ and $S_{\text{tot}} = s + s^* + x$ are constant. If these are determined by the reactions (1) alone, that is, they are independent of the choice of influences and rate functions, then they are said to be *conservation laws*.

For each reaction r_u, consider the vector v_u in $\mathbb{R}^n$ with i-th component $\beta_{i,u} - \alpha_{i,u}$. Then the *stoichiometric matrix*, A, is the $n \times m$ matrix with columns v_u, $u = 1, \ldots, m$. The rank l of A is the dimension of the stoichiometric space. Any vector w that lies in the left kernel of A, that is, fulfills $wA = 0$ is a conservation law and $w \cdot (c_1, \ldots, c_n) = \sum_{i=1}^{n} w_i c_i$ is independent of time. Therefore, a set of linearly independent conservation laws is obtained by choosing a basis $\{w^1, \ldots, w^d\}$ of the left kernel of A. Because A has rank l, there are $d = n - l$ linearly independent conservation laws. Given *total amounts* $A^1_{\text{tot}}, \ldots, A^d_{\text{tot}}$ in $\mathbb{R}$, the associated *stoichiometric class* is defined by

$$\{c \in \mathbb{R}^n \mid \omega^j \cdot c = A^j_{\text{tot}}, \text{ for all } j = 1, \ldots, d\}. \tag{4}$$

The dynamics takes place in a fixed stoichiometric class determined by the initial concentrations of the system.

In example (2), the stoichiometric matrix is

$$A = \begin{pmatrix} -1 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 \\ -1 & 1 & 1 & -1 \\ 1 & -1 & -1 & 1 \end{pmatrix}.$$

A basis of the left kernel of A is given by the vectors $\omega^1 = (1, 1, 0, 1)$ and $\omega^2 = (0, 0, 1, 1)$, which give the equations $\omega^1 \cdot c = s + s^* + x$ and $\omega^2 \cdot c = e + x$.

2.4 Injectivity and multistationarity

An interaction network is said to have the *capacity for multiple positive steady states* if there is a compatible dynamical system (3) that has more than one positive steady state in some stoichiometric class. In other words, it has the capacity for multiple positive steady states if there exist rate functions K_u, compatible with the influences, and total amounts $A^1_{\text{tot}}, \ldots, A^d_{\text{tot}}$, such that the system

$$\sum_{u=1}^{m} (\beta_{i,u} - \alpha_{i,u}) K_u(c_1, \ldots, c_n) = 0, \quad i = 1, \ldots, n, \tag{5}$$

$$\omega^j \cdot c = A^j_{\text{tot}}, \quad j = 1, \ldots, d, \tag{6}$$

has more than one positive solution (the choice of basis $\{\omega^1, \ldots, \omega^d\}$ does not affect the outcome). If the rank of A is maximal ($d=0$), then there are no conservation laws and (6) is disregarded. For $i = 1, \ldots, n$, let

$$g_i(c_1, \ldots, c_n) = \sum_{u=1}^{m} (\beta_{i,u} - \alpha_{i,u}) K_u(c_1, \ldots, c_n). \quad (7)$$

If the function $g = (g_1, \ldots, g_n)$ is injective (one-to-one) over $\mathbb{R}^n_+$ restricted to a given stoichiometric class (4) then the system cannot have multiple positive solutions within that class. An interaction network is said to be *injective* if this is the case for all stoichiometric classes and all rate functions compatible with the influences (that is, all compatible dynamical systems). In other words, an interaction network is injective if the function defined by the left-hand sides of (5) and (6) is injective for all choices of compatible rate functions K_u. The function maps c into $\mathbb{R}^{n+d}$.

Clearly, an injective interaction network does not have the capacity for multiple positive steady states in any stoichiometric class. We provide here a simple characterization of injective interaction networks. Failure of the criterion is thus a necessary condition for the existence of multiple positive steady states in a dynamical system compatible with the given interaction network (Wiuf and Feliu, 2013).

3 RESULTS

A matrix Y with symbolic entries $y_{*,*}$ is called *sign–non-singular* if the determinant of Y is a non-zero homogeneous polynomial in $y_{*,*}$ with all coefficients being positive or all being negative. For the matrices considered here, Y is sign–non-singular if its determinant has constant non-zero sign for positive values of $y_{*,*}$.

3.1 Characterization of injective interaction networks

The *influence matrix*, Z, is an $m \times n$ symbolic matrix where the non-zero entries are variables. The (u, i)-th entry is defined as

- $z_{u,i}$ if there is a positive edge (S_i, r_u).
- $-z_{u,i}$ if there is a negative edge (S_i, r_u).
- 0 if there is no edge between S_i and r_u.

Define the $n \times n$ matrix M as the product of A and Z, $M = AZ$. Let $\{\omega^1, \ldots, \omega^d\}$ be a basis of the left kernel of A and let $i_1, \ldots, i_d$ be indices corresponding to d rows of A that are linearly dependent of the remaining l rows (these will be linearly independent). An easy way to determine the indices is to compute a basis of the left kernel of A and perform Gaussian elimination to obtain a new basis $\{\omega^1, \ldots, \omega^d\}$. Then i_j can be taken to be the index of the first non-zero entry of ω^j (Feliu and Wiuf, 2012). We define a new $n \times n$ matrix, M^*, by replacing the i_j-th row of M by ω^j.

The matrix M^* has d rows with real entries and l rows whose non-zero entries are linear polynomials in $z_{*,*}$. Hence, the determinant of M^* is either zero or a homogeneous polynomial in $z_{*,*}$ of degree l. Further, no variable has an exponent greater than 1.

THEOREM 1. (Wiuf and Feliu, 2013) An interaction network is injective if and only if M^* is sign–non-singular.

The criterion is easy to check, e.g. the influence matrix of (2) is

$$Z = \begin{pmatrix} z_{1,1} & 0 & z_{3,1} & 0 \\ 0 & 0 & 0 & z_{4,2} \\ 0 & 0 & 0 & z_{4,3} \\ 0 & z_{2,4} & z_{3,4} & 0 \end{pmatrix}.$$

The matrix M^* is AZ with the first row changed to $(1, 1, 0, 1)$ and the third row to $(0, 0, 1, 1)$ (according to the first non-zero entry):

$$M^* = \begin{pmatrix} 1 & 1 & 0 & 1 \\ 0 & -z_{2,4} & -z_{3,4} & z_{4,3} \\ 0 & 0 & 1 & 1 \\ z_{1,1} & z_{2,4} & z_{3,1} + z_{3,4} & -(z_{4,2} + z_{4,3}) \end{pmatrix}.$$

The determinant of M^* is

$$z_{1,1}z_{2,4} + z_{2,4}z_{3,1} + z_{1,1}z_{3,4} + z_{2,4}z_{4,2} + z_{1,1}z_{4,3},$$

which is a non-zero polynomial with all non-zero coefficients being positive. The interaction network is therefore injective and there can at most be one positive steady state in any stoichiometric class.

When the determinant of M^* is identically zero, M^* is not sign–non-singular and hence the network is not injective. In this case, all steady states are degenerate, that is, the Jacobian of the system is singular at the steady state (Feliu and Wiuf, 2012).

Existence of two terms of opposite signs in the determinant of M^* implies that there are two cycles of opposite signs in the so-called species–reaction graph (SR-graph) (Banaji and Craciun, 2009; Craciun and Feinberg, 2006; Wiuf and Feliu, 2013). These correspond to two feedback loops, one negative and one positive, in the SR-graph. Our method is thus a refinement of Thomas' rule (Kaufman *et al.*, 2007; Soulé, 2003; Thomas, 1981), applied in the particular setting. In fact, a pair of positive cycles intersecting in a particular way is required for a network to be non-injective (Banaji and Craciun, 2009; Craciun and Feinberg, 2006).

3.2 Power-law kinetics

Power-law kinetics is a general class of kinetics that includes mass-action kinetics. Typically, they appear as approximations to actual kinetics, for example, in chemical mass-action systems where some reactions are fast, or in systems with spatially constrained reactions (non-homogeneous media) (Bajzer *et al.*, 2008; Kopelman, 1998; Schnell and Turner, 2004). They have been advocated as reasonable approximations to the kinetics in general (Savageau, 1998). The reaction rates take the functional form

$$K_u(c) = k_u c_1^{v_{u,1}} \cdot \ldots \cdot c_n^{v_{u,n}},$$

where $k_u > 0$ is a constant and $v_u \in \mathbb{R}^n$. Note that the exponents v_u are allowed to be non-integer and also negative. Mass-action kinetics is a power-law kinetics with positive integer exponents specified by the stoichiometric coefficients of the reactant complexes. Theorem 1 can be refined to determine if the function g is injective over any stoichiometric class for any choice of k_u and fixed $v_{*,*}$. The *kinetic order* matrix V is the $m \times n$ matrix with the vector v_u in the u-th row. Let $\mathrm{diag}(a_1, \ldots, a_r)$ denote the diagonal matrix with diagonal entries $a_1, \ldots, a_r$. Consider symbolic vectors $k = (k_1, \ldots, k_n)$ and $z = (z_1, \ldots, z_m)$ and let $M = A\mathrm{diag}(z)V\mathrm{diag}(k)$. Let $\{\omega^1, \ldots, \omega^d\}$ be a basis of the left kernel of A and $i_1, \ldots, i_d$ row indices as above. We define an $n \times n$ matrix, M^*, by replacing the i_j-th row of M by ω^j. The matrix M^* is a symbolic matrix in z_* and k_*.

THEOREM 2. (Wiuf and Feliu, 2013) The interaction network with power-law kinetics and fixed kinetic orders is injective if and only if M^* is sign–non-singular.

Consider example (2) again and assume mass-action kinetics. Then $k = (k_1, \ldots, k_4)$, $z = (z_1, \ldots, z_4)$,

$$V = \begin{pmatrix} 1 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \\ 0 & 1 & 1 & 0 \end{pmatrix},$$

and

$$M^* = \begin{pmatrix} 1 & 1 & 0 & 1 \\ 0 & -k_2 z_4 & -k_3 z_4 & k_4 z_3 \\ 0 & 0 & 1 & 1 \\ k_1 z_1 & k_2 z_4 & k_3(z_1 + z_4) & -k_4(z_2 + z_3) \end{pmatrix}.$$

The matrix M^* in the mass-action setting and in the qualitative setting is similar. The essential difference is that the entries $z_{*,*}$ in the qualitative setting decompose into a product of two vectors z, k in the mass-action setting. The determinant of M^* is $k_1k_2z_1z_4 + k_2k_3z_1z_4 + k_1k_3z_1z_4 + k_2k_4z_2z_4 + k_1k_4z_1z_3$ and the network is injective, in agreement with the conclusion of the qualitative analysis.

4 IMPLEMENTATION

The conditions of the theorems can be checked with software for symbolic manipulation, such as Mathematica or Maple. This software has efficient built-in functions to compute the rank, kernel and determinant of a matrix, as well as functions to manipulate polynomials and perform Gaussian elimination.

The algorithm proceeds through the following steps:

(i) Compute the matrix $M = AZ$ or $M = A\mathrm{diag}(z)V\mathrm{diag}(k)$.
(ii) Find a basis $\{\omega^1, \ldots, \omega^d\}$ of the left kernel of A and reduce it by Gaussian elimination.
(iii) Construct M^*: For each j determine the first non-zero entry i_j of the ω^j and replace the i_j-th row of M by ω^j.
(iv) Compute the determinant of M^* as a function of $z_{*,*}$ or z_*:
   (a) If it is identically zero, then the network is not injective.
   (b) If there are non-zero terms in the determinant, extract the signs of the coefficients. If all signs are the same, the interaction network is injective. If they are not, then the interaction network is not injective.

The generic script in Maple is provided and exemplified with model (8) (see Section 5) in the Supplementary Data S1 (or Supplementary Data S2 for the pdf version of the code). The computational cost of the algorithm depends on the computation of the determinant of a symbolic matrix of size n and its expansion as a polynomial in $z_{*,*}$ or z_*. The first step is fast for sparse matrices, which is often the case, as each species usually is involved in few reactions. The cost of the second step increases with the number of terms in the entries of M^*

Laplace expansion of the determinant can be used to reduce the cost of computation. Let $I = \{i_1, \ldots, i_k\} \subseteq \{1, \ldots, n\}$ be a set of row indices and $|I| = \sum_{j=1}^{k} i_j$. For any $n \times n$ matrix B, we have

$$\det(B) = (-1)^{|I|} \sum_{J \subseteq \{1,\ldots,n\}} (-1)^{|J|} \det(B_{I,J}) \det(B_{I^c,J^c})$$

where $I^c = \{1, \ldots, n\} \backslash I$ (similarly for J^c) and $B_{I,J}$ is the matrix B restricted to the rows in I and columns in J (similarly for B_{I^c,J^c}). Hence, $\det(B)$ can be computed from $n!/(k!(n-k)!)$ pairs of determinants of size, k and $n-k$, respectively. Additionally, if a sign contradiction is reached after inspecting set J then the computation can be interrupted as the network cannot be injective in this case.

It is worth emphasizing that for a specific system there might be row/column operations that can be done on M^* to simplify the computation of $\det(M^*)$.

5 TEST

We tested the algorithm on a multisite phosphorylation system with r sites and influences derived from mass-action kinetics (Wang and Sontag, 2008). The system has $m = 6r$ reactions and $n = 3(r+1)$ species (Supplementary Data S1). For $1 < r < 8$ (resp. $r = 1$), the algorithm easily concludes that the system is not injective (resp. injective). However, the algorithm collapses on a common computer for $r = 8$ due to memory allocation problems (Table 1). This indicates that the algorithm is not suited for large networks but can be applied efficiently for moderately sized networks.

The matrix M^* has $n-3$ rows with symbolic entries and three rows with integer entries, corresponding to the conservation laws. We expand $\det(M^*)$ along the three rows with integer entries. Then, $b := \det(B_{I,J})$ is a 3×3 numerical determinant and the symbolic determinant $\det(B_{I^c,J^c})$ is only computed if $b \neq 0$.

Table 1 shows the running time using direct computation of the determinant and using expansion along the rows of the conservation laws. We first expand the determinant before concluding on injectivity. The second method can be stopped as soon as two terms are found with contradicting signs, in which case we conclude that the system is non-injective.

By expanding along the rows of the conservation laws, we can decide on injectivity for up to $r = 17$, in which case M^* is a 54×54 matrix. Expansion of the determinant generates a memory allocation error for $r = 18$. We could proceed to expand along more rows (or columns) but these would be symbolic now. The running time would increase as the number of determinants to compute increases.

6 APPLICATIONS

The method is suited for screening large sets of interaction networks to detect those that have the potential for multistationarity. This is illustrated in two different examples. In the first, we generate all possible small motifs of sRNA-mediated gene regulation. In the second, we consider two databases of models of biological systems.

6.1 RNA interference motifs

sRNAs have been demonstrated to regulate gene expression in RNA interference (Bartel, 2004; Cullen, 2005), but the mechanism is not fully understood and only few mathematical models

Table 1. Running time for the r-site phosphorylation system

r	n	m	Method 1	Method 2	Method 3	Minors
1	6	6	0.001	0.004	0.004	20
2	9	12	0.005	0.065	0.075	84
3	12	18	0.020	0.391	0.044	220
4	15	24	0.199	1.275	0.081	455
5	18	30	3.161	4.293	0.191	816
6	21	36	29.24	17.18	0.256	1330
7	24	42	625.9	99.39	0.444	2024
8	27	48	X	613.3	0.795	2925
9	30	54	X	3811	1.169	4060
10	33	60	X	X	2.195	5456
11	36	66	X	X	3.998	7140
12	39	72	X	X	7.696	9139
13	42	78	X	X	15.18	11480
14	45	84	X	X	32.18	14190
15	48	90	X	X	67.74	17296
16	51	96	X	X	171.7	20825
17	54	102	X	X	1199	24804

m =number of reactions; n = number of species; 'Method 1', direct computation of the determinant; 'Method 2', computing the signs of the coefficients of the determinant by Laplace expansion along conservation laws; 'Method 3', same as Method 2 but stopped if a sign contradiction is reached. The last column shows the number of minors along the conservation laws computed for Method 2. Maple 16 was used on a Macbook Pro, Lion Mac OS X. Processor: 2.2 GHz, Intel core i7. Memory: 4 GB.

have been proposed (Cuccato *et al*., 2011; Liu *et al*., 2011; Mitarai *et al*., 2007; Zhdanov, 2008, 2009, 2011).

In Mitarai *et al*. (2007), the sRNA R negatively regulates the mRNA M by binding to it, and the degradation of the complex is triggered. In addition, there is a protein complex F that represses the transcription of R and, consequently, F acts as an activator of M through a double negative loop. The model incorporates consumption of F, enhanced by the presence of M. The species concentrations change according to the following system of ODEs:

$$\dot{f} = \alpha_F - \delta_F f - \frac{\gamma_M m f}{f+K}, \quad \dot{r} = \frac{\alpha_R}{1+f} - \delta_R r - \gamma r m, \quad \dot{m} = \alpha_M - \delta_M m - \gamma r m, \tag{8}$$

with $\alpha_*, \delta_*, \gamma_*, K > 0$. Note that F influences negatively the production of R, that is, the reaction $0 \to R$. The interaction network of this model is shown in Figure 2b.

We focus on the negative regulatory mechanism induced by repression of either mRNA or its product (Liu *et al*., 2011) and build qualitative models. In post-transcriptional repression, sRNA binds to mRNA and the complex is degraded. In translational repression, sRNA binds to the protein F and blocks its function. We generate all interaction networks consisting of three species: sRNA (R), mRNA (M) and a molecule F, which is either the protein blocked by sRNA or a molecule involved in regulation of sRNA or mRNA.

The generated interaction networks share a fixed backbone, depending on whether post-transcriptional or translational repression is considered (Fig. 2a). Each species has positive influence on its own degradation, that is, the reaction (degradation) rate increases with increasing concentration. This is also the case for the joint degradation of M and R, and of R and F. For the two modes of repression, we allow F to influence (positively, negatively or neutrally) the formation of M and R, and M and R to influence positively the degradation of F. The possibility that M enhances the production of F is optional in post-transcriptional repression and assumed in translational repression. In the latter, F is the protein translated from M. This leads to two scenarios A and B (Fig. 2c).

In total, there are 243 interaction networks, 162 for scenario A and 81 for scenario B (Fig. 2c). We find that 56 (reps. 15) of the interaction networks in scenario A (reps. B).

Non-injectivity is preserved when adding edges to a non-injective interaction network. In Figure 2c, we show the minimal non-injective interaction networks for each scenario. An interaction network that does not contain any of these motifs is injective and cannot have multiple positive steady states, whatever the choice of rate functions. The models in Mitarai *et al*. (2007) and Zhdanov (2009, Section 4) are both injective. In the latter, multistationarity does not exist for the specific choice of rate functions (Zhdanov, 2009, Section 4). Here we conclude that the reason for this is independent of the choice and is, in fact, a property of the interaction network. The main model in Zhdanov (2009) is motif $a.1$ and the two models in Liu *et al*. (2011) contain motifs $a.2$ and $b.3$. These findings are consistent with the results in Zhdanov (2009) and Liu *et al*. (2011), where it is shown that the systems exhibit multistationarity for specific rate functions. The motifs in Liu *et al*. (2011) are not minimal with this property.

6.2 Screening models in Biomodels and KEGG

In this section, we apply the method to screen selected models in two publicly available databases: KEGG [Kanehisa and Goto (2000), http://www.kegg.jp/] and Biomodels [Li *et al*. (2010), http://www.ebi.ac.uk/biomodels-main/]. Specifically, we use the models in the database PoCaB (Samal *et al*., 2012), which consists of 365 models from Biomodels and 103 models from KEGG with organism code *hsa* (*Homo sapiens*). The database PoCaB contains pre-computed stoichiometric matrices, mass-action exponent matrices and kinetic data from the selected models.

We analyzed the models from Biomodels in two different ways. Firstly, we imposed mass-action kinetics on all models and checked whether they are injective or not. Secondly, using Maple functionality, we automatically extracted the influence matrices of the reported kinetics. This step is not possible for all models because some kinetics are not monotone. There are 323 models (out of 365) for which the influence matrix could be computed.

The models from KEGG have no associated kinetic data. We analyzed the models first assuming mass-action kinetics and afterward assuming that the influences are derived from mass-action kinetics.

Both databases contain models of varying size (Fig. 3). The injectivity test is easily computed for the smallest systems using the direct approach. A rough cut-off for being small is that the number of reactions is below 33. The computation of $\det(M^*)$ for the larger models requires expansion of the determinant. We noticed that the matrix M^* often contains rows/columns with

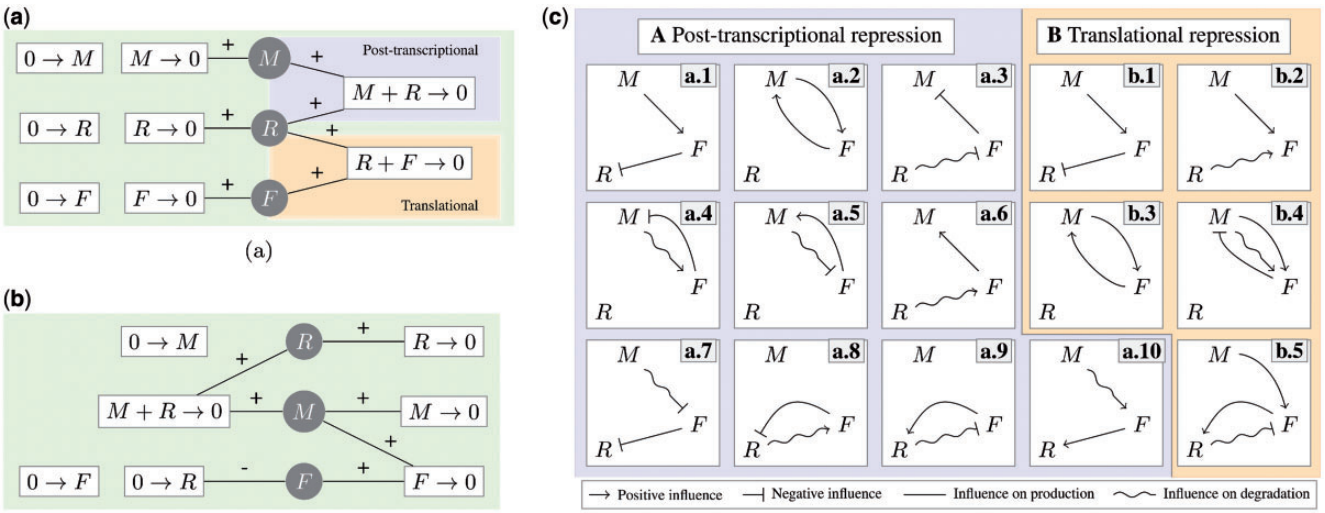

Fig. 2. sRNA-mediated repression. (**a**) Common backbone for the interaction networks with sRNA-mediated repression. The post-transcriptional repression backbone does not include the orange box, and the translational repression backbone does not include the blue box. (**b**) Example of sRNA-mediated repression for the model in (8). (**c**) Minimal non-injective motifs for the interplay sRNA–mRNA–protein/molecule. The common backbone is not redrawn here. The solid lines indicate influence on the production rate and the snake lines indicate influence on the degradation rate. The two different arrow tips indicate whether the influence is positive or negative. Symmetric networks are not removed, for example, *a*.3 and *a*.7 are symmetric by interchanging M and R

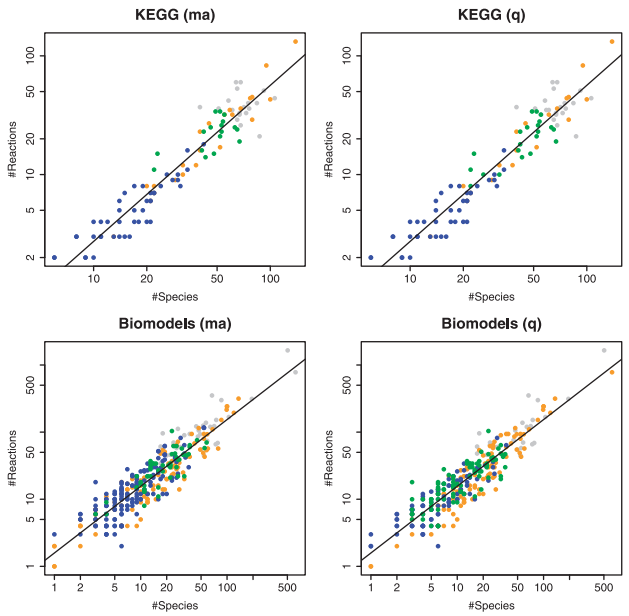

Fig. 3. The number of reactions is plotted against the number of species for the analyzed models in KEGG and analyzed models in Biomodels (log–log scale). 'ma' refers to mass-action kinetics and 'q' to the qualitative influence (see main text for details). One hundred three models were used from KEGG in both analyses. Three hundred sixty-five (323) models were used from Biomodels for the 'ma' analysis ('q' analysis). Blue: Injective, Orange: Non-injective with identically zero determinant, Green: Non-injective with non-identically zero determinant, Gray: Analysis failed. The data used in the figure is provided in the Supplementary Data S3

only one non-zero entry. If the non-zero entry is a polynomial with coefficients with distinct signs, then the network is not injective. If this is not the case, then the row and column corresponding to this non-zero entry can be removed from M^*, thereby simplifying M^* by one dimension. We repeat this procedure iteratively until no row/column can be removed. This process reduces the size of M^*, but it also makes the matrix less sparse. Hence the computation of the determinant can still be computationally expensive.

Table 2 and Figure 3 provide a summary of the results. Detailed information is given in Supplementary Data S3. Non-injective networks for which $\det(M^*) = 0$ identically are reported separately. For the Biomodels database, in all but 14 cases, $\det(M^*)$ is identically zero because there are species in the model that do not influence any reaction (contributing a zero column in M) and this is not compensated by the conservation laws. A detailed analysis of each specific model might reveal that the model should be appropriately modified.

The results in Table 2 show that the method could decide on injectivity on a large fraction of the networks. A network can be injective when taken with mass-action kinetics but non-injective when taken with general rate functions compatible with mass-action kinetics. This is the case for the KEGG data, but not the Biomodels data, where the general influences not necessarily are compatible with mass-action kinetics. Hence the injectivity tests for mass-action kinetics and general influences are not necessarily related.

7 DISCUSSION

We have developed and implemented a computationally efficient and simple method to qualitatively assert whether a network

cannot exhibit multistationarity. The approach embraces a broad class of dynamical systems by allowing for conservation laws and arbitrary influences. Our method decides whether an interaction network is injective or not. If it is injective, it can at most have one positive steady state within each stoichiometric class.

Qualitative inference relies solely on the structure of the interaction network. This approach to inference has the particular strength of freeing the analysis from any specific ODE system and to highlight the generality of a conclusion (or property). In our context, it is surprising that the exclusion of multistationarity can be so strongly encoded in the network structure (reactions and influences) alone and be independent of the specific form of the rate functions, even if these involve complicated and non-linear terms.

An overview of different methods to preclude and/or assert multistationarity is provided in Table 3. Our algorithm is in the class of *injectivity-based* criteria to preclude multistationarity. In the context of chemical reaction network theory, these criteria generally fall in two groups, Jacobian-based methods (Banaji *et al.*, 2007; Craciun and Feinberg, 2005, 2010; Feliu and Wiuf, 2012; Gnacadja, 2012; Joshi and Shiu, 2012) and graphical methods (Banaji and Craciun, 2010, 2009; Craciun and Feinberg, 2006; Soulé, 2003). These apply to different specializations of networks and rate functions, such as mass-action kinetics or specific influences, and can be seen as specializations of our method (Wiuf and Feliu, 2013). The graphical conditions are derived from conditions on the Jacobian and would therefore preclude multistationarity in fewer cases than a corresponding Jacobian-based method.

A different criterion (a sign condition) to characterize injective networks is given in Shinar and Feinberg (2012) for some specific influences. Their definition of a compatible rate function is less restrictive than ours and corresponds, in our setting, to treat classes of influences together (Wiuf and Feliu, 2013).

If the kinetics is restricted to mass-action, there exist methods that complement injectivity-based methods in that multistationarity not only can be precluded, but also asserted. The main methods in this class are given in Table 3. Other methods include an injectivity-based method applicable to weakly reversible networks (Otero-Muras *et al.*, 2012) and a study of embedded networks (Joshi and Shiu, 2013).

We applied our method to a large class of models of gene silencing by RNA interference. The method ran efficiently on this set. Further, we observed that the non-existence of multistationarity reported for some of the motifs for specific choices of rate functions (Zhdanov, 2009) has a qualitative origin. Subsequently, we applied our method to two databases of biological models and showed that the method could decide on injectivity for a large fraction of the models. It is remarkable that a large proportion of the networks is injective and hence cannot exhibit multistationarity.

Existence of multistationarity is often asserted from random parameter search, assuming the rate functions take a generic form. However, exclusion of multistationarity cannot be decided from a finite number of sampled parameter values alone. Further, the result might depend strongly on how the parameters are sampled. Our method provides an automatized procedure to assert that multistationarity cannot occur. It provides an additional tool to various other available softwares to address multistationarity (Table 3). For mass-action kinetics, other softwares exist to extract various network characteristics, e.g. Szederkényi *et al.* (2012).

Table 2. For each category (row) the percentage of networks and the average number of species in the networks are shown

Networks	Injective	Non-injective				Analysis		
		Zero		Non-zero		Failed		
KEGG (ma)	42.7%	17.9	22.3%	52.9	16.5%	48.7	18.5%	70.4
KEGG (q)	40.7%	17.1	18.5%	55.2	20.4%	45.2	20.4%	69.1
BioM (ma)	45.8%	10.5	30.4%	22.8	14.8%	20.5	9.0%	82.3
BioM (q)	31.6%	8.3	32.2%	31.0	27.9%	13.5	8.3%	71.3

In total, there are 103, 103, 365 and 323 networks in each category, respectively. BioM refers to Biomodels, 'ma' refers to mass-action kinetics and 'q' to the qualitative influence (see main text for details).

Table 3. Overview of methods to preclude and/or assert multistationarity with description, availability of software and main reference

Preclusion of multistationarity. Arbitrary kinetics		
Determinant-based injectivity test[a]	Maple script	This manuscript
Concordant networks	CRNT toolbox[b]	Shinar and Feinberg (2012)
DSR-graph	ERNEST[c] and CoNtRol[d]	Banaji and Craciun (2009)
Interaction graph		Soulé (2003)
Assertion of multistationarity. Mass-action kinetics		
Deficiency-based	CRNT toolbox[b] and ERNEST[c]	Feinberg (1987)
Subnetwork analysis		Conradi *et al.* (2007)
Sign pattern analysis		Conradi and Flockerzi (2012)
Toric steady states		Pérez Millán *et al.* (2012)

Most methods are developed in several references or apply to certain specializations. Only the most general or a representative one is given in the table.
[a]The method specializes when the kinetics is power-law or mass-action. A determinant-based condition is given in Craciun and Feinberg (2005) to assert multistationarity for mass-action networks when production and degradation of all species are assumed.
Links to software: [b]Ellison *et al.* (2012), [c]Soranzo and Altafini (2009), [d]Donnell *et al.* (2013).

Non-injectivity is a necessary condition for an interaction network to exhibit multiple positive steady states. Hence, it is possible that a network is non-injective without being multistationary. In practice, however, we have observed that a non-injective interaction network generally enables a choice of rate functions and parameters for which multistationarity occurs. Prospective work will focus on investigating under what conditions this is true and, if it is the case, how the rate functions and parameters can be constructed.

Typical ODE models, such as SIR models and the Lotka–Volterra model, can be put in the framework of an interaction network, even though the model is derived from a different perspective. Interpretation of ODE models as interaction networks with potential non-realistic (hypothetical) reactions might seem artificial. However, it highlights an important aspect, namely that of freeing the system from a specific choice of ODEs. Bearing in mind the difficulties in choosing the 'correct' system of ODEs, we see this aspect as a strong advantage of our method and encourage the modeling community to consider qualitative inference broadly with the aim of separating model specificities from structural properties.

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