JOINING AND DECOMPOSING REACTION NETWORKS

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Abstract. In systems and synthetic biology, much research has focused on the behavior and design of single pathways, while, more recently, experimental efforts have focused on how cross-talk (coupling two or more pathways) or inhibiting molecular function (isolating one part of the pathway) affects systems-level behavior. However, the theory for tackling these larger systems in general has lagged behind. Here, we analyze how joining networks (e.g., cross-talk) or decomposing networks (e.g., inhibition or knock-outs) affects three properties that reaction networks may possess—identifiability (recoverability of parameter values from data), steady-state invariants (relationships among species concentrations at steady state, used in model selection), and multistationarity (capacity for multiple steady states, which correspond to multiple cell decisions). Specifically, we prove results that clarify, for a network obtained by joining two smaller networks, how properties of the smaller networks can be inferred from or can imply similar properties of the original network. Our proofs use techniques from computational algebraic geometry, including elimination theory and differential algebra.

Keywords: reaction network, mass-action kinetics, multistationarity, identifiability, steady-state invariant, Gröbner basis

1. Introduction

Cells transmit information via molecular interactions which are complicated and numerous: a typical eukaryotic cell contains approximately 8×10^9 molecules. Understanding the function and behavior of such a large number of molecules is challenging and often intractable. Therefore, much effort in the field of systems biology focuses on first understanding and predicting the behavior of smaller sets of interacting molecular species, called signaling pathways. Advances in experimental technology have enabled the possibility of measuring more species, prompting questions about what happens when two or more specific pathways interact [17]. This problem of predicting the effect of joining pathways is the focus of our work.

Whenever two or more pathway models are combined, it is reasonable to expect that some model properties of the larger model may be inferred predictably from properties of the component models. Within this context, our work focuses on three important properties of pathway models: *identifiability*, whether the parameter values can be determined from data, *steady-state invariants*, which characterize a model and provide a framework for hypothesis testing with limited data, and *multistationarity*, which is the capacity for multiple positive steady states. We prove results on how these properties are affected when we combine two or more models. We consider, first, linear models, and then extend our results, where possible, to nonlinear models.

A biological example to motivate our study is signaling in apoptosis (programmed cell death). Activation of the death signal can be initiated by either the intrinsic pathway (via

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stress) or the extrinsic pathway (via ligand-receptor binding). Mathematical models of each pathway have been developed [19, 48], and analyses of these models have revealed that both pathways have the capacity for two steady-states, which correspond to a cell-death state and a cell-alive state [3, 19, 48, 40], meaning that the models are multistationary. Analyses of cell death models have also focused on identifiability [22] and steady-state invariants [40]. Since [19] and [48], additional models have been constructed with a focus on the molecular network between the intrinsic and extrinsic pathways at the mitochondrial membrane [3, 1, 11] as well as joining both pathways into a single model [37, 29]. However, predicting how joining pathways affects cell death checkpoints and other model properties is difficult. Pursuing this question for general pathway networks is similar in some respects to analyzing retroactivity and modules within a larger network [13, 52].

In this work, we are interested in signaling pathway models that describe molecular interactions via biochemical reactions. In particular, we will study chemical reaction networks, directed graphs in which the nodes are molecular complexes and the edges are reactions weighted by rate constants (parameters). Under the assumption of mass-action kinetics, each reaction graph gives rise to a system of polynomial differential equations. Thus, in essence, we are interested in how this polynomial system of differential equations changes as we construct larger networks from smaller ones. Since our emphasis is on the structure of the equations, not the value of the parameters, our analysis focuses on properties that hold in general.

Reaction networks can be joined naturally in various ways; two such ways are shown in Figure 1. As shown in Figure 1A, one way we can *glue* together two networks X and Y is via a new or shared edge. Networks obtained by gluing over new or shared edges arise naturally when considering linear compartmental models and are central to Section 3. Another way to glue together X and Y is via a shared node (Figure 1B); such gluing allows us to investigate *cross-talk*, interactions between signaling pathways X and Y that have at least one shared molecule. Currently, cross-talk is an active area of research in biology, especially for predicting the effects of drug targets on cells. Networks obtained by gluing over shared nodes are analyzed in terms of their steady-state invariants in Section 4.

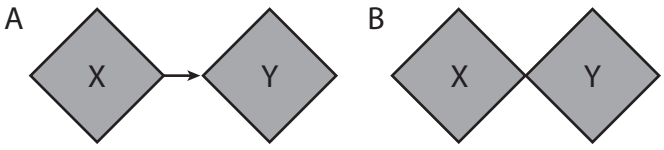


FIGURE 1. (A) Gluing two networks via a new edge. Biologically, this may correspond to distinct networks of the same pathway. (B) Gluing two motifs that have a shared species (node). Biologically, this may be called cross-talk.

The outline of our work is as follows. Section 2 introduces the background and definitions. Next, Sections 3, 4, and 5 each correspond to a property of interest: identifiability, steady-state invariants, and multistationarity (respectively). The proofs of our results rely on techniques from computational algebraic geometry, such as elimination theory and differential algebra; indeed, algebraic tools are increasingly used in the analyses of reaction networks (see the survey [15]). Finally, a discussion appears in Section 6.

2. Background

Valuable information may be obtained by translating a chemical reaction network into a system of differential equations. In our setting, we form a polynomial dynamical system which is amenable to algebraic analysis described in the subsequent sections. First, we begin with an example of a *chemical reaction*: $A+B \to 3A+C$, where A, B, and C are chemical *species*. These species could represent various proteins modifying one another. In this reaction, the *reactant* forms the left hand side of the reaction (one species A and one of B), which react to form the *product* (three A and one C).

We follow convention and denote concentrations of the species by lower case x_A, x_B, and x_C, which will change in time as the reaction occurs. Here, we assume *mass-action kinetics*, that is, species A and B react at a rate proportional to the product of their concentrations, where the proportionality constant is the *reaction rate constant* κ. Noting that the reaction yields a net change of two units in the amount of A, we obtain the differential equation $\frac{d}{dt}x_A = 2\kappa x_A x_B$, where t is time. The other two equations arise similarly: $\frac{d}{dt}x_B = -\kappa x_A x_B$ and $\frac{d}{dt}x_C = \kappa x_A x_B$.

A *chemical reaction network* consists of finitely many reactions (see Definition 2.1 below). The mass-action differential equations that a network defines are a sum of the monomial contributions from the reactants of each chemical reaction in the network; these differential equations will be defined in equation (2).

2.1. Chemical reaction networks.

We now provide precise definitions.

Definition 2.1. A *chemical reaction network* $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ consists of three finite sets $\mathcal{S}, \mathcal{C}$, and $\mathcal{R}$.

(1) A set of chemical *species* $\mathcal{S} = \{A_1, A_2, \ldots, A_n\}$, where $n \in \mathbb{N}$ denotes the number of species.
(2) A set $\mathcal{C} = \{y_1, y_2, \ldots, y_p\}$ of *complexes* (finite nonnegative-integer combinations of the species), where $p \in \mathbb{N}$ denotes the number of complexes.
(3) A set of *reactions*, ordered pairs of the complexes: $\mathcal{R} \subseteq (\mathcal{C} \times \mathcal{C}) \setminus \{(y_i, y_i) \mid y_i \in \mathcal{C}\}$.

Throughout this work, the integer unknown r denotes the number of reactions. A *subnetwork* of a network $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ is a network $\widetilde{G} = (\widetilde{\mathcal{S}}, \widetilde{\mathcal{C}}, \widetilde{\mathcal{R}})$ with $\widetilde{\mathcal{R}} \subseteq \mathcal{R}$.

We also make a simplifying assumption: every complex in $\mathcal{C}$ must appear in at least one reaction in $\mathcal{R}$, and every species in $\mathcal{S}$ must appear in at least one complex in $\mathcal{C}$. This assumption does not restrict the class of networks we can study, just how they are represented.

A network can be viewed as a directed graph whose nodes are complexes and whose edges correspond to the reactions. Like for all network analysis, properties of the connectedness of the graph can be useful. A reaction $y_i \to y_j$ is *reversible* if it is bi-directional, i.e., the reverse reaction $y_j \to y_i$ is also in $\mathcal{R}$; these reactions are depicted by $y_i \rightleftharpoons y_j$.

Writing the i-th complex as $y_{i1}A_1 + y_{i2}A_2 + \cdots + y_{in}A_n$ (where $y_{ij} \in \mathbb{Z}_{\geq 0}$, for $j = 1, 2, \ldots, n$, are the *stoichiometric coefficients*), we introduce the following monomial:

$$x^{y_i} \;:=\; x_1^{y_{i1}} x_2^{y_{i2}} \cdots x_n^{y_{in}} \;.$$

(By convention, the *zero complex* yields the monomial $x^{(0,\ldots,0)} = 1$.) For example, the two complexes in the reaction $A+B \to 3A+C$ considered earlier give rise to the monomials $x_A x_B$ and $x_A^3 x_C$, which determine the vectors $y_1 = (1,1,0)$ and $y_2 = (3,0,1)$. These vectors define the rows of a $p \times n$-matrix of nonnegative integers, which we denote by $Y = (y_{ij})$. Next, the unknowns $x_1, x_2, \ldots, x_n$ represent the concentrations of the n species in the network, and we regard them as functions $x_i(t)$ of time t.

We distinguish between monomolecular complexes (e.g., A or B), bimolecular complexes (e.g., $2A$ or $A+B$), and others (e.g., 0 or $A+2B$), as follows. A complex $y_{i1}A_1 + y_{i2}A_2 + \cdots + y_{in}A_n$ is *monomolecular* if exactly one stoichiometric coefficient y_{ij} equals 1, and all other y_{ik}'s are 0. A complex $y_{i1}A_1 + y_{i2}A_2 + \cdots + y_{in}A_n$ is *at-most-bimolecular* if the sum of the stoichiometric coefficients y_{ij} is at most 2. A reaction network is itself *monomolecular* (respectively, *at-most-bimolecular*) if all its complexes are monomolecular or the zero complex (respectively, all its complexes are at-most-bimolecular). The reaction systems arising from monomolecular networks are known as linear compartmental models (see §2.4).

For a reaction $y_i \to y_j$ from the i-th complex to the j-th complex, the *reaction vector* $y_j - y_i$ encodes the net change in each species that results when the reaction takes place. The *stoichiometric matrix* Γ is the $n \times r$ matrix whose k-th column is the reaction vector of the k-th reaction i.e., it is the vector $y_j - y_i$ if k indexes the reaction $y_i \to y_j$.

We associate to each reaction a positive parameter κ_{ij}, the *rate constant* of the reaction. In this article, we will treat the rate constants κ_{ij} as positive unknowns in order to analyze the entire family of dynamical systems that arise from a given network as the κ_{ij}'s vary.

2.2. **Chemical reaction systems.** The *reaction kinetics system* defined by a reaction network G and reaction rate function $R : \mathbb{R}^n_{\geq 0} \to \mathbb{R}^r$ is given by the following system of ODEs:

$$\frac{dx}{dt} \;=\; \Gamma \cdot R(x)\ . \tag{1}$$

A *steady state* of a reaction kinetics system (1) is a nonnegative concentration vector $x^* \in \mathbb{R}^n_{\geq 0}$ at which the ODEs (1) vanish: $\Gamma \cdot R(x^*) = 0$.

For *mass-action kinetics*, which is the setting of this paper, the coordinates of R are $R_k(x) = \kappa_{ij} x^{y_i}$, if k indexes the reaction $y_i \to y_j$. A *chemical reaction system* refers to the system of differential equations (1) arising from a specific chemical reaction network $(\mathcal{S}, \mathcal{C}, \mathcal{R})$ and a choice of rate constants $(\kappa_{ij}) \in \mathbb{R}^r_{>0}$ (recall that r denotes the number of reactions) where the reaction rate function R is that of mass-action kinetics. Specifically, the mass-action ODEs are:

$$\frac{dx}{dt} \quad = \quad \sum_{y_i \to y_j \text{ is in } \mathcal{R}} \kappa_{ij} x^{y_i} (y_j - y_i) \quad =: \quad f_\kappa(x)\ . \tag{2}$$

The *stoichiometric subspace* is the vector subspace of $\mathbb{R}^n$ spanned by the reaction vectors $y_j - y_i$, and we will denote this space by S:

$$S \;:=\; \mathbb{R}\{y_j - y_i \mid y_i \to y_j \text{ is in } \mathcal{R}\}\ . \tag{3}$$

Note that in the setting of (1), one has $S = \mathrm{im}(\Gamma)$. For the network consisting of the single reaction $A+B \to 3A+C$, we have $y_2 - y_1 = (2,-1,1)$, which means that with each occurrence of the reaction, two units of A and one of C are produced, while one unit of B is consumed. This vector $(2,-1,1)$ spans the stoichiometric subspace S for the network. Note that the

vector $\frac{dx}{dt}$ in (1) lies in S for all time t. In fact, a trajectory $x(t)$ beginning at a positive vector $x(0) = x^0 \in \mathbb{R}^n_{>0}$ remains in the *stoichiometric compatibility class*, which we denote by

$$\mathcal{P} \;:=\; (x^0 + S) \cap \mathbb{R}^n_{\geq 0} \;, \tag{4}$$

for all positive time. In other words, $\mathcal{P}$ is forward-invariant with respect to the dynamics (1).

2.3. **Combining networks.** Here we introduce operations that allow two or more networks to be 'glued' together to form a single network. These operations encompass many natural operations that arise in biological modeling, for instance, connecting two networks by a one-way flow, or extending a model to include additional pathways. The aim of this work is to investigate how these operations affect three properties of networks: identifiability, steady-state invariants, and multistationarity.

Definition 2.2. The *union* of reaction networks $N_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1)$ and $N_2 = (\mathcal{S}_2, \mathcal{C}_2, \mathcal{R}_2)$ is

$$N_1 \cup N_2 \;:=\; (\mathcal{S}_1 \cup \mathcal{S}_2,\; \mathcal{C}_1 \cup \mathcal{C}_2,\; \mathcal{R}_1 \cup \mathcal{R}_2) \;.$$

The union of finitely many reaction networks N_i is defined similarly.

Next, we classify the union $N_1 \cup N_2$ according to whether their respective sets of complexes (or reactions) of N_i are disjoint. The possible relationships among these sets are constrained by the following implications:

$$\mathcal{S}_1 \cap \mathcal{S}_2 = \emptyset \quad \Rightarrow \quad \mathcal{C}_1 \cap \mathcal{C}_2 = \emptyset \text{ or } \mathcal{C}_1 \cap \mathcal{C}_2 = \{0\} \quad \Rightarrow \quad \mathcal{R}_1 \cap \mathcal{R}_2 = \emptyset \;.$$

If the two species sets are disjoint ($\mathcal{S}_1 \cap \mathcal{S}_2 = \emptyset$), then the networks N_1 and N_2 are completely disjoint, so analyzing their union is equivalent to analyzing N_1 and N_2 separately. Thus, we are interested in the three remaining cases:

Definition 2.3. The union $N_1 \cup N_2$ of $N_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1)$ and $N_2 = (\mathcal{S}_2, \mathcal{C}_2, \mathcal{R}_2)$ is formed by:

(1) *gluing complex-disjoint networks* if $\mathcal{S}_1 \cap \mathcal{S}_2 \neq \emptyset$ and the two networks have no complex in common except possibly the zero complex, i.e., $\mathcal{C}_1 \cap \mathcal{C}_2 \subseteq \{0\}$ (and thus $\mathcal{R}_1 \cap \mathcal{R}_2 = \emptyset$),
(2) *gluing over complexes* if the two networks have at least one non-zero complex in common (i.e., $\mathcal{C}_1 \cap \mathcal{C}_2 \nsubseteq \{0\}$) but no reactions in common (i.e., $\mathcal{R}_1 \cap \mathcal{R}_2 = \emptyset$),
(3) *gluing over reactions* if the two networks have at least one reaction in common (i.e., $\mathcal{R}_1 \cap \mathcal{R}_2 \neq \emptyset$).

Notation. We will denote the species of $N_1 \cup N_2$ as $\mathbf{x} = x_1, \ldots, x_n$, and the species of N_1 and N_2 as $\mathbf{x}(1) = \{x_1, \ldots, x_j\}$ and $\mathbf{x}(2) = \{x_k, \ldots, x_n\}$, respectively. Here, $k \leq j$, because the species sets overlap. We let $\kappa(1)$ and $\kappa(2)$ be the rate constants of the reactions in $\mathcal{R}_1$ and $\mathcal{R}_2$, respectively, and we let $\kappa = \kappa(1) \cup \kappa(2)$ denote the rate constants of N.

Remark 2.4. If networks N_1 and N_2 are monomolecular, then they are complex-disjoint if and only if they are species-disjoint ($\mathcal{S}_1 \cap \mathcal{S}_2 = \emptyset$). Thus, we can not glue complex-disjoint networks that are monomolecular.

We introduce more operations, in which N_1 and N_2 may have disjoint species sets:

Definition 2.5. Consider networks $N_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1)$ and $N_2 = (\mathcal{S}_2, \mathcal{C}_2, \mathcal{R}_2)$.

(1) Let $\{y \to y'\}$ denote a network that consists of a single reaction that is not in $\mathcal{R}_1 \cup \mathcal{R}_2$ and for which $y \in \mathcal{C}_1$ and $y' \in \mathcal{C}_2$. The network obtained by *joining* N_1 *and* N_2 *by a new reaction* $y \to y'$ is:

$$N_1 \cup N_2 \cup \{y \to y'\} \ .$$

(2) Let $\mathcal{R}'$ and $\mathcal{R}''$ be sets of reactions for which $\mathcal{R}' \subseteq (\mathcal{R}_1 \cup \mathcal{R}_2)$ and $\mathcal{R}'' \cap (\mathcal{R}_1 \cup \mathcal{R}_2) = \emptyset$, and every reaction $y \to y'$ in $\mathcal{R}''$ satisfies $y \in \mathcal{C}_1$ and $y' \in \mathcal{C}_2$. Let N_3 denote the network that consists of the reactions in $\mathcal{R}''$. The network obtained by *joining* N_1 *and* N_2 *by replacing reactions* $\mathcal{R}'$ *by* $\mathcal{R}''$ is:

$$(\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1 \setminus \mathcal{R}') \ \cup \ (\mathcal{S}_2, \mathcal{C}_2, \mathcal{R}_2 \setminus \mathcal{R}') \ \cup \ N_3 \ .$$

Joining by a new reaction, in Definition 2.5(1), adds a one-way flow from one network to another. As for replacing reactions, in Definition 2.5(2), we describe an instance of this. Suppose that a large network is formed by two subnetworks M_1 and M_2, plus a reaction $X \to Y$ from M_1 to M_2. Then, to study each subnetwork separately, we might consider $N_1 = M_1 \cup \{X \to 0\}$ and $N_2 = M_2 \cup \{0 \to Y\}$. Later, when we want to put these two networks together, we *join* N_1 *and* N_2 *by replacing reactions* $\{X \to 0 \to Y\}$ *by* $\{X \to Y\}$.

Example 2.6. Consider the following networks:

$$N_1 \ = \ \{0 \to A \to B\} \ , \quad N_2 \ = \ \{A \to B \leftrightarrow C\} \ , \quad \text{and} \quad N_3 \ = \ \{C \leftrightarrow D\} \ .$$

The network $N_1 \cup N_2$ formed by gluing over the shared reaction $A \to B$ is $\{0 \to A \to B \leftrightarrow C\}$. Also, the network obtained by joining N_1 and N_3 by a new reaction $B \to C$ is $\{0 \to A \to B \to C \leftrightarrow D\}$.

Remark 2.7. Using the definitions above and recalling our assumption that networks include only those species or complexes that take part in reactions, we see that a network N is a *subnetwork* of G if there exists a network N' for which $G = N \cup N'$. In this case, to obtain the mass-action ODEs (2) for N from those of G, simply set all rate constants to zero for those reactions not in N. As for the ODEs obtained by gluing networks as in Definition 2.3, we clarify them in Lemma 2.8.

The next result follows from the fact that the mass-action ODEs are a sum over reactions.

Lemma 2.8. *Consider networks* $N_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1)$ *and* $N_2 = (\mathcal{S}_2, \mathcal{C}_2, \mathcal{R}_2)$*, and denote their mass-action ODEs* (2) *by, respectively,* $dx/dt = f$ *and* $dx/dt = g$*. Define* $f_i := 0$ *(respectively,* $g_i := 0$*) for species* $i \in \mathcal{S}_2 \setminus \mathcal{S}_1$ *(respectively,* $i \in \mathcal{S}_1 \setminus \mathcal{S}_2$*). Let* $N = N_1 \cup N_2$ *be the reaction network obtained by gluing* N_1 *and* N_2*. Then the mass-action ODEs for* N *are given by:*

(1) $dx/dt = f + g$*, if* $\mathcal{R}_1 \cap \mathcal{R}_2 = \emptyset$ *(i.e., gluing complex-disjoint networks or over complexes).*
(2) $dx/dt = f + \widetilde{g}$*, if* $\mathcal{R}_1 \cap \mathcal{R}_2 \neq \emptyset$ *(i.e., gluing over reactions), where* $dx/dt = \widetilde{g}$ *denotes the mass-action ODEs for the subnetwork of* N_2 *comprising only reactions in* $\mathcal{R}_2 \setminus \mathcal{R}_1$*.*

Remark 2.9. A related approach to gluing networks, introduced by Johnston [42], involves "translating" some of the complexes in such a way that the "translated" networks (taken with certain general kinetics) define the same dynamical systems as the original network (taken with mass-action kinetics). We do not consider translated networks in this work.

	Identifiability	Steady-state invariants
Glue over complexes		Theorem 4.7 Theorem 4.10
Glue over reactions		Theorem 4.9
Join by a new reaction	Theorem 3.30 Theorem 3.33	
Join by replacing reactions	Theorem 3.15 Theorem 3.23 Theorem 3.28	

TABLE 1. Summary of results on joining networks

	Identifiability	Steady-state invariants
Unglue over complexes		Theorem 4.7 Theorem 4.10
Unglue over reactions		Theorem 4.9
Decompose via a lost reaction	Theorem 3.33	
Decompose by replacing reactions	Theorem 3.28	

TABLE 2. Summary of results on decomposing networks

Our results on joining and "decomposing" networks are summarized in Tables 1 and 2. Additionally, examples pertaining to multistationarity and gluing over complexes or joining by a new reaction are given in Sections 5.5 and 5.4, respectively. Some of our results on identifiability are in the context of monomolecular networks, which can be viewed as "linear compartmental models". We turn to this topic now.

2.4. **Monomolecular networks and linear compartmental models.** A special class of reaction networks that we will consider is that of monomolecular networks. Recall that this means that each complex of the network is either a single species (e.g., X_1 or X_2) or the zero complex. The associated differential equations (2) therefore are linear; the general form is:

$$\frac{dx(t)}{dt} = A\ x(t) + u\ , \tag{5}$$

where A is a matrix with nonnegative off-diagonal entries, and u is a nonnegative vector of inflow rates. Both A and u are composed of rate-constant parameters.

Monomolecular networks have many applications in areas such as pharmacokinetics, cell biology, and ecology, and are more commonly called *linear compartmental models* [31]. In this setting, the *input* vector u is viewed as a control vector $u(t)$ (at least one component of u is assumed to be controlled, and the non-controllable components $u_i(t)$ are constants).

Thus, equation (5) becomes[1]:

$$\frac{dx(t)}{dt} = A\ x(t) + u(t)\ , \tag{6}$$

and the matrix A is called the *compartmental matrix*. Also, each species concentration $x_i(t)$ is called a *state variable* in this setting, representing the concentration of material in *compartment* i. Note that $u_i(t) \equiv 0$ when there is no inflow of material to compartment i (i.e., no *inflow* reaction $0 \to X_i$). *Outflow* reactions of the form $X_i \to 0$ are called *leaks*. The dictionary between these terms is in Table 3.

Reaction networks	**Compartmental models**
Monomolecular network	Linear compartmental model
Species	Compartment
Species concentration	State variable
Inflow reaction (production)	Input
Outflow reaction (degradation)	Leak

TABLE 3. Dictionary between reaction networks and compartmental models.

For identifiability problems, we assume as part of the setup that some of the species concentrations $x_i(t)$ can be observed. This is summarized as an *output* (or measurement) vector $z(t)$, in which each coordinate[2] is one of the observed species concentrations $x_i(t)$. In literature, the vector $y(t)$ is usually used, but we use $z(t)$ as we reserve y for complexes.

Alternatively, we can define a linear compartmental model in terms of a directed graph $\mathfrak{G} = (V, E)$ with vertex set V and set of directed edges E, and three sets $In, Out, Leak \subseteq V$. Each vertex $i \in V$ is a compartment in the model, while each edge $j \to i$ in E represents the flow of material (reaction) from the j-th to the i-th compartment. The sets $In, Out, Leak$ are the sets of input (inflow-reaction), output, and leak (outflow-reaction) compartments, respectively. Thus, we can write a linear compartmental model $\mathcal{M}$ as $\mathcal{M} = (\mathfrak{G}, In, Out, Leak)$.

Remark 2.10. We use the convention in this paper that, for linear compartmental models, the rate constant describing the reaction from the j-th compartment to the i-th compartment is written as a_{ij}, whereas for monomolecular networks (and for chemical reaction networks, in general) we use κ_{ji} to describe the reaction rate constant from species X_j to species X_i.

Example 2.11. The chemical reaction network $\left\{ 0 \xrightarrow{u_1} X_1 \underset{\kappa_{12}}{\overset{\kappa_{21}}{\leftrightarrows}} X_2 \xrightarrow{\kappa_{20}} 0 \right\}$ is a monomolecular network with ODEs as follows:

$$\begin{pmatrix} x_1' \\ x_2' \end{pmatrix} = \begin{pmatrix} -\kappa_{12} & \kappa_{21} \\ \kappa_{12} & -\kappa_{20} - \kappa_{21} \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix} + \begin{pmatrix} u_1(t) \\ 0 \end{pmatrix} .$$

If we view the network as a linear compartmental model, we use the following notation:

[1]The standard definition of a linear compartmental model incorporates an extra matrix B as follows: $\frac{dx(t)}{dt} = A\ x(t) + B\ u(t)$; our work therefore considers the case when B is the identity matrix.

[2]The standard definition of a linear compartmental model incorporates an extra matrix C as follows: $z(t) = C\ x(t)$; our work therefore considers the case when C is a diagonal matrix with only ones and zeroes along the diagonal, and $z_i(t)$ is only defined when the corresponding diagonal element of C is one.

$$\begin{pmatrix} x_1' \\ x_2' \end{pmatrix} = \begin{pmatrix} -a_{21} & a_{12} \\ a_{21} & -a_{02} - a_{12} \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix} + \begin{pmatrix} u_1(t) \\ 0 \end{pmatrix} .$$

If we assume a measurement (output) from the first compartment, we have an additional equation $z_1(t) = x_1(t)$, which we call an *output equation*.

3. Identifiability

We are interested in two identifiability problems for linear and nonlinear state space models. The first concerns joining two identifiable submodels. The second concerns restricting a model to smaller components (subnetworks).

3.1. **Background: identifiability and input-output equations.** Structural identifiability, which was introduced by Bellman and Astrom in 1970 [5], concerns whether it is possible to uniquely recover the parameter values of a model given perfect input-output data. Numerous techniques to address this question have been developed [7, 14, 21, 41, 63], and a particularly fruitful approach involves using differential algebra. This approach, which was introduced by Ljung and Glad [49] and Ollivier [55], is described briefly below.

The setup for an identifiability problem is as follows. A *model* consists of the following:

(i) parametrized differential equations – in our setting, mass-action differential equations (2) arising from a network G where the parameters are the rate constants, and
(ii) a specification of which compartments (e.g., species) have inflow rates that are controlled by the experimenter (these rates $u_i(t)$ are called *input* variables) and which are *output* variables (there must be at least one output variable). The reactions associated to the inflows are incorporated in the differential equations, while the specification of output variables yields additional equations called the *output equations*.

We assume that the resulting output vector $z(t)$ can be measured. That is, we assume perfect (noiseless) input-output data $(u(t), z(t))$.

The first step of the differential-algebra approach transforms the state space equations (that is, the differential equations of the model in which $u(t)$ is the vector of inflow-rate constants for all input vectors) into a system of differential equations, called the input-output equations, that involve only the parameters, input variables, output variables, and their derivatives. More precisely, the parametrized differential equations, the output equations, and each of their M derivatives (where M is the number of output variables) generate an ideal, and then, using Gröbner bases, all species concentrations (equivalently, state variables) except the input and output variables are eliminated (equivalently, the ideal is intersected with the subring with only input and output variables and their derivatives) [53].

Equations in this elimination ideal, the *input-output equations*, involve only the parameters, input variables, output variables, and their derivatives. Each input-output equation therefore has the following form:

$$\sum_i c_i(\kappa)\ \psi_i(u, z) = 0 \ ,$$

where the sum is finite, the coefficients $c_i(\kappa)$ are rational functions in the parameter vector $\kappa = (\kappa_1, \ldots, \kappa_r)$, and the $\psi_i(u, z)$'s are differential monomials in $u(t)$ and $z(t)$.

Another method for finding the input-output equations is to form the *characteristic set* [58]. This is a triangular system that generates the same dynamics as the original system. The equations in this triangular system that involve only the input variables, output variables, and parameters, generate the input-output equations. Also, if the derivatives of the state variables do not appear in the last n equations of the characteristic set (here n is the number of state variables), the model is *algebraically observable* [58], i.e., the last n equations of the characteristic set involve polynomials purely in $u(t), u'(t), ..., z(t), z'(t), ..., \kappa$, and $x_i(t)$ for each state variable $x_i(t)$. In this case, as stated in the literature, "one can, in principle, solve for $x_1, ..., x_n$ in the triangular set of algebraic equations recovering the state as an (instantaneous) function of the input-output variables and their derivatives" [58]. One can also define algebraic observability without reference to the characteristic set [16].

Regardless of the method of obtaining input-output equations, we choose M algebraically independent input-output equations (where M is again the number of output variables) [55], and then consider the vector of *all* of their coefficients $c = (c_1(\kappa), ..., c_T(\kappa))$. This induces a map $c : \mathbb{R}^r \to \mathbb{R}^T$, called the *coefficient map*.

The next step of the differential-algebra approach assumes that the coefficients $c_i(\kappa)$ of the input-output equations can be recovered uniquely from input-output data, and thus are presumed to be known quantities [62]. This assumption is reasonable because, given perfect data, we have values for $u(t), u'(t), u''(t), ...$ and $z(t), z'(t), z''(t), ...$ at many time instances. This results in a system of linear equations in the coefficients $c_i(\kappa)$, and so, for a general input function $u(t)$ and generic parameters, there is a unique solution for the coefficients $c_i(\kappa)$.

Therefore, the identifiability question is: can the parameters of the model be recovered from the coefficients of the input-output equations?

Definition 3.1 (Preliminary definition of identifiability)**.** Consider a model, and let c denote its coefficient map.

- The model is *generically globally identifiable* if there is a dense open subset $\Omega \subseteq \mathbb{R}^r$ such that $c : \Omega \to \mathbb{R}^T$ is one-to-one.
- The model is *generically locally identifiable* if there is a dense open subset $\Omega \subseteq \mathbb{R}^r$ such that around every $\kappa \in \Omega$ there is an open neighborhood $U_\kappa \subseteq \Omega$ such that $c : U_\kappa \to \mathbb{R}^T$ is one-to-one.
- The model is *generically unidentifiable* if there is a dense subset $\Omega \subseteq \mathbb{R}^r$ such that $c^{-1}(c(\kappa))$ is infinite for all $\kappa \in \Omega$.

This ability to distinguish between local and global identifiability sets the differential-algebra approach apart from other methods to analyze identifiability, such as the transfer function or similarity transformation approaches [7, 21], which can detect local identifiability only.

Identifiability is well defined:

Proposition 3.2 (Ollivier [55])**.** *Definition 3.1 does not depend on the choice of the algebraically independent input-output equations that define the coefficient map.*

Remark 3.3. In this paper, we focus on generic identifiability, so we will say "globally identifiable" in place of "generically globally identifiable". Similarly, "locally identifiable" or "unidentifiable" will mean generically so. Furthermore, for brevity, we will simply say "identifiable" when we mean "locally (respectively, globally) identifiable." The locus of non-generic parameters, for linear compartmental models, was analyzed in [34].

Remark 3.4. In many applications, it is reasonable to restrict the domain of the coefficient map c to some natural, open, biologically relevant parameter space $\Theta \subseteq \mathbb{R}^r$. For instance, $\Theta = \mathbb{R}^r_{>0}$ is an appropriate parameter space for the vector of rate constants κ. Here, however, we use $\mathbb{R}^r$ to be consistent with the literature on compartmental models.

In several results, we will use a notion of identifiability that generalizes Definition 3.1 in two ways. We now explain the motivation behind these two generalizations. First, we wish to allow for identifiability under "changes of variables" as follows. Consider two models $\mathcal{M}$ and $\mathcal{M}'$, where $\mathcal{M}'$ is identifiable. Assume also that starting from the ODEs of M, after replacing input variables u_i of $\mathcal{M}$ with some known functions $\hat{u}_i$ of measurable quantities (e.g., output variables), we obtain precisely the ODEs of $\mathcal{M}'$. Then, if we have input-output data $(u(t), u'(t), \ldots, z(t), z'(t), \ldots)$ at many time points for $\mathcal{M}$, we can compute $(\hat{u}(t), \hat{u}'(t), \ldots)$, and then use this as part of input-output data for $\mathcal{M}'$, thereby recovering the parameters. It is therefore reasonable to say that $\mathcal{M}$ is identifiable. Such an argument was used, for instance, in the proof of [54, Proposition 6].

Secondly, we will extend the definition of identifiability to allow for adding inputs. The motivation is as follows. Suppose a model $\mathcal{M}$ is obtained from a model $\mathcal{N}$ by adding one or more inputs. Then an experimenter could collect data from $\mathcal{M}$ *without* using the extra inputs, so these data would effectively arise from model $\mathcal{N}$. So, if $\mathcal{N}$ is identifiable, we also want to say that $\mathcal{M}$ is identifiable.

Accordingly, we allow both types of extension in the following recursive definition.

Definition 3.5. A model $\mathcal{M}$ is *locally (respectively, globally) identifiable* if $\mathcal{M}$ is locally (respectively, globally) identifiable as in Definition 3.1 or if there exist:

(1) a subset $\{\lambda_1, \ldots, \lambda_k\}$ of the set of parameters $\{\kappa_1, \ldots, \kappa_r\}$ of $\mathcal{M}$ (as shorthand, we write $\kappa = (\lambda, \mu) \in \mathbb{R}^k \times \mathbb{R}^{r-k}$),
(2) a dense open subset $\Omega \subseteq \mathbb{R}^r$, such that for all $\kappa^* = (\lambda^*, \mu^*) \in \Omega$, there exist only finitely many (respectively, a unique) $\lambda^{**} \in \mathbb{R}^k$ such that
$$c(\lambda^{**}, \mu^*) \;=\; c(\lambda^*, \mu^*)\ ,$$
where $c : \mathbb{R}^r \to \mathbb{R}^T$ is the coefficient map of $\mathcal{M}$,
(3) nested subsets $\{x_{i_1}, \ldots, x_{i_k}\} \subseteq \{x_{j_1}, \ldots, x_{j_\ell}\}$ of the state variables $\{x_1, \ldots, x_n\}$ of $\mathcal{M}$, such that $x_{i_1}, \ldots, x_{i_k}$ are *not* input variables of $\mathcal{M}$,
(4) an $\mathbb{R}^\ell$-valued function $g(\gamma, \widetilde{u};\ x_{j_1}, \ldots, x_{j_\ell})$ that depends on (a) a vector γ of some parameters of $\mathcal{M}$ that are disjoint from λ, (b) a vector $\widetilde{u}$ of some of the inputs of $\mathcal{M}$, and (c) the variables $x_{j_1}, \ldots, x_{j_\ell}$,
(5) a non-constant function f_i (for every $i = 1, \ldots, p$) of the input and output variables of $\mathcal{M}$, their derivatives, and also the λ_i's,

such that the following hold:

(i) the ODEs of $\mathcal{M}$ for the state variables $x_{j_1}, \ldots, x_{j_\ell}$ are as follows:
$$\begin{pmatrix} x'_{j_1} \\ \vdots \\ x'_{j_\ell} \end{pmatrix} \;=\; g(\gamma, \widetilde{u};\ x_{j_1}, \ldots, x_{j_\ell}) + (f_1 \mathbf{e}_{i_1} + \cdots + f_\ell \mathbf{e}_{i_k})\ , \tag{7}$$
where $\mathbf{e}_i$ denotes the i-th canonical basis vector in $\mathbb{R}^r$,

(ii) when each f_p in the equations (7) is replaced by a new variable $\hat{u}_{i_p}$, then the resulting ODEs are those of a model $\mathcal{M}'$ (with state variables $x_{j_1}, \ldots, x_{j_\ell}$, parameters γ, and inputs $\widetilde{u}$ and $\hat{u}$), and
(iii) when $\mathcal{M}'$ is taken so that the output variables are precisely those of $\mathcal{M}$ in $\{x_{j_1}, \ldots, x_{j_\ell}\}$, then $\mathcal{M}'$ is locally (respectively, globally) identifiable or can be obtained from some locally (respectively, globally) identifiable model by adding one or more inputs.

We do not know whether Definition 3.5 encompasses more models than Definition 3.1, so we pose the question here.

Question 3.6. Is there a model that is identifiable in the sense of Definition 3.5, but not in the sense of Definition 3.1?

The differential-algebra approach to identifiability has been used to analyze models in systems biology, e.g., via the software `DAISY` of Bellu *et al.* [6] (see also software comparisons in [41]), but has received surprisingly little attention in the reaction network community. That is not to say that few identifiability analyses have been performed on reaction networks, only that such investigations used other techniques [8, 12], focused on somewhat different questions, or both [32]. One such work is that of Craciun and Pantea, which we describe now.

Craciun and Pantea answered the following questions: when can the rate constants of a reaction network be recovered given its dynamics, and also when can the reaction network itself (the set of reactions, but not their rate constants) be recovered from its dynamics [10]? For the former question, the "dynamics" refers to time-course data $x(t)$ (all variables are therefore viewed as output, i.e., measurable, variables). This is a natural starting point when considering identifiability problems arising from reaction networks. Also, their results yield sufficient conditions for a network to be unidentifiable (in the sense of Definition 3.1), i.e. if the network is unidentifiable with all state variables measured, then the network is unidentifiable when only a subset of state variables are measured. These results, to our knowledge, are the only general results pertaining to identifiability of reaction networks.

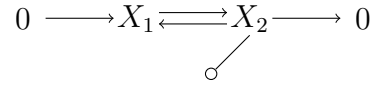
In this section, we prove more results that apply to general networks. Note, however, that our setup differs from that of [10]: we assume the network is known, but that only some of the concentrations $x_i(t)$ can be measured, and then aim to recover the rate constants.

More precisely, we focus on models $(G, \mathcal{I}, \mathcal{O})$ defined by a reaction network $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$, input set $\mathcal{I} \subseteq \mathcal{S}$, and output set $\mathcal{O} \subseteq \mathcal{S}$. Also, we make the following assumption:

the set of input species consists of all inflow-reaction species, i.e.:
$$\mathcal{I} \;=\; \{X_i \mid 0 \to X_i \text{ is a reaction in } G\}.$$

A model therefore is specified by a network G and its output-species set $\mathcal{O}$, and so we will write $(G, \mathcal{O})$ in place of $(G, \mathcal{I}, \mathcal{O})$.

Notation 3.7. Following the literature, we indicate output species, when depicting reaction networks, by this symbol: $\checkmark$. For instance, the monomolecular network depicted below, which arises from the network $G = \{0 \to X_1 \rightleftarrows X_2 \to 0\}$, has one input species ($\mathcal{I} = \{X_1\}$) and one output species ($\mathcal{O} = \{X_2\}$):



Thus, the inflow rate of the reaction $0 \to X_1$, denoted by $u_1(t)$, is assumed to be controllable, whereas the other three reaction rates are fixed constants:

$$0 \xrightarrow{u_1(t)} X_1 \underset{\kappa_{21}}{\overset{\kappa_{12}}{\rightleftarrows}} X_2 \xrightarrow{\kappa_{20}} 0$$

Remark 3.8. In contrast with the general setup for identifiability analysis, the leaks in our setting are specified by the network G itself, and thus need not be specified separately.

3.2. **Prior results.** This subsection compiles two results, from our work [33], on identifiability of monomolecular reaction networks (i.e., linear compartmental models). We will use these results to prove results on joining networks.

Proposition 3.11, which is [33, Theorem 3.8], states that an input-output equation involving an output variable z_i corresponds to an input-output equation arising from the "output-reachable subgraph" to z_i.

Definition 3.9. For a linear compartmental model $\mathcal{M} = (\mathfrak{G}, In, Out, Leak)$, let $i \in Out$. The *output-reachable subgraph to* i (or *to* z_i) is the induced subgraph of $\mathfrak{G}$ containing all vertices j for which there is a directed path in $\mathfrak{G}$ from j to i.

Definition 3.10. For a linear compartmental model $\mathcal{M} = (\mathfrak{G}, In, Out, Leak)$, let $H = (V_H, E_H)$ be an induced subgraph of G that contains at least one output. The *restriction* of $\mathcal{M}$ to H, denoted by $\mathcal{M}_H$, is obtained from $\mathcal{M}$ by removing all incoming edges to $\mathfrak{G}$, retaining all leaks and outgoing edges (which become leaks), and retaining all inputs and outputs in $\mathfrak{G}$; that is,

$$\mathcal{M}_H \;:=\; (H,\; In_H,\; Out_H,\; Leak_H)\;,$$

where $In_H := In \cap V_H$ and $Out_H := Out \cap V_H$, and the leak set is

$$Leak_H \;:=\; (Leak \cap V_H) \cup \{i \in V_H \mid (i,j) \in E(\mathfrak{G}) \text{ for some } j \notin V_H\}\;.$$

Also, the labels of edges in H are inherited from those of $\mathfrak{G}$, and labels of leaks are:

$$\text{label of leak from } k^{\text{th}} \text{ compartment} \;=\; \begin{cases} a_{0k} + \sum_{\{j \notin V_H \mid (k,j) \in E(\mathfrak{G})\}} a_{jk} & \text{if } k \in Leak \cap V_H \\ \sum_{\{j \notin V_H \mid (k,j) \in E(\mathfrak{G})\}} a_{jk} & \text{if } k \notin Leak \cap V_H\;. \end{cases}$$

Proposition 3.11 (Input-output equations [33])**.** *Let $\mathcal{M} = (\mathfrak{G}, In, Out, Leak)$ be a linear compartmental model. Let $i \in Out$, and assume that there exists a directed path in $\mathfrak{G}$ from some input compartment to compartment-i. Let $H = (V_H, E_H)$ denote the output-reachable subgraph to z_i, and let A_H denote the compartmental matrix for the restriction $\mathcal{M}_H$. Assume $In \cap V_H$ is nonempty. Define ∂I to be the $|V_H| \times |V_H|$ matrix in which every diagonal entry is the differential operator d/dt and every off-diagonal entry is 0. Then the following is an input-output equation for $\mathcal{M}$:*

$$\det(\partial I - A_H) z_i \;=\; \sum_{j \in In \cap V_H} (-1)^{i+j} \det\left(\partial I - A_H\right)_{ji} u_j\;, \tag{8}$$

where $(\partial I - A_H)_{ji}$ denotes the matrix obtained from $(\partial I - A_H)$ by removing the row corresponding to compartment-j and the column corresponding to compartment-i. Thus, this input-output equation (8) *involves only the output-reachable subgraph to z_i.*

The next result, which is [33, Theorem 4.3], analyzes the effect of adding an outflow.

Definition 3.12. The *non-flow subnetwork* of a reaction network G is the subnetwork obtained by removing from G the zero complex, all outflow reactions (leaks), and inflows.

Lemma 3.13 (Adding one outflow [33])**.** *Let $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a monomolecular reaction network with no outflow reactions and at least one inflow reaction. Assume that the non-flow subnetwork of G is strongly connected. Let $\mathcal{O} \subseteq \mathcal{S}$, and let $\widetilde{G}$ be obtained from G by adding one outflow reaction. Then, if $(G, \mathcal{O})$ is generically locally identifiable, then so is $(\widetilde{G}, \mathcal{O})$.*

3.3. **Joining by replacing reactions.** This section considers the question, *After joining two identifiable networks by replacing reactions, is the resulting network identifiable?* Theorem 3.15 states that the answer is 'yes' if the two networks are joined by a "one-way flow" (see Definition 3.14), the two networks have disjoint sets of species, and the first network is algebraically observable.

Let us explain what we mean by a "one-way flow". There are four scenarios. In the first, one or more outflow reactions (leaks) $X_i \to 0$ in one network correspond to some $0 \to X_j$'s in the other network, i.e. each leak in the first network is an input in the second. Joining these networks therefore creates new reactions $X_i \to X_j$, as summarized here:

SCENARIO 1: Joining $\boxed{\begin{array}{c} X_i \to 0 \\ \vdots \end{array}}$ and $\boxed{\begin{array}{c} 0 \to X_j \\ \vdots \end{array}}$ yields $\boxed{\begin{array}{c} X_i \to X_j \\ \vdots \end{array}}$

In the second scenario, certain reactions $X_i \to 0$ are replaced by new reactions $X_i \to X_j$:

SCENARIO 2: Joining $\boxed{\begin{array}{c} X_i \to 0 \\ \vdots \end{array}}$ and $\boxed{\begin{array}{c} X_j \\ \vdots \end{array}}$ yields $\boxed{\begin{array}{c} X_i \to X_j \\ \vdots \end{array}}$

In the third scenario, the new reactions $X_i \to X_j$ are added, and none are replaced:

SCENARIO 3: Joining $\boxed{\begin{array}{c} X_i \\ \vdots \end{array}}$ and $\boxed{\begin{array}{c} X_j \\ \vdots \end{array}}$ yields $\boxed{\begin{array}{c} X_i \to X_j \\ \vdots \end{array}}$

In the fourth scenario, certain reactions $0 \to X_j$ are replaced by new reactions $X_i \to X_j$:

SCENARIO 4: Joining $\boxed{\begin{array}{c} X_i \\ \vdots \end{array}}$ and $\boxed{\begin{array}{c} 0 \to X_j \\ \vdots \end{array}}$ yields $\boxed{\begin{array}{c} X_i \to X_j \\ \vdots \end{array}}$

Here we define these scenarios precisely:

Definition 3.14. Let $N_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1)$ and $N_2 = (\mathcal{S}_2, \mathcal{C}_2, \mathcal{R}_2)$ be reaction networks with disjoint sets of species $\mathcal{S}_1 = \{X_1, \dots, X_m\}$ and $\mathcal{S}_2 = \{X_{m+1}, \dots, X_n\}$. A network G is obtained by *joining N_1 and N_2 by a one-way flow* if there exist a nonempty subset $\mathfrak{I} \subseteq [m]$ and a function $\phi : \mathfrak{I} \to \{m+1, \dots, n\}$ such that one of the following holds:

- SCENARIO 1: The set $\mathcal{R}_1' := \{X_i \to 0 \mid i \in \mathfrak{I}\}$ is a set of outflow reactions of N_1, the set $\mathcal{R}_2' := \{0 \to X_{\phi(i)} \mid i \in \mathfrak{I}\}$ is a set of inflow reactions of N_2, and G is obtained by joining N_1 and N_2 by replacing $\mathcal{R}_1' \cup \mathcal{R}_2'$ by $\{X_i \to X_{\phi(i)} \mid i \in \mathfrak{I}\}$.

- SCENARIO 2: The set $\mathcal{R}_1' := \{X_i \to 0 \mid i \in \mathfrak{I}\}$ is a set of outflow reactions of N_1, and G is obtained from N_1 and N_2 by replacing $\mathcal{R}_1'$ by $\{X_i \to X_{\phi(i)} \mid i \in \mathfrak{I}\}$.
- SCENARIO 3: G is obtained by joining N_1 and N_2 by the new reactions $\{X_i \to X_{\phi(i)} \mid i \in \mathfrak{I}\}$.
- SCENARIO 4: The set $\mathcal{R}_2' := \{0 \to X_{\phi(i)} \mid i \in \mathfrak{I}\}$ is a set of inflow reactions of N_2, and G is obtained from N_1 and N_2 by replacing $\mathcal{R}_2'$ by $\{X_i \to X_{\phi(i)} \mid i \in \mathfrak{I}\}$.

Recall our assumption that the set of input species in a model consists of all inflow-reaction species. Then this set, for the network obtained by joining by a one-way flow (Definition 3.14), is as follows. Let $\mathcal{I}_i \subseteq \mathcal{S}_i$ be the input-species set for species set $\mathcal{S}_i$ for $i \in \{1, 2\}$. Let

$$\mathcal{I}_2' \;:=\; \begin{cases} \mathcal{I}_2 - \{X_{\phi(i)} \mid i \in \mathfrak{I}\} & \text{if } G \text{ is obtained via Scenario 1 or 4} \\ \mathcal{I}_2 & \text{if } G \text{ is obtained via Scenario 2 or 3.} \end{cases}$$

Then the input-species set for the joined network G is $\mathcal{I}_1 \cup \mathcal{I}_2'$.

Consider a network G obtained by joining $N_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1)$ and $N_2 = (\mathcal{S}_2, \mathcal{C}_2, \mathcal{R}_2)$ by a one-way flow (via a joining function $\phi : \mathfrak{I} \to \{m+1, \ldots, n\}$). Let $\mathcal{O}_1 \subseteq \mathcal{S}_1$ and $\mathcal{O}_2 \subseteq \mathcal{S}_2$ be nonempty. Then $(G, \mathcal{O}_1 \cup \mathcal{O}_2)$ is the *model obtained by joining* $(N_1, \mathcal{O}_1)$ *and* $(N_2, \mathcal{O}_2)$ (via ϕ).

Our first main result generalizes [54, Proposition 6], which analyzed a subcase of Scenario 1.

Theorem 3.15. *Let $N_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1)$ and $N_2 = (\mathcal{S}_2, \mathcal{C}_2, \mathcal{R}_2)$ be reaction networks with disjoint sets of species. Let $\mathcal{O}_1 \subseteq \mathcal{S}_1$ and $\mathcal{O}_2 \subseteq \mathcal{S}_2$ be nonempty. Assume $(N_1, \mathcal{O}_1)$ is algebraically observable. Let G be a network obtained by joining N_1 and N_2 by a one-way flow via Scenario 1 or 2. Then, if $(N_1, \mathcal{O}_1)$ and $(N_2, \mathcal{O}_2)$ are identifiable, then $(G, \mathcal{O}_1 \cup \mathcal{O}_2)$ is identifiable.*

Proof. Let N_1, N_2, and G be as in the statement of the theorem. Then network G arises, as in Definition 3.14, by way of a set $\mathfrak{I}$ and a joining function ϕ.

We consider first the case of Scenario 1. We write the ODEs of N_1 as follows:

$$\begin{pmatrix} x_1' \\ \vdots \\ x_m' \end{pmatrix} \;=\; f(\alpha, u^{(1)};\; x_1, \ldots, x_m) - \sum_{i \in \mathfrak{I}} \beta_i x_i \mathbf{e}_i \;, \tag{9}$$

where $u^{(1)} = u^{(1)}(t)$ is the input vector (that is, the experimenter-controlled vector of inflow rates for the species in $\mathcal{I}_1$), α is the vector of non-inflow rate constants for reactions *not* in $\mathcal{R}_1' = \{X_i \to 0 \mid i \in \mathfrak{I}\}$, and β_i, for $i \in \mathfrak{I}$, denotes the rate constant for the outflow reaction $X_i \to 0$ in $\mathcal{R}_1'$. Also, $\mathbf{e}_i$ denotes the i-th canonical basis vector.

Similarly, we write the ODEs of N_2 as follows (recall that we are in Scenario 1):

$$\begin{pmatrix} x_{m+1}' \\ \vdots \\ x_n' \end{pmatrix} \;=\; g(\gamma, u^{(2)};\; x_{m+1}, \ldots, x_n) + \sum_{j \in \phi(\mathfrak{I})} \widetilde{u}^{(2)}_{0 \to X_j} \mathbf{e}_j \;, \tag{10}$$

where γ is the input vector of non-inflow rate constants, $\widetilde{u}^{(2)}_{0 \to X_j} = \widetilde{u}^{(2)}_{0 \to X_j}(t)$, for $j \in \phi(\mathfrak{I})$, is the (controlled) rate for the to-be-replaced reaction $0 \to X_j$, and $u^{(2)} = u^{(2)}(t)$ is the vector of all remaining inflow rates.

The joined network G has ODEs as follows:

$$\begin{pmatrix} x_1' \\ \vdots \\ x_n' \end{pmatrix} = \begin{pmatrix} f(\alpha, u^{(1)};\ x_1, \ldots, x_m) \\ g(\gamma, u^{(2)};\ x_{m+1}, \ldots, x_n) \end{pmatrix} - \sum_{i \in \mathfrak{I}} \beta_i x_i (\mathbf{e}_i - \mathbf{e}_{\phi(i)}) \ . \tag{11}$$

Notice that the first m of the ODEs of G are equal to the ODEs of N_1, as given in (9).

We claim that identifiability of $(N_1, \mathcal{O}_1)$ implies identifiability of the rate constants of the vectors α and β of G. To see this, we consider a coefficient map c_{N_1} for N_1 arising from a choice of $|\mathcal{O}_1|$ algebraically independent input-output equations of N_1 (which are also input-output equations of G), and then extend it to a coefficient map $(c_{N_1}, \widetilde{c})$ for G by extending to a set of $|\mathcal{O}_1 \cup \mathcal{O}_2|$ algebraically independent input-output equations of G. Thus, since c_{N_1} is generically locally (respectively, globally) one-to-one, thereby allowing the vectors α and β to be recovered for N_1, we conclude that α and β can be recovered for G.

Thus, to finish the proof in Scenario 1, we need only show that identifiability of $(N_2, \mathcal{O}_2)$ implies identifiability of the rate constants γ for G. The last $(n-m)$ ODEs of G, from equation (11), are:

$$\begin{aligned} \begin{pmatrix} x_{m+1}' \\ \vdots \\ x_n' \end{pmatrix} &= g(\gamma, u^{(2)};\ x_{m+1}, \ldots, x_n) + \sum_{i \in \mathfrak{I}} \beta_i x_i \mathbf{e}_{\phi(i)} \\ &= g(\gamma, u^{(2)};\ x_{m+1}, \ldots, x_n) + \sum_{j=m+1}^{n} \left(\sum_{\{i \in \mathfrak{I} | \phi(i) = j\}} \beta_i x_i \right) \mathbf{e}_j \ . \end{aligned} \tag{12}$$

As N_1 is algebraically observable, the state variables $x_1, \ldots, x_m$ can be written as a function of $u^{(1)}, z^{(1)}, \alpha$, and β. Therefore, for $j \in \phi(\mathfrak{I})$, the sum $\sum_{\{i \in \mathfrak{I} | \phi(i) = j\}} \beta_i x_i$ is a function of $u^{(1)}, z^{(1)}, \alpha$, and β, and so we may treat these sums as known quantities or as controlled inflow rates, thereby recovering the parameters γ. More precisely, for $j \in \phi(\mathfrak{I})$, letting $\hat{u}_j := \sum_{\{i \in \mathfrak{I} | \phi(i) = j\}} \beta_i x_i$, then the last $(n-m)$ ODEs of G, in (12), match those of the identifiable network N_2, in (10). Hence, by Definition 3.5, G is identifiable.

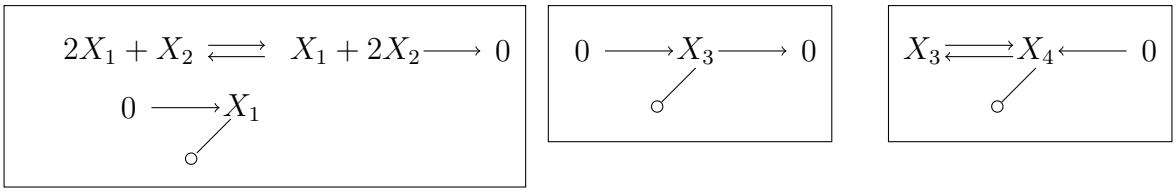
For Scenario 2, let N_3 be obtained from N_2 by adding inflows $0 \to X_j$ (inputs) for all $j \in \phi(\mathfrak{I})$. Then, by definition, N_3 is identifiable, and G is obtained from N_1 and N_3 by a one-way flow via Scenario 1. So, following the above proof (for Scenario 1), G is identifiable. □

We define inductively what it means to join several networks by a one-way flow. A network is obtained by *joining networks $N_1, \ldots, N_p$ by a one-way flow* if it results from joining, by a one-way flow, N_1 and a network obtained by joining $N_2, \ldots, N_p$ by a one-way flow. Similarly, a model obtained by *joining models $(N_1, \mathcal{O}_1), \ldots, (N_p, \mathcal{O}_p)$ by a one-way flow* arises from a network obtained by joining $N_1, \ldots, N_p$ by a one-way flow, and the output set is $\mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p$.

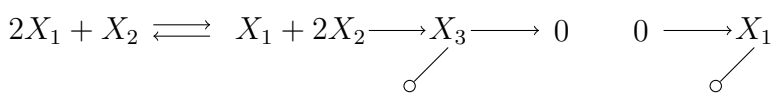
Now the following result is immediate from Theorem 3.15:

Corollary 3.16. *Let $N_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1), \ldots, N_p = (\mathcal{S}_p, \mathcal{C}_p, \mathcal{R}_p)$ be reaction networks with pairwise disjoint sets of species. Let $\mathcal{O}_i \subseteq \mathcal{S}_i$ be nonempty for $i = 1, ..., p$. Assume $(N_1, \mathcal{O}_1), \ldots, (N_{p-1}, \mathcal{O}_{p-1})$ are algebraically observable. Let G be a network obtained by joining $N_1, ..., N_p$ by a one-way flow via Scenario 1 or 2. Then, if $(N_1, \mathcal{O}_1), ..., (N_p, \mathcal{O}_p)$ are identifiable, then $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ is identifiable.*

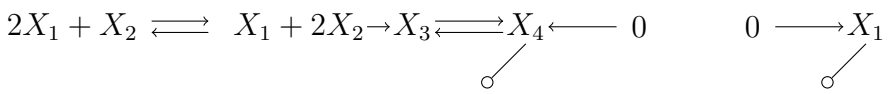
Example 3.17. Consider three networks, which we call $(N_1, \mathcal{O}_1)$, $(N_2, \mathcal{O}_2)$, and $(N_3, \mathcal{O}_3)$:



Each *model* is globally identifiable and $(N_1, \mathcal{O}_1)$ is algebraically observable (e.g., using DAISY [6]). So, by Theorem 3.15, the model depicted below, which is obtained by joining N_1 and N_2 via Scenario 1 (by replacing the reactions $X_1 + 2X_2 \to 0$ and $0 \to X_3$ by the reaction $X_1 + 2X_2 \to X_3$), is also globally identifiable:

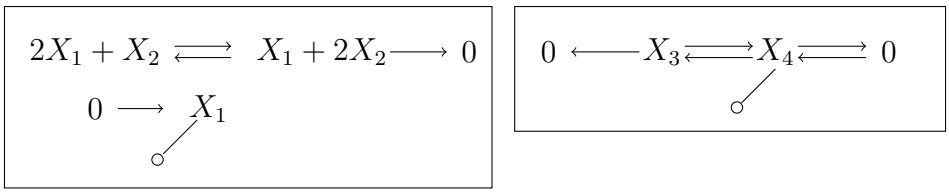


Similarly, by the same theorem, joining N_1 and N_3 via Scenario 2 (by replacing $X_1+2X_2 \to 0$ by $X_1 + 2X_2 \to X_3$), yields a model that is globally identifiable:

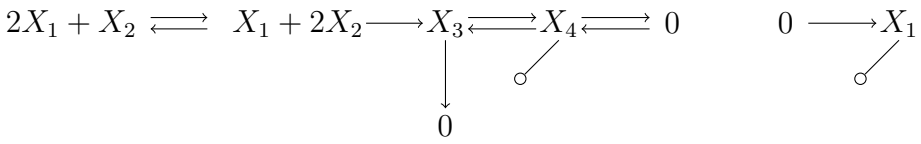


Informally, Theorem 3.15 above stated the following: assuming that $(N_1, \mathcal{O}_1)$ is algebraically observable, if identifiable networks N_1 and N_2 are joined via Scenario 1 or 2, then the result is still identifiable. We now consider the converse: If the joined model is identifiable, can we conclude that $(N_1, \mathcal{O}_1)$ and $(N_2\mathcal{O}_2)$ are also identifiable? For N_2, in general, we can not (see Example 3.18 below and Example 3.26 in the next subsection); but under extra hypotheses, we can (see Theorem 3.28 in the next subsection). As for N_1, we give a counterexample in the next subsection (see Example 3.25).

Example 3.18. Consider two models, which we call $(N_1, \mathcal{O}_1)$ and $(N_2, \mathcal{O}_2)$:



The first model is the same as in the previous example, which we noted is algebraically observable. The model below, obtained by joining N_1 and N_2 via Scenario 2 (by replacing $X_1 + 2X_2 \to 0$ with $X_1 + 2X_2 \to X_3$) is globally identifiable (e.g., using DAISY [6]):



However, $(N_2, \mathcal{O}_2)$ is unidentifiable [54].

3.4. **Monomolecular networks.** The previous subsection focused on networks G formed by joining two networks by a one-way flow via Scenario 1 or 2. We examined the extent to which identifiability can be "transferred" from subnetworks N_i to G (Theorem 3.15).

The current subsection considers the case when all networks are monomolecular (the case of linear compartmental models). In this setting, we obtain stronger conclusions than in Theorem 3.15 (see Theorems 3.23 and 3.28). We also consider more scenarios for joining by a one-way flow (Theorem 3.30 and Theorem 3.33). We informally summarize our results as follows: *Let G be obtained by joining monomolecular networks N_1 and N_2 by a one-way flow via Scenario 1, 2, 3, or 4. Then (1) if N_1 and N_2 are identifiable, then G is identifiable, and (2) if N_1 and G are identifiable in the case of Scenario 1 or 4, then N_2 is identifiable.* (For the precise statements, see Theorems 3.23, 3.28, 3.30, and 3.33 and Corollary 3.34).

Remark 3.19. The results in the rest of this section pertain to monomolecular networks that have at least one inflow reaction (i.e., at least one input). This requirement allows us to use a prior result pertaining to input-output equations (Proposition 3.11). (Recall that we already required, in Section 3.1, that every model has at least one output.)

3.4.1. *Joining output connectable, monomolecular networks via Scenario 1 or 2.* The results in the previous subsection required some of the models $(N_i, \mathcal{O}_i)$ to be algebraically observable. This condition is in general difficult to verify, but automatically holds for monomolecular networks that satisfy a condition that is easier to check, namely, being "output connectable" (Definition 3.20 and Lemma 3.21). Therefore, we can state a version of Corollary 3.16 for monomolecular networks (see Theorem 3.23).

Definition 3.20. A linear compartmental model is *output connectable* if every compartment has a directed path leading from it to an output compartment [30].

Thus, a monomolecular-reaction-network model $(G, \mathcal{O})$ is output connectable if for every species X_i there is a directed path in G from X_i to some output species $X_j \in \mathcal{O}$. Such models are algebraically observable:

Lemma 3.21. *Let $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a monomolecular reaction network, and let $\mathcal{O} \subseteq \mathcal{S}$ be nonempty. If $(G, \mathcal{O})$ is output connectable, then $(G, \mathcal{O})$ is algebraically observable.*

We prove Lemma 3.21 in Appendix A, where the lemma is restated as follows: *Every output connectable linear compartmental model is algebraically observable* (Corollary A.3).

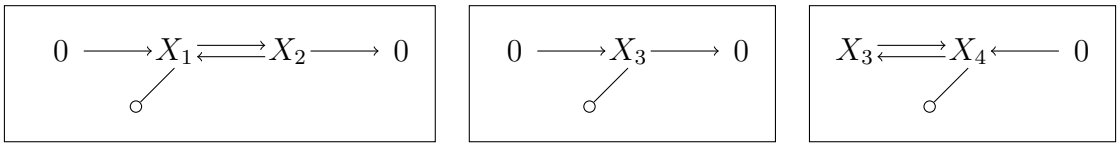
Remark 3.22. A linear compartmental model is output connectable if and only if it is structurally observable [30]. Lemma 3.21 therefore extends this result to algebraic observability. In fact, for such models, we give explicit algebraic-observability relationships for each state variable in terms of inputs, outputs, and parameters (see Proposition 3.27 and its proof).

Theorem 3.23. *Let $N_1, \ldots, N_p$ be monomolecular networks with pairwise disjoint sets of species $\mathcal{S}_1, \ldots, \mathcal{S}_p$. Let $\mathcal{O}_i \subseteq \mathcal{S}_i$ be nonempty for $i = 1, \ldots, p$. Assume that, for $i = 1, \ldots, p-1$, the network N_i has at least one inflow reaction and $(N_i, \mathcal{O}_i)$ is output connectable. Let G be a network obtained by joining $N_1, \ldots, N_p$ by a one-way flow via Scenario 1 or 2. Then, if $(N_1, \mathcal{O}_1)$, $\ldots$, $(N_p, \mathcal{O}_p)$ are identifiable, then $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ is identifiable.*

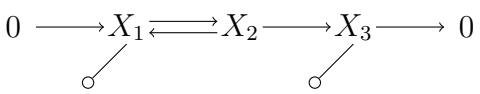
Proof. This follows directly from Corollary 3.16 and Lemma 3.21. □

Output connectable models include models arising from strongly connected graphs (more precisely, when the non-flow subnetwork is strongly connected). See the following examples.

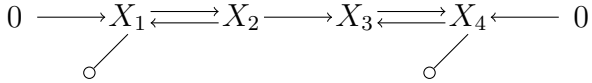
Example 3.24. Consider three models, which we call $(N_1, \mathcal{O}_1)$, $(N_2, \mathcal{O}_2)$, and $(N_3, \mathcal{O}_3)$:



Each model is identifiable [54], has one inflow reaction, and has strongly connected non-flow subnetwork. So, by Theorem 3.23, the model depicted below, which is obtained by joining N_1 and N_2 via Scenario 1 (by replacing the reactions $X_2 \to 0$ and $0 \to X_3$ by the reaction $X_2 \to X_3$), is also identifiable:

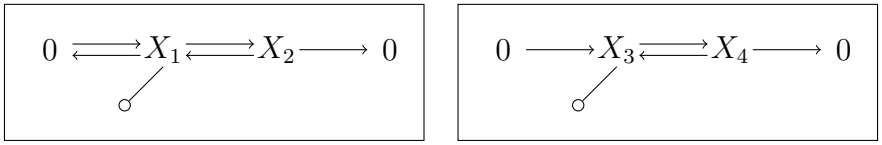


Similarly, by the same theorem, joining N_1 and N_3 via Scenario 2 (by replacing $X_2 \to 0$ by $X_2 \to X_3$), yields a model that is identifiable:

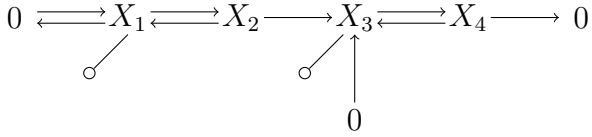


The next examples show that partial converses to Theorem 3.23 do *not* hold: in Scenario 2, if $(G, \mathcal{O}_1 \cup \mathcal{O}_2)$ is identifiable, it does not follow that N_1 is identifiable, nor N_2.

Example 3.25. Consider two models, which we call $(N_1, \mathcal{O}_1)$ and $(N_2, \mathcal{O}_2)$:

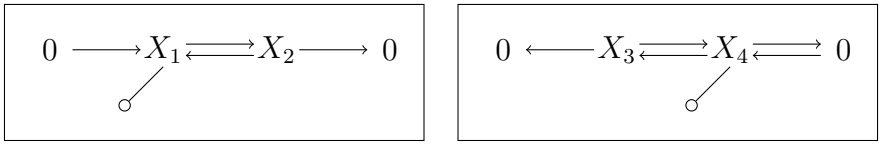


Each N_i has one inflow reaction and has strongly connected non-flow subnetwork. The model below, obtained by joining N_1 and N_2 via Scenario 2 (by replacing $X_2 \to 0$ with $X_2 \to X_3$) is at least locally identifiable [54]:

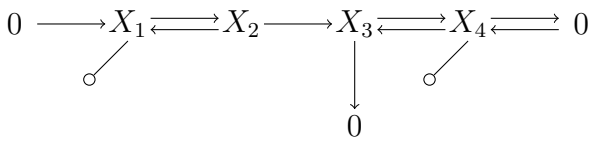


However, $(N_1, \mathcal{O}_1)$ is unidentifiable [54]. (On the other hand, it is straightforward to check that $(N_2, \mathcal{O}_2)$ is globally identifiable.)

Example 3.26. Consider two models, which we call $(N_1, \mathcal{O}_1)$ and $(N_2, \mathcal{O}_2)$:



Each N_i has one inflow reaction, with strongly connected non-flow subnetwork. The model below, obtained by joining N_1 and N_2 via Scenario 2 (by replacing $X_2 \to 0$ with $X_2 \to X_3$), is at least locally identifiable [54]:



However, $(N_2, \mathcal{O}_2)$ is unidentifiable [54]. (The model $(N_1, \mathcal{O}_1)$ is globally identifiable, as it is equivalent to the model $(N_1, \mathcal{O}_2)$ in Example 3.25.)

In Theorem 3.23, we saw that if identifiable, output connectable, monomolecular networks N_i are joined by a one-way flow (via Scenario 1 or 2), then the result is still identifiable. The next main result, Theorem 3.28, states that if N_1 and each of the inductively joined networks N_1 and N_2, N_1 and N_2 and N_3, etc., are identifiable, we also conclude that $N_2, N_3, ...$ are identifiable – as long as we are in Scenario 1 and the joining is "in a row" over a single reaction. In contrast, in Scenario 2, we can not obtain the same conclusion (recall Example 3.26).

To prove Theorem 3.28, we need the following strengthening of [54, Lemma 3].

Proposition 3.27 (Equations for algebraic observability)**.** *Let $G = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a monomolecular network, and let $\mathcal{O} \subseteq \mathcal{S}$ be nonempty. Assume that there exists a species $i \in \mathcal{S}$ such that for every species $X_j \in \mathcal{S} \setminus \{X_i\}$, there exists a sequence of reactions $X_j \to \cdots \to X_i$ in G from X_j to X_i. Then for every such $X_j \in \mathcal{S} \setminus \{X_i\}$, there exists an equation of the form $x_j = g$ that holds (for generic values of the rate constants) along all solutions of $(G, \mathcal{O})$, where g is a $\mathbb{Q}(\{\kappa_{lk} \mid l \to k \text{ is a reaction in } G\})$-linear combination of x_i and the inflow-reaction variables u_p (for inflow reactions $0 \to X_p$) and their derivatives $x_i^{(q)}$ and $u_p^{(q)}$, and the coefficient of at least one of the $x_i^{(q)}$'s is nonzero.*

We prove Proposition 3.27 in the appendix.

The next result pertains to networks joined by a one-way flow "in a row". For networks $N_1, \ldots, N_p$ joined by a one-way flow, we say they are joined *in a row* if the new reactions are from N_1 to N_2, from N_2 to N_3, and so on; more precisely, the joining functions $\phi_q : \mathfrak{I}_q \to \{i \mid X_i \in \mathcal{S}_{q+1} \cup \cdots \cup \mathcal{S}_p\}$ (for $q = 1, \ldots, p-1$) satisfy $\phi(\mathfrak{I}_q) \subseteq \{i \mid X_i \in \mathcal{S}_{q+1}\}$.

We also require a stronger condition than output connectable, where each of the networks formed by joining N_1, N_2, ..., N_k, for $k = 1, ..., p-1$, is output connectable, which can be considered as *inductively output connectable*.

Theorem 3.28. *Let G be a network obtained by joining, in a row, monomolecular networks $N_1, \ldots, N_p$ with pairwise disjoint sets of species $\mathcal{S}_1, \ldots, \mathcal{S}_p$ by a one-way flow – but only via Scenario 1. Let $\mathcal{O}_1 \subseteq \mathcal{S}_1, \ldots, \mathcal{O}_p \subseteq \mathcal{S}_p$ be nonempty. Assume the following:*

(1) each joining by a one-way flow is over a single reaction,
(2) every N_i (for $i = 1, \ldots, p$) has at least one inflow reaction,
(3) for every $X_\ell \in \mathcal{O}_i$ (for any $i = 1, \ldots, p$) there is a directed path in N_i from an inflow-reaction (input) species to X_ℓ,
(4) for $q = 1, \ldots, p-1$, there exists a species $X_{i_q} \in \mathcal{O}_q$ such that for every species $X_j \in \mathcal{S}_1 \cup \cdots \cup \mathcal{S}_q \setminus \{X_{i_q}\}$, there exists a sequence of reactions $X_j \to \cdots \to X_{i_q}$ in G from X_j to X_{i_q},

(5) *the following* $p-1$ *models are identifiable:* $(N_1, \mathcal{O}_1)$*, the model obtained by joining* $(N_1, \mathcal{O}_1)$ *and* $(N_2, \mathcal{O}_2)$*, ..., and the model obtained by joining* $(N_1, \mathcal{O}_1)$*,* $(N_2, \mathcal{O}_2)$*, ...* $(N_{p-1}, \mathcal{O}_{p-1})$ *(via the same joining functions as for* G*).*

Then $(N_2, \mathcal{O}_2), \ldots, (N_p, \mathcal{O}_p)$ *are all identifiable if and only if* $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ *is identifiable.*

Proof. The forward direction ("⇒") follows from Theorem 3.23.

For the backward direction ("⇐"), assume that $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ is identifiable. We prove by induction that $(N_2, \mathcal{O}_2)$, ..., $(N_p, \mathcal{O}_p)$ are identifiable. By assumption $(N_1, \mathcal{O}_1)$ is identifiable. So, for induction, assume that $(N_{r-1}, \mathcal{O}_{r-1})$ is identifiable for some $2 \leq r \leq p$. We must show that $(N_r, \mathcal{O}_r)$ is identifiable.

The N_i's are joined "in a row", so we let M denote the network obtained by joining $N_1, \ldots, N_{r-1}$ by a one-way flow, and let $\widetilde{M}$ be obtained from joining M and N_r (via the same joining functions as for G). By hypothesis, $\widetilde{M}$ is obtained from joining M and N_r over a single reaction: for some species X_i and $X_{j'}$, the outflow reaction $X_i \to 0$ in M and the inflow (input) reaction $0 \to X_{j'}$ are replaced by the new reaction $X_i \to X_{j'}$. Also by hypothesis, $(\widetilde{M}, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_r)$ is identifiable.

Let n and m denote the number of species of, respectively, $\widetilde{M}$ and M. Following the proof of Theorem 3.15, specifically, from equation (10), the ODEs of N_r are as follows:

$$\begin{pmatrix} x'_{m+1} \\ \vdots \\ x'_n \end{pmatrix} \;=\; g(\gamma, u^{(2)};\; x_{m+1}, \ldots, x_n) + \widetilde{u}^{(2)}_{0 \to X_{j'}} \mathbf{e}_{j'} \;, \tag{13}$$

where γ is the input vector of non-inflow rate constants, and $\widetilde{u}^{(2)}_{0 \to X_{j'}}$ is the rate for the reaction $0 \to X_{j'}$ and $u^{(2)} = u^{(2)}(t)$ is the vector of all remaining inflow rates.

Similarly, using equation (12), the last $(n-m)$ ODEs of $\widetilde{M}$ are:

$$\begin{pmatrix} x'_{m+1} \\ \vdots \\ x'_n \end{pmatrix} \;=\; g(\gamma, u^{(2)};\; x_{m+1}, \ldots, x_n) + \kappa_{i0} x_i \mathbf{e}_{j'} \;. \tag{14}$$

Here, κ_{i0} denotes the rate constant for the outflow reaction $X_i \to 0$ in M.

By assumption, there exists $X_{i_{r-1}} \in \mathcal{O}_{r-1}$ such that for every species $X_j \in \mathcal{S}_1 \cup \cdots \cup \mathcal{S}_{r-1} \setminus \{X_{i_{r-1}}\}$, there exists a sequence of reactions $X_j \to \cdots \to X_{i_{r-1}}$ in $\widetilde{M}$ (and thus in M) from X_j to $X_{i_{r-1}}$. Hence, M and $X_{i_{r-1}}$ together satisfy the hypotheses of Proposition 3.27.

Thus, there exists an equation of the form $x_i = g_i$ that holds (for generic choices of the rate constants) along solutions of M, where g_i is a $\mathbb{Q}(\{\kappa_{lk} \mid l \to k \text{ is a reaction in } M\})$-linear combination of $x_{i_{r-1}}$ and the inflow-reaction variables and their derivatives, and the coefficient of at least one $x^{(q)}_{i_{r-1}}$ is nonzero. Thus, from equations (13) and (14), when we make the following substitution into the ODEs of N_r:

$$\widetilde{u}^{(2)}_{0 \to X_j} \;:=\; \kappa_{i0} g_i \;, \tag{15}$$

we get differential equations satisfied by solutions of the dynamical system defined by $\widetilde{M}$.

Hence, any input-output equation for N_r can be transformed into an input-output equation for $\widetilde{M}$ by making the substitution (15). Specifically, when we make this substitution into the following input-output equations for N_r (one for each $X_\ell \in \mathcal{O}_r$) from Proposition 3.11 (which applies because of hypothesis (3) in the statement of Theorem 3.28):

$$\det(\partial I - A_{H_\ell}) z_\ell \;=\; \sum_{j \in In_2 \cap V_{H_\ell}} (-1)^{\ell+j} \det\left(\partial I - A_{H_\ell}\right)_{j\ell} u_j \;, \tag{16}$$

we obtain the following input-output equations for $\widetilde{M}$ (one for each $\ell \in \mathcal{O}_r$):

$$\begin{aligned} \det(\partial I - A_{H_\ell}) z_\ell \;=\; & \sum_{j \in (In_2 \cap V_{H_\ell}) \setminus \phi(\mathfrak{I}_{r-1})} (-1)^{\ell+j} \det\left(\partial I - A_{H_\ell}\right)_{j\ell} u_j \\ & +\; (-1)^{\ell+j'} \det\left(\partial I - A_{H_\ell}\right)_{j'\ell} \kappa_{i0} g_i \;, \end{aligned} \tag{17}$$

where $H_\ell = (V_{H_\ell}, E_{H_\ell})$ is the output-reachable subgraph (of the directed graph underlying the non-flow subnetwork of N_r) to ℓ, and A_{H_ℓ} is the corresponding compartmental matrix. Also, In_r denotes the set of all inflow species in N_r.

Next, we claim that the input-output equations for M obtained from Proposition 3.11 are also input-output equations for $\widetilde{M}$. Indeed, there are no reactions in $\widetilde{M}$ from outside of M into M, so for any output variable $X_i \in \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_{r-1}$ in M, the output-reachable subgraph (of $\widetilde{M}$) to X_i is contained in M. Thus, our claim follows from Proposition 3.11.

Thus, the following are $|\mathcal{O}_1| + \cdots + |\mathcal{O}_r|$ input-output equations for $\widetilde{M}$:

(1) the $|\mathcal{O}_1| + \cdots + |\mathcal{O}_{r-1}|$ input-output equations for M obtained from Proposition 3.11, and
(2) the $|\mathcal{O}_r|$ equations in (17).

These input-output equations are algebraically independent, because they each involve a distinct output. Thus, as we have $|\mathcal{O}_1 \cup \cdots \cup \mathcal{O}_r| = |\mathcal{O}_1| + \cdots + |\mathcal{O}_r|$ algebraically independent input-output equations, we get a coefficient map for $\widetilde{M}$, which we denote by $c = (c^M; c^{(r)})$. By hypothesis, $c = (c^M; c^{(r)})$ is finite-to-one. Here and in the remainder of this proof, we write "finite-to-one" to mean "generically finite-to-one (respectively, generically one-to-one)".

Let c^{N_r} denote the coefficient map for N_r arising from the input-output equations (16). We claim that $(c^M; c^{N_r})$ is finite-to-one. Indeed, comparing equations (16) and (17), we see that for each coefficient in (the expansion of) equation (16) (i.e., each coordinate of c^{N_r}), either this coefficient also appears as a coefficient in (17), or a (nonzero) $\mathbb{F}$-multiple of it is a coefficient of some $x^{(q)}_{i_{r-1}}$ in (17), where $\mathbb{F} := \mathbb{Q}\left(\{\kappa_{lk} \mid l \to k \text{ is a reaction in } M\}\right)$. Conversely, each coefficient in (the expansion of) equation (17) (i.e., each coordinate of $c^{(r)}$), if not also a coordinate of c^{N_r}, is an $\mathbb{F}$-multiple of a coordinate in c^{N_r}. From generic input-output data, any rational function in $\mathbb{F}$ can be recovered (up to finitely many values) using c^M, and so the fact that $(c^M; c^{(r)})$ is finite-to-one implies that $(c^M; c^{N_r})$ is finite-to-one, as we claimed.

The function c^M depends only on the parameters in M, and similarly c^{N_r} depends only on the parameters in N_r. So, the fact that $(c^M; c^{N_r})$ is finite-to-one implies that c^{N_r} is finite-to-one. Hence N_r is identifiable. □

Example 3.29. In Example 3.24, the model formed by joining N_1 with N_2 is identifiable. We also know that N_1 is identifiable. Hence, by Theorem 3.28, N_2 is also identifiable.

3.4.2. *Joining strongly connected, monomolecular networks via Scenario 3 or 4.* In this subsection, we show that joining certain monomolecular networks by new reactions – namely, strongly connected networks without leaks – preserves identifiability (Theorem 3.30).

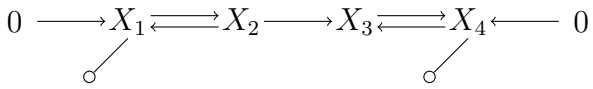
Theorem 3.30. *Let $N_1, \ldots, N_p$ be monomolecular networks with pairwise disjoint sets of species $\mathcal{S}_1, \ldots, \mathcal{S}_p$. Let $\mathcal{O}_1 \subseteq \mathcal{S}_1, \ldots, \mathcal{O}_p \subseteq \mathcal{S}_p$ be nonempty. Assume, for $i = 1, \ldots, p-1$, that N_i has no outflows and at least one inflow reaction, and that the non-flow subnetwork of N_i is strongly connected. Let G be obtained by joining $N_1, \ldots, N_p$ by a one-way flow via Scenario 3 or 4. Assume, moreover, that each joining by a one-way flow is over a single reaction. Then, if $(N_1, \mathcal{O}_1)$, $\ldots$, $(N_p, \mathcal{O}_p)$ are all identifiable, then $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ is identifiable.*

Proof. For $i = 1, \ldots, p-1$, let $\widetilde{N}_i$ denote the network obtained from N_i by adding an outflow reaction (leak) at the compartment from which a new one-way-flow reaction emerges in G. By construction, G is obtained by joining $\widetilde{N}_1, \ldots, \widetilde{N}_{p-1}$, and N_p by Scenario 1 or 2.

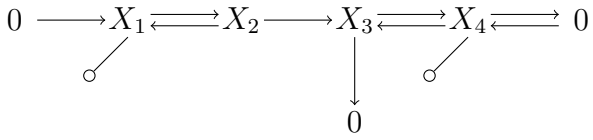
Assume that $(N_1, \mathcal{O}_1)$, $\ldots$, $(N_p, \mathcal{O}_p)$ are identifiable. Then (for $i = 1, \ldots, p-1$), by Lemma 3.13, the model $(\widetilde{N}_i, \mathcal{O}_i)$ is identifiable (here we use the fact that N_i has no outflow reactions and is strongly connected). So, by Theorem 3.23, $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ is identifiable. □

Example 3.31. Both linear compartmental models below are at least locally identifiable [54]:

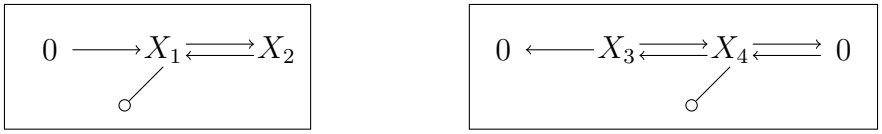
Thus, by Theorem 3.30, joining the networks by Scenario 3 yields a model, below, that is at least locally identifiable:



Example 3.32. Like Theorem 3.23 earlier, Theorem 3.30 can *not* be extended to conclude that, if $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ is identifiable, then $(N_2, \mathcal{O}_2), \ldots, (N_p, \mathcal{O}_p)$ are also. We can see this by modifying Example 3.26. In that example, we saw that the following model is locally identifiable:



This model is formed by joining the following models, $(N_1', \mathcal{O}_1')$ and $(N_2, \mathcal{O}_2)$, by Scenario 3:



As noted earlier in Example 3.26, model $(N_2, \mathcal{O}_2)$ is unidentifiable.

Our final theorem in this section is a partial converse to Theorem 3.30: If N_1 and each of the inductively joined networks N_1 and N_2, N_1 and N_2 and N_3, etc., are all identifiable (in Scenario 4), then each N_i is identifiable.

Theorem 3.33. *Let G be a network obtained by joining, in a row, monomolecular networks $N_1, \ldots, N_p$ with pairwise disjoint sets of species $\mathcal{S}_1, \ldots, \mathcal{S}_p$ by a one-way flow – but only via Scenario 4. Let $\mathcal{O}_1 \subseteq \mathcal{S}_1, \ldots, \mathcal{O}_p \subseteq \mathcal{S}_p$ be nonempty. Assume the following:*

(1) each joining by a one-way flow is over a single reaction,
(2) every N_i (for $i = 1, \ldots, p$) has at least one inflow reaction,
(3) for $i = 1, \ldots, p-1$, the network N_i has no outflows and the non-flow subnetwork of N_i is strongly connected,
(4) for every $\ell \in \mathcal{O}_p$, there is a directed path in N_p from an inflow-reaction species (input) to X_ℓ,
(5) the following $p-1$ models are identifiable: $(N_1, \mathcal{O}_1)$, the model obtained by joining $(N_1, \mathcal{O}_1)$ and $(N_2, \mathcal{O}_2)$, ..., and the model obtained by joining $(N_1, \mathcal{O}_1)$, $(N_2, \mathcal{O}_2)$, ... , and $(N_{p-1}, \mathcal{O}_{p-1})$ (via Scenario 4 and the same joining functions as for G).

Then $(N_2, \mathcal{O}_2), \ldots, (N_p, \mathcal{O}_p)$ are all identifiable if and only if $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ is identifiable.

Proof. The forward direction ("$\Rightarrow$") follows from Theorem 3.30.

We now prove the backward direction ("$\Leftarrow$"). For $i = 1, \ldots, p-1$, let $\widetilde{N}_i$ denote the network obtained from N_i by adding an outflow reaction (leak) at the compartment from which a new one-way-flow reaction emerges in G. It follows, by construction, that for $1 \leq j < k \leq p$, the model obtained by joining $(N_j, \mathcal{O}_j)$, $(N_{j+1}, \mathcal{O}_{j+1})$, ... , and $(N_k, \mathcal{O}_k)$ (via Scenario 4 and the same joining functions as for G) equals the model obtained by joining $(\widetilde{N}_j, \mathcal{O}_j)$, $(\widetilde{N}_{j+1}, \mathcal{O}_{j+1})$, ... ,$(\widetilde{N}_{k-1}, \mathcal{O}_{k-1})$, and $(N_k, \mathcal{O}_k)$ via Scenario 1 (and the same joining functions as for G). We use this fact below.

We prove the following (stronger) claim: *For $i = 1, \ldots, p$,*

(a) the model $(N_i, \mathcal{O}_i)$ is identifiable (and hence, by Lemma 3.13, $(\widetilde{N}_i, \mathcal{O}_i)$ also is, if $i \leq p-1$), and
(b) if $i \leq p-1$, the model $(M_i, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_i)$ is identifiable, where M_i denotes the network obtained by joining $(\widetilde{N}_1, \mathcal{O}_1), \ldots, (\widetilde{N}_i, \mathcal{O}_i)$ via Scenario 1 (and the same joining functions as for G).

We prove this claim by strong induction on i. For the base case, $i = 1$, part (a) holds by assumption, and (b) follows, as noted above, from Lemma 3.13.

For the inductive hypothesis, assume that (a) and (b) hold for $i = 1, 2, \ldots, m-1$ for some $2 \leq m \leq p$. We prove the $i = m$ case of the claim by showing that Theorem 3.28 (the "$\Leftarrow$" direction) applies to the networks $\widetilde{N}_1, \ldots, \widetilde{N}_{i-1}$, and N_i. As noted above, the network obtained by joining the networks $(\widetilde{N}_1, \mathcal{O}_1)$, $(\widetilde{N}_2, \mathcal{O}_2)$, ... , $(\widetilde{N}_{i-1}, \mathcal{O}_{i-1})$, and $(N_i, \mathcal{O}_i)$ by Scenario 1 equals the network obtained by joining $(N_1, \mathcal{O}_1)$, ... , and $(N_i, \mathcal{O}_i)$ by Scenario 4, which by hypothesis is identifiable. Also, by the inductive hypothesis, the models $(M_1, \mathcal{O}_1), \ldots, (M_{i-1}, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_{i-1})$ are all identifiable. Finally, hypotheses (3) and (4) in the statement of Theorem 3.28 apply to the networks $\widetilde{N}_1, \ldots, \widetilde{N}_{i-1}$, and N_i, because of hypotheses (3) and (4) in the statement of Theorem 3.33. Therefore, Theorem 3.28 (the "$\Leftarrow$" direction) applies, and so N_i is identifiable. This verifies (a).

For (b), assume $i \leq p-1$. By part (a) of the inductive hypothesis, the networks $\widetilde{N}_1, \ldots, \widetilde{N}_i$ are identifiable. Hence, by Theorem 3.23, the model $(M_i, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_i)$ is identifiable. □

Strongly connected networks are output connectable, so we obtain the following unifying corollary to Theorems 3.23, 3.28, 3.30, and 3.33.

Corollary 3.34. *Let $N_1, \ldots, N_p$ be monomolecular networks with pairwise disjoint sets of species $\mathcal{S}_1, \ldots, \mathcal{S}_p$. Assume, for $i = 1, \ldots, p$, that N_i has at least one inflow reaction and that the non-flow subnetwork of N_i is strongly connected. Let $\mathcal{O}_i \subseteq \mathcal{S}_i$ be nonempty for $i = 1, \ldots, p$.*

(1) Let G be a network obtained by joining $N_1, \ldots, N_p$ by a one-way flow via Scenario 1, 2, 3, or 4. If the joining is by Scenario 3 or 4, assume additionally that each joining is over a single reaction, and that, for $i = 1, \ldots, p-1$, the network N_i has no outflows. Then, if $(N_1, \mathcal{O}_1)$, …, $(N_p, \mathcal{O}_p)$ are identifiable, then $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ is identifiable.

(2) Let G be a network obtained by joining, in a row, $N_1, \ldots, N_p$ by a one-way flow via Scenario 1 or 4. Assume that each joining is over a single reaction and that the following models are identifiable: $(N_1, \mathcal{O}_1)$, the model obtained by joining $(N_1, \mathcal{O}_1)$ and $(N_2, \mathcal{O}_2)$, …, and the model obtained by joining $(N_1, \mathcal{O}_1)$, … , and $(N_{p-1}, \mathcal{O}_{p-1})$ (via the same joining functions as for G). If the joining is by Scenario 4, assume that, for $i = 1, \ldots, p-1$, the network N_i has no outflows. Then, $(N_2, \mathcal{O}_2)$, …, $(N_p, \mathcal{O}_p)$ are all identifiable if and only if $(G, \mathcal{O}_1 \cup \cdots \cup \mathcal{O}_p)$ is identifiable.

4. Steady-State Invariants

In this section, we move away from identifiability and toward the problem of understanding how steady-state invariants of networks obtained by gluing are related to the steady-state invariants of the joined networks before gluing. Steady-state invariants are polynomial equations satisfied by the species concentrations at steady state [35, 51]. These polynomials are used for model comparison and are particularly useful when only incomplete data are available [38, 39, 50]. Specifically, when only some of the species concentrations are measurable, we compute an ideal obtained by eliminating non-measurable species variables from the steady-state equations, and then the generators of this ideal are used to test goodness-of-fit.

However, eliminating the unobservable variables to obtain a set of steady-state invariants can be computationally challenging, and the resulting Gröbner basis, when it can be computed, is often large and difficult to interpret. One of our aims, therefore, is to determine how the steady-state invariants of a large network can be built from those of smaller subnetworks.

Our progress toward this aim is as follows. Consider a network N obtained by gluing two networks N_1 and N_2 over complexes or reactions. We are interested in determining how the steady-state invariants of N, after projecting them to involve only species and reactions in N_i, are related to the steady-state invariants of N_i. First, we show that every steady-state invariant of N_i arises as such a projection (Proposition 4.4). However, in general, some of the projected steady-state invariants of N are not steady-state invariants of N_i. This motivates us to find conditions when these projections are always steady-state invariants. We succeed for certain monomolecular networks obtained by gluing two networks over a species (Theorem 4.7) or a single reaction (Theorem 4.9). Moreover, in the case of monomolecular networks glued over a species, under some hypotheses, we recover the entire elimination ideal from the elimination ideals of the smaller networks N_i (Theorem 4.10).

4.1. **Connection to related work.** Steady-state invariants are not the only situation in which species variables of a reaction network are eliminated. Another context arises when analyzing a network's steady states, specifically its capacity for multistationarity. Here certain variables (usually intermediate complexes) often can be eliminated (usually linearly) so that there are effectively fewer steady-state equations to solve [9]. Thomson and Gunawardena performed such eliminations for post-translational modification networks [65], and subsequently Feliu and Wiuf and co-authors extended these ideas to signaling networks [23, 24, 26, 28], gave graphical criteria for when such elimination succeeds [60], and proved that the Gröbner basis of the steady-state ideal of a network extends to one that includes intermediates [59].

Here we too are interested in eliminating species from the steady-state equations that are experimentally unobservable. However, our setup and the questions we ask differ from those in the above references. For us, the set of variables to eliminate is given, and we would like to know how joining networks affects these eliminations. Earlier authors, in contrast, focused on eliminating as many species as possible.

A second situation involving elimination in reaction networks pertains to quasi-steady state and other approximations [56, 64]. Here, elimination is performed to obtain a lower-dimensional approximation of the system, which is valid when certain assumptions on the rate constants are met [36]. In our work, however, we are interested in steady states of the full system, not a reduced system.

4.2. **Setup.** We begin by introducing steady-state ideals and steady-state invariants.

Definition 4.1. Let $N = (\mathcal{S}, \mathcal{C}, \mathcal{R})$ be a network with mass-action ODEs:

$$\frac{dx}{dt} \;=\; \sum_{y_i \to y_j \text{ is in } \mathcal{R}} \kappa_{ij} x^{y_i}(y_j - y_i) \;=:\; (f_1(x), f_2(x), \ldots, f_n(x)) \;.$$

We call $f_1, f_2, \ldots, f_n$ the *system polynomials* of N, and they generate the *steady-state ideal*:

$$I_N \;:=\; \left\langle f_1(x),\; f_2(x),\; \ldots,\; f_n(x) \right\rangle \;\subseteq\; \mathbb{Q}[\boldsymbol{\kappa}; \mathbf{x}] \;.$$

Every $g \in I_N$ vanishes at steady state and so we say that g is a *steady-state invariant*. As mentioned earlier, we are interested in steady-state invariants that involve certain observable variables $\mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}$, namely, elements in the elimination ideal:

$$I_N^{\text{elim}} \;:=\; I_N \cap\; \mathbb{Q}[\boldsymbol{\kappa};\; \mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}] \;.$$

When eliminating a single species X_ℓ, we use the notation:

$$I_N^{\text{elim}(x_\ell)} \;:=\; I_N \cap\; \mathbb{Q}[\boldsymbol{\kappa};\; \mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_{\ell-1}, \mathbf{x}_{\ell+1}, \mathbf{x}_{\ell+2}, \ldots, \mathbf{x}_n] \;.$$

We consider the following setup: a set of observable variables $\mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}$, and a network N obtained by gluing two networks N_1 and N_2 over complexes or reactions. We consider the corresponding elimination ideals: $I_{N_i}^{\text{elim}} = I_{N_i} \cap\; \mathbb{Q}[\boldsymbol{\kappa}(i);\; \mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}]$, for $i = 1, 2$, where $\boldsymbol{\kappa}(i)$ denotes the vector of rate consants for network N_i.

We aim to investigate how I_N^{elim} is related to $I_{N_1}^{\text{elim}}$ and $I_{N_2}^{\text{elim}}$. Specifically, we compare $\phi_i(I_N^{\text{elim}})$ to $I_{N_i}^{\text{elim}}$ (for $i = 1, 2$), where ϕ_i is the projection to the species variables and rate

constants of network N_i. More precisely, ϕ_i is the ring homomorphism defined on generators as follows:

$$\begin{aligned}\phi_i \ : \ \mathbb{Q}[\boldsymbol{\kappa}, \mathbf{x}] \ &\to \ \mathbb{Q}[\boldsymbol{\kappa}(i), \mathbf{x}(i)] \\ \kappa_a \ &\mapsto \ \begin{cases} \kappa_a & \text{if } \kappa_a \in \boldsymbol{\kappa}(i) \\ 0 & \text{if } \kappa_a \notin \boldsymbol{\kappa}(i) \end{cases} \\ x_a \ &\mapsto \ \begin{cases} x_a & \text{if } x_a \in \mathbf{x}(i) \\ 0 & \text{if } x_a \notin \mathbf{x}(i) \end{cases}\end{aligned}$$

Remark 4.2. Recall from Lemma 2.8 that each system polynomial h_j of N can be written in the form $h_j = f_j + \widetilde{g}_j$ where f_j is the j-th system polynomial of N_1 and $\widetilde{g}_j$ is the j-th system polynomial of the network obtained from N_2 by removing reactions in N_1. It follows that $\phi_1(h_j) = f_j$ and $\phi_2(h_j) = g_j$.

We will prove the containment $\phi_i(I_N^{\text{elim}}) \subseteq I_{N_i}^{\text{elim}}$ (Proposition 4.4), and then investigate when the containment is an equality.

4.3. **Results.** We begin by showing that, before elimination, our ideals of interest, $\phi_i(I_N^{\text{elim}})$ and $I_{N_i}^{\text{elim}}$, are in fact equal.

Lemma 4.3. *Let N be the reaction network obtained by gluing two networks N_1 and N_2 over a set of complexes or a set of reactions. Then, for $i = 1, 2$, we have the following equality:*

$$I_{N_i} \ = \ \phi_i(I_N) \ \subseteq \ \mathbb{Q}[\boldsymbol{\kappa}(i), \mathbf{x}(i)] \ .$$

Proof. Let $f_1, f_2, \ldots, f_n$ be the system polynomials of $N_1 = (\mathcal{S}_1, \mathcal{C}_1, \mathcal{R}_1)$, and $g_1, g_2, \ldots, g_n$ the system polynomials of $N_2 = (\mathcal{S}_2, \mathcal{C}_2, \mathcal{R}_2)$, where $f_i := 0$ (respectively, $g_i := 0$) for species $i \in \mathcal{S}_2 \setminus \mathcal{S}_1$ (respectively, $i \in \mathcal{S}_1 \setminus \mathcal{S}_2$). Let $h_1, h_2, \ldots, h_n$ be the system polynomials of N.

By symmetry, we may assume $i = 1$. As ϕ_1 is surjective, we have

$$\phi_1(I_N) = \phi_1(\langle h_1, h_2, \ldots, h_n \rangle) \ = \ \langle \phi_1(h_1), \phi_1(h_2), \ldots, \phi_1(h_n) \rangle \ = \ \langle f_1, f_2, \ldots, f_n \rangle \ = \ I_{N_1} \ ,$$

where we also used Remark 4.2. □

Proposition 4.4. *Let N be a reaction network obtained by gluing two networks N_1 and N_2 over a set of complexes or a set of reactions. Consider a set of (observable) variables $\mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}$. For $i = 1, 2$, we have the following containment:*

$$\phi_i(I_N \cap \mathbb{Q}[\boldsymbol{\kappa}; \mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}]) \ \subseteq \ I_{N_i} \cap \mathbb{Q}[\boldsymbol{\kappa}(i); \mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}] \ . \tag{18}$$

In other words, $\phi_i(I_N^{\text{elim}}) \ \subseteq \ I_{N_i}^{\text{elim}}$.

Proof. Let $h \in I_N \cap \mathbb{Q}[\boldsymbol{\kappa}; \mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}]$. We must show $\phi_i(h) \in I_{N_i} \cap \mathbb{Q}[\boldsymbol{\kappa}(i); \mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}]$. To see this, first note that $\phi_i(h) \in \phi_i(I_N) = I_{N_i}$, by Lemma 4.3. Also, $h \in \mathbb{Q}[\boldsymbol{\kappa}; \mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}]$ implies that $\phi_i(h) \in \mathbb{Q}[\boldsymbol{\kappa}(i); \mathbf{x}_{j_1}, \mathbf{x}_{j_2}, \ldots, \mathbf{x}_{j_l}]$, and this completes the proof. □

Here we give two counterexamples to equality of the containment (18) in Proposition 4.4.

Example 4.5 (Gluing over complexes)**.** Consider the networks $N_1 = \{X_1 \overset{\kappa_1}{\to} X_2\}$ and $N_2 = \{X_2 \overset{\kappa_2}{\to} X_1\}$. Then by gluing over complexes, we obtain $N = N_1 \cup N_2 = \{X_1 \underset{\kappa_1}{\overset{\kappa_2}{\leftrightarrows}} X_2\}$. The corresponding steady-state ideals are:

$$I_N = \langle -\kappa_1 x_1 + \kappa_2 x_2 \rangle, \quad I_{N_1} = \langle k_1 x_1 \rangle, \quad I_{N_2} = \langle \kappa_2 x_2 \rangle \ .$$

Elimination of x_1 gives:

$$I_N^{\mathrm{elim}(x_1)} = \langle 0 \rangle, \quad I_{N_1}^{\mathrm{elim}(x_1)} = \langle 0 \rangle, \quad I_{N_2}^{\mathrm{elim}(x_1)} = \langle \kappa_2 x_2 \rangle \ .$$

Then $\phi_2\left(I_N^{\mathrm{elim}(x_1)}\right) = \langle 0 \rangle \subsetneq I_{N_2}^{\mathrm{elim}(x_1)}$, so the containment (18) in general is not an equality for gluing over complexes.

Example 4.6 (Gluing over reactions)**.** Let $N_1 = \{X_3 \overset{\kappa_1}{\to} X_1 + X_3,\ X_4 \overset{\kappa_2}{\to} X_2\}$ and $N_2 = \{X_4 \overset{\kappa_2}{\to} X_2,\ X_2 \overset{\kappa_3}{\to} X_1 + X_2\}$. Gluing over the reaction $X_4 \overset{\kappa_2}{\to} X_2$ yields

$$N \ = \ N_1 \cup N_2 \ = \ \{X_3 \overset{\kappa_1}{\to} X_1 + X_3, \quad X_4 \overset{\kappa_2}{\to} X_2, \quad X_2 \overset{\kappa_3}{\to} X_1 + X_2\}.$$

The corresponding steady-state ideals are:

$$I_N = \langle \kappa_1 x_3 + \kappa_3 x_2, \kappa_2 x_4 \rangle, \quad I_{N_1} = \langle \kappa_2 x_4, \kappa_1 x_3 \rangle, \quad I_{N_2} = \langle \kappa_2 x_4, \kappa_3 x_2 \rangle.$$

Elimination of x_3 gives:

$$I_N^{\mathrm{elim}(x_3)} = \langle \kappa_2 x_4 \rangle, \quad I_{N_1}^{\mathrm{elim}(x_3)} = \langle \kappa_2 x_4 \rangle, \quad I_{N_2}^{\mathrm{elim}(x_3)} = \langle \kappa_2 x_4, \kappa_3 x_2 \rangle.$$

Again, we find $\phi_2\left(I_N^{\mathrm{elim}(x_3)}\right) = \langle \kappa_2 x_4 \rangle \subsetneq I_{N_2}^{\mathrm{elim}(x_3)}$, so equality of the containment (18) does not hold in general for gluing over reactions.

These counterexamples prompt the question: *Are there combinatorial conditions on N that guarantee equality of the containment $\phi_i(I_N^{\mathrm{elim}}) \subseteq I_{N_i}^{\mathrm{elim}}$ in* (18)*?* Some positive results in this direction are the focus of the next subsections.

4.3.1. *Monomolecular networks.* In this section, we prove three results for monomolecular networks. Throughout the section, we make the following simplifying assumption:

monomolecular networks do not involve the zero complex.

For such a network N, the mass-action ODEs (and hence the system polynomials) are linear and can be written in matrix notation as

$$x' \ = \ A_\kappa^T x \ ,$$

where A_κ is the negative Laplacian of the reaction graph of N.

In this monomolecular (i.e., linear) setting, computing a Gröbner basis with respect to an elimination order is equivalent to performing Gaussian elimination on A_κ^T where the first columns correspond to variables to be eliminated. *Hence, a set of generators for the elimination ideal $I_N^{\mathrm{elim}(x_\ell)}$ can be obtained by performing Gaussian elimination on a matrix obtained from A_κ^T by moving the column corresponding to x_ℓ to the first column, and then considering only those rows with zero in the first entry.* Our proofs will rely on this fact.

Another fact we will use often is that an $n \times n$ Laplacian matrix has rank at most $n - 1$. Indeed, the column vectors of a Laplacian matrix always sum to the zero vector. Hence, before performing Gaussian elimination, we can always delete a row of the matrix A_κ^T.

Theorem 4.7. *Let N be a network obtained by gluing monomolecular networks N_1 and N_2 over a single species, say, X_j. Then for every species X_ℓ, the following holds for $i = 1, 2$:*

$$\phi_i(I_N^{\mathrm{elim}(x_\ell)}) \;=\; I_{N_i}^{\mathrm{elim}(x_\ell)} \;.$$

Proof. Let A_κ denote the negative Laplacian of the reaction graph of N. The rows and columns of A_κ correspond to the vertices of N, or equivalently, the complexes (which are individual species, as N is monomolecular) of N. Partitioning the complexes of N into the following parts, $\mathcal{C}_1 \setminus \{X_j\}$, $\{X_j\}$, and $\mathcal{C}_2 \setminus \{X_j\}$, and thus the rows of A_κ^T, we can write

$$A_\kappa^T \;=\; \begin{pmatrix} M_1 & \mathbf{m_{1_j}} & \mathbf{0} \\ \mathbf{m_1} & m_{1_j} + m_{2_j} & \mathbf{m_2} \\ \mathbf{0} & \mathbf{m_{2_j}} & M_2 \end{pmatrix} \tag{19}$$

where

$$A_{\kappa(1)}^T \;=\; \begin{pmatrix} M_1 & \mathbf{m_{1_j}} \\ \mathbf{m_1} & m_{1_j} \end{pmatrix} \text{ and } A_{\kappa(2)}^T \;=\; \begin{pmatrix} m_{2_j} & \mathbf{m_2} \\ \mathbf{m_{2_j}} & M_2 \end{pmatrix} \tag{20}$$

are the $j \times j$ and $(n-j+1) \times (n-j+1)$ negative transposes of the Laplacians of the reaction graphs of N_1 and N_2, respectively.

We may assume that $i = 1$. With an eye toward performing Gaussian elimination on the matrices (19) and (20), we delete the row that corresponds to X_j to obtain:

$$\widetilde{A}_\kappa^T \;=\; \begin{pmatrix} M_1 & \mathbf{m_{1_j}} & \mathbf{0} \\ \mathbf{0} & \mathbf{m_{2_j}} & M_2 \end{pmatrix}, \qquad \text{and} \qquad \widetilde{A}_{\kappa(1)}^T \;=\; \begin{pmatrix} M_1 & \mathbf{m_{1_j}} \end{pmatrix}.$$

The top rows of $\widetilde{A}_\kappa^T$ are obtained by appending zeroes to the ends of the rows of the matrix $\widetilde{A}_{\kappa(1)}^T$. Hence, performing Gaussian elimination on $\widetilde{A}_{\kappa(1)}^T$ (after moving the column for X_l to the first column) can be done equally well in $\widetilde{A}_\kappa^T$. Hence, $I_{N_1}^{\mathrm{elim}(x_l)} \subseteq \phi_1(I_N^{\mathrm{elim}(x_l)})$. The reverse containment, $I_{N_1}^{\mathrm{elim}(x_l)} \supseteq \phi_1(I_N^{\mathrm{elim}(x_l)})$, is Proposition 4.4, and so the desired equality holds. □

Remark 4.8. Theorem 4.7 concerns monomolecular networks, so the invariants obtained by Gaussian elimination in the proof are the *type 1 complex-linear invariants* from [47].

Theorem 4.9. *Let N be obtained by gluing two monomolecular networks N_1 and N_2 over a single reaction $X_{j_1} \to X_{j_2}$ or over a pair of reversible reactions $X_{j_1} \rightleftharpoons X_{j_2}$. If X_{j_1} does not belong to any other reaction in N_2, and X_{j_2} does not belong to any other reaction in N_1, then for every species X_ℓ, the following holds for $i = 1, 2$:*

$$\phi_i(I_N^{\mathrm{elim}(x_\ell)}) \;=\; I_{N_i}^{\mathrm{elim}(x_\ell)} \;.$$

Proof. We treat the irreversible case $X_{j_1} \to X_{j_2}$ and reversible case $X_{j_1} \rightleftharpoons X_{j_2}$ simultaneously, by introducing rate constants κ_{j_1,j_2} and κ_{j_2,j_1}, and noting that none of our arguments depend on whether $\kappa_{j_1,j_2} = 0$ or $\kappa_{j_2,j_1} = 0$. Thus, we also may assume that $i = 1$.

Partitioning the complexes of N into the following parts, $\mathcal{C}_1 \setminus \{X_{j_1}, X_{j_2}\}$, $\{X_{j_1}\}$, $\{X_{j_2}\}$, and $\mathcal{C}_2 \setminus \{X_{j_1}, X_{j_2}\}$, we can write the matrix A_κ^T in the following block form:

$$\begin{pmatrix} M_1 & \mathbf{m_{j_1}} & \mathbf{0} & \mathbf{0} \\ \mathbf{p_{j_1}} & s_1 - \kappa_{j_1,j_2} & \kappa_{j_2,j_1} & \mathbf{0} \\ \mathbf{0} & \kappa_{j_1,j_2} & s_2 - \kappa_{j_2,j_1} & \mathbf{q_{j_2}} \\ \mathbf{0} & \mathbf{0} & \mathbf{m_{j_2}} & M_2 \end{pmatrix} \tag{21}$$

where

$$A_{\kappa(1)}^T = \begin{pmatrix} M_1 & \mathbf{m_{j_1}} & \mathbf{0} \\ \mathbf{p_{j_1}} & s_1 - \kappa_{j_1,j_2} & \kappa_{j_2,j_1} \\ \mathbf{0} & \kappa_{j_1,j_2} & -\kappa_{j_2,j_1} \end{pmatrix} \quad \text{and} \quad A_{\kappa(2)}^T = \begin{pmatrix} -\kappa_{j_1,j_2} & \kappa_{j_2,j_1} & \mathbf{0} \\ \kappa_{j_1,j_2} & s_2 - \kappa_{j_2,j_1} & \mathbf{q_{j_2}} \\ \mathbf{0} & \mathbf{m_{j_2}} & M_2 \end{pmatrix} \tag{22}$$

are the transposes of the negative Laplacians for N_1 and N_2, respectively.

With an eye toward performing Gaussian elimination on the matrices (21) and (22), we delete the row that corresponds to X_{j_1}:

$$\widetilde{A}_\kappa^T = \begin{pmatrix} M_1 & \mathbf{m_{j_1}} & \mathbf{0} & \mathbf{0} \\ \mathbf{p_{j_1}} & s_1 - \kappa_{j_1,j_2} & \kappa_{j_2,j_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{m_{j_2}} & M_2 \end{pmatrix}, \quad \text{and} \quad \widetilde{A}_{\kappa(1)}^T = \begin{pmatrix} M_1 & \mathbf{m_{j_1}} & \mathbf{0} \\ \mathbf{p_{j_1}} & s_1 - \kappa_{j_1,j_2} & \kappa_{j_2,j_1} \end{pmatrix}.$$

The top rows of $\widetilde{A}_\kappa^T$ are obtained by appending zeroes to the ends of the rows of the matrix $\widetilde{A}_{\kappa(1)}^T$. Hence, performing Gaussian elimination on $\widetilde{A}_{\kappa(1)}^T$ (after moving the column for X_l to the first column) can be done equally well in $\widetilde{A}_\kappa^T$. Hence, $I_{N_1}^{\mathrm{elim}(x_l)} \subseteq \phi_1(I_N^{\mathrm{elim}(x_l)})$. The reverse containment, $I_{N_1}^{\mathrm{elim}(x_l)} \supseteq \phi_1(I_N^{\mathrm{elim}(x_l)})$, is Proposition 4.4, and so the desired equality holds. □

The following result concerns networks for which we can use the elimination ideals of N_1 and N_2 to directly compute the elimination ideal of N.

Theorem 4.10. *Let N be obtained by gluing two monomolecular networks N_1 and N_2 over a single species, say, X_k. If the flow through X_k is unidirectional (i.e., whenever X_k is the product of a reaction, the reactant is in N_1, and whenever X_k is the reactant, the product is in N_2; or vice-versa), then*

$$I_N^{\mathrm{elim}(x_j)} = I_{N_1}^{\mathrm{elim}(x_j)} \oplus I_{N_2}^{\mathrm{elim}(x_j)}.$$

Proof. We may assume that all reactions to X_k are from N_1 and all reactions from X_k are towards N_2. Let A_κ denote the negative Laplacian of the reaction graph of N. Similarly, let $A_{\kappa(1)}$ and $A_{\kappa(2)}$ denote the corresponding matrices for N_1 and N_2, respectively. The columns of A_κ^T correspond to the complexes of N. Partitioning the complexes of N into three parts $\{X_k\}$, $\mathcal{C}_1 \setminus \{X_k\}$, and $\mathcal{C}_2 \setminus \{X_k\}$ induces the following block structure on A_κ^T:

$$A_\kappa^T = \begin{pmatrix} a & \mathbf{u} & \mathbf{v} \\ \mathbf{0} & M_1 & \mathbf{0} \\ \mathbf{w} & \mathbf{0} & M_2 \end{pmatrix} \tag{23}$$

where

$$A_{\kappa(1)}^T = \begin{pmatrix} 0 & \mathbf{u} \\ \mathbf{0} & M_1 \end{pmatrix} \quad \text{and} \quad A_{\kappa(2)}^T = \begin{pmatrix} a & \mathbf{v} \\ \mathbf{w} & M_2 \end{pmatrix} \tag{24}$$

are the $j \times j$ and $(n-j+1) \times (n-j+1)$ negative transposes of the Laplacians of the reaction graphs of N_1 and N_2, respectively. (Here, M_1 and M_2 are, respectively, $(j-1) \times (j-1)$ and $(n-j) \times (n-j)$ matrices).

After removing the first row of (23), we see that the matrix has a block form prescribed by the subnetworks. Hence, the generators of $I_N^{\mathrm{elim}(x_j)}$ obtained by Gaussian elimination on the matrix (23) are the same generators obtained by performing Gaussian elimination on the matrices (24) (again with the first row removed) and then taking the union of the results. □

4.3.2. *Beyond monomolecular networks.* In the future, we hope to generalize results we proved for monomolecular networks to the non-monomolecular setting. Specifically, we pose the following problem:

Problem 4.11. Find conditions that guarantee the equality $\phi_i(I_N^{\mathrm{elim}}) = I_{N_i}^{\mathrm{elim}}$.

We end this subsection with two examples involving non-monomolecular networks, which may point the way toward progress on Problem 4.11.

Example 4.12. Consider the networks $N_1 = \{X_3 \stackrel{\kappa_1}{\to} X_1 + X_3,\ X_4 \stackrel{\kappa_2}{\to} X_2\}$ and $N_2 = \{X_4 \stackrel{\kappa_2}{\to} X_2,\ X_2 \stackrel{\kappa_3}{\to} X_1 + X_2\}$. Then by gluing over the shared reaction $X_4 \stackrel{\kappa_2}{\to} X_2$, we obtain

$$N \ = \ N_1 \cup N_2 \ = \ \{X_3 \stackrel{\kappa_1}{\to} X_1 + X_3\ , \quad X_4 \stackrel{\kappa_2}{\to} X_2\ , \quad X_2 \stackrel{\kappa_3}{\to} X_1 + X_2\}\ .$$

The corresponding steady-state ideals are:

$$I_N = \langle \kappa_1 x_3 + \kappa_3 x_2, \kappa_2 x_4 \rangle, \quad I_1 = \langle \kappa_2 x_4, \kappa_1 x_3 \rangle, \quad I_2 = \langle \kappa_2 x_4, \kappa_3 x_2 \rangle.$$

Elimination of x_4 gives:

$$I_N^{\mathrm{elim}(x_4)} = \langle \kappa_1 x_3 + \kappa_3 x_2 \rangle, \quad I_{N_1}^{\mathrm{elim}(x_4)} = \langle \kappa_1 x_3 \rangle, \quad I_{N_2}^{\mathrm{elim}(x_4)} = \langle \kappa_3 x_2 \rangle.$$

Notice that (for $i = 1, 2$) we have the equality $\phi_i\left(I_N^{\mathrm{elim}(x_4)}\right) = I_{N_i}^{\mathrm{elim}(x_4)}$.

Example 4.13 (Phosphorylation)**.** Protein modification plays a crucial role in protein activation and de-activation. Generally, an enzyme binds to a substrate, forms an enzyme-substrate complex, and then modifies the substrate by adding, for instance, a phosphate (phosphorylation) or removing one (dephosphorylation). Consider two one-site phosphorylation cycles $N_1 = \{S_0 + E \leftrightarrows X \to S_1 + E,\ S_1 + F \leftrightarrows Y \to S_0 + F\}$ and $N_2 = \{S_1 + E \leftrightarrows X_1 \to S_2 + E,\ S_2 + F \leftrightarrows Y_2 \to S_1 + F\}$. Identifying the shared complexes $S_1 + E$ and also $S_1 + F$ in each of the networks and gluing over them, we obtain $N = N_1 \cup N_2$, a two-site phosphorylation cycle [25]. For every species j of N and for both networks, i.e., $i = 1, 2$, we have $\phi_i\left(I_N^{\mathrm{elim}(\mathrm{x_j})}\right) = I_{N_i}^{\mathrm{elim}(\mathrm{x_j})}$. This result is surprising, and it prompts us to ask, *For which protein modification networks does the equality* $\phi_i(I_N^{\mathrm{elim}}) = I_{N_i}^{\mathrm{elim}}$ *hold?*

4.4. **Discussion.** Decomposition results like the ones in this section are a common theme in algebraic statistics and phylogenetic algebraic geometry [2, 18, 20], and thus one of our aims is to deepen the interaction between the fields of algebraic statistics and algebraic systems biology. A guiding question for the future therefore is as follows: Can we use techniques from algebraic statistics to analyze the steady-state invariants in larger classes of models?

Additionally, we hope that our results set the stage for obtaining more than just steady-state invariants. Specifically, just as elimination techniques helped build a framework for

understanding a network's capacity for multistationarity (multiple steady states) [27], in the future our results may also contribute to understanding this topic, which we turn to next.

5. Multistationarity

For a network with a decomposition into two subnetworks, the previous sections related its identifiability properties and steady-state invariants to that of the two subnetworks. Now we turn to a third topic, multistationarity, and show through several examples that this property is sometimes preserved and sometimes lost when going from a subnetwork to a network.

5.1. **Background.** Recall that a *steady state* of a reaction kinetics system is a nonnegative concentration vector $x^* \in \mathbb{R}^n_{\geq 0}$ at which the ODEs (1) vanish. We are interested in networks that admit multiple steady states, and if so whether these multiple positive states are stable (i.e., accessible). This is of particular biological importance for cellular decision making. If a system has two positive steady states, but only one is ever stable, the system cannot choose between states, for example, cell fate.

Definition 5.1.

(1) A steady state x^* is *nondegenerate* if $\mathrm{Im}\,(df_\kappa(x^*)|_S) = S$. (Here, $df_\kappa(x^*)$ is the Jacobian matrix of f_κ at x^*.)
(2) A nondegenerate steady state is *exponentially stable* if each of the $\sigma := \dim(S)$ nonzero eigenvalues of $df_\kappa(x^*)$ has negative real part.

Also, we distinguish between *positive steady states* $x^* \in \mathbb{R}^n_{>0}$ and *boundary steady states* $x^* \in \left(\mathbb{R}^n_{\geq 0} \setminus \mathbb{R}^n_{>0}\right)$.

Definition 5.2.

(1) A reaction network is *multistationary* if, for some choice of positive rate constants κ_{ij}, the resulting mass-action kinetics system (2) admits two or more positive steady states in some stoichiometric compatibility class (4). Otherwise, the network is *monostationary*.
(2) Analogously, a network is *nondegenerately multistationary* or *multistable* if it admits multiple nondegenerate or exponentially stable, respectively, positive steady states.

5.2. **Monomolecular networks are *not* nondegenerately multistationary.** We begin by showing that monomolecular networks are *not* nondegenerately multistationary.

Proposition 5.3. *If G is a reaction network in which each reactant complex is either monomolecular or the zero complex, then G is not nondegenerately multistationary.*

Proof. Let G be a network in which all nonzero reactants are monomolecular. Let $\mathcal{P} = (x^0 + S) \cap \mathbb{R}^s_{\geq 0}$ be a stoichiometric compatibility class of G, and let $\{\kappa_{ij}\}$ be any choice of positive rate constants. We must show that the resulting system does *not* admit more than one nondegenerate positive steady state in $\mathcal{P}$. The steady states in $\mathcal{P}$ are the solutions of the system comprising the following equations:

(1) the equations obtained by setting all right-hand sides of the ODEs to zero (these are linear because the reactants of G are at-most-monomolecular), and

(2) the linear equations $\langle x - x^0,\ v(i)\rangle = 0$, where $v(1), v(2), \ldots, v(T)$ form a basis of $S^\perp$.

Thus, the steady states in $\mathcal{P}$ form the solution set of a system of linear equations; hence there are 0, 1, or infinitely many. If there are infinitely many, then the set of steady states in $\mathcal{P}$ is a positive-dimensional affine subset of $\mathcal{P}$, and so every steady state in $\mathcal{P}$ is degenerate. □

Remark 5.4. We can not remove 'nondegenerately' from the statement of Proposition 5.3. This fact was illustrated in [46] via the network $G = \{0 \leftarrow A \rightarrow 2A\}$. Its only reactant, A, is monomolecular. If the two rate constants are equal, then every positive value of x_A is a degenerate steady state. When the two rate constants differ, then the resulting system admits no positive steady states. Thus, G is multistationary, but only degenerately so.

5.3. **"Lifting" multistationarity from subnetworks and other networks.** When can we "lift" multiple steady states from a subnetwork to the full network? That is, from simply knowing that a subnetwork (or other related network) is multistationary, when can we conclude that the full network is, too? Investigating this question is currently an active area of research. A typical result in this area, described informally, is as follows: if N is a subnetwork of G and both networks contain all possible flow reactions, then if N is multistationary then G is as well [44]. Another is the following: if N is obtained from G by removing "intermediate" complexes, then if N is multistationary then G is too [27]. A survey of these types of results is in [45, §4], and additional results appear in recent work of Banaji and Pantea [4].

We end this subsection with a cautionary example, which illustrates why results in this area are nontrivial. If a subnetwork of a given network is multistable, it is tempting to conclude that the larger network is as well. As explained above, in some cases we have results that guarantee that this will work, but this does not hold in general:

Example 5.5 (Having a multistable subnetwork does *not* imply multistability.)**.** The following network is multistable [43]:

$$0 \leftrightarrow A \qquad 2A \leftrightarrow 3A\ .$$

However, adding the reaction $A \rightarrow B$ to the network yields a network with no positive steady states (for any choice of rate constants). Indeed, the concentration of B goes to ∞.

The main question guiding the remainder of this section is: *for two networks N_1 and N_2 that are joined together in some way, how is the capacity for multistationarity of the overall network related to that of N_1 and N_2?* We are interested in two ways of joining the networks: adding a single reaction from N_1 to N_2 (Section 5.4), and "gluing" over a (unique) complex that is common to both N_1 and N_2 (Section 5.5).

5.4. **Joining two networks by a new reaction.** We show by example that by joining multistationary networks N_1 and N_2 (with no complex in common) by a new reaction (from a complex in N_1 to one in N_2), the new network may be non-multistationary or multistationary.

Example 5.6 (Resulting network is *not* multistationary)**.** The idea behind this example is the following: if we add a new reaction to join one multistationary network N_1 to another one N_2, then if both networks are mass-preserving and their respective species sets are disjoint, then the new network "drains" all species concentrations from N_1 and hence no positive steady states exist. Concretely, let $N_1 = \{3A \leftrightarrows 2A + B,\ A + 2B \leftrightarrows 3B\}$, and let $N_2 = \{3C \leftrightarrows 2C + D,\ C + 2D \leftrightarrows 3D\}$. Clearly, the two networks are equivalent. Each network

is multistationary (multistable, in fact [61]). However, adding the reaction $3A \to 3C$ to join the two networks yields a network with no positive steady states (for any choice of reaction rate constants).

Example 5.7 (Resulting network is multistationary)**.** Let $N_1 = \{0 \leftarrow A,\ 2A \to 3A \leftarrow 4A\}$, and let $N_2 = \{5A \leftarrow 6A,\ 7A \to 8A \leftarrow 9A\}$. Each network N_i admits 2 positive steady states [46, §3]. Adding the reaction $4A \to 5A$ to join the two networks yields a network that admits 5 positive steady states [46].

Example 5.8 (Resulting network is multistationary, even if species sets of N_1 and N_2 are disjoint)**.** Let $N_1 = \{0 \leftrightarrows A,\ 2A \to 3A\}$, and let $N_2 = \{B \leftrightarrows 2B,\ 3B \to 4B\}$. Each network N_i admits 2 positive steady states [46, §3]. Adding the reaction $A \to B$ to join the two networks yields a network that admits 4 positive steady states (networks N_1 and N_2 are decoupled, so the maximum number of positive steady states multiplies).

5.5. **Joining two networks by gluing over a complex.** The following examples show that by joining two multistationary networks N_1 and N_2 by a single shared complex, the resulting network may be non-multistationary or multistationary.

Example 5.9 (Resulting network is *not* multistationary)**.** Let $N_1 = \{0 \leftarrow A + B,\ 3A \to 4A + B\}$, and let $N_2 = \{A + B \to 2A,\ 2A + 3B \leftarrow 3A + 2B\}$. Each network N_i admits multiple positive steady states [46]. Gluing the networks over the unique shared complex, $A + B$, yields a network that (it is easy to check) always has a unique positive steady state.

Example 5.10 (Resulting network is multistationary)**.** Let $N_1 = \{0 \leftrightarrows A,\ 2A \to 3A\}$, and let $N_2 = \{3A \leftarrow 4A,\ 5A \leftrightarrows 6A\}$. Each network N_i admits 2 positive steady states [46]. Gluing the two networks over the unique shared complex $3A$ yields a network that admits 5 positive steady states [46].

The above examples motivate some problems for future work.

Problem 5.11. Formulate necessary or sufficient conditions under which two multistationary networks, when joined by a new reaction or glued over a complex, yield another multistationary network.

We are also interested in obtaining a Bézout-type upper bound on the maximum number of positive steady states arising when two networks are joined. Specifically, if N_1 admits m_1 positive steady states, and N_2 admits m_2, does it follow that the joined network admits at most $m_1 m_2$ positive steady states? Finally, the biological interest goes beyond multistationarity, to multistability, so we ask, *when does joining two multistable networks yield another multistable network?*

6. Discussion

As mentioned earlier, systems biology is in need of theory pertaining to what happens when biological pathways are joined or decomposed. Accordingly, this work contributes to starting such a theory. Our results and examples investigated the effects of joining or decomposing networks on three properties: identifiability, steady-state invariants, and multistationarity. Many of our results focused on monomolecular networks, and we also provided initial steps for systems with higher molecularity. Going forward, the techniques presented in this work

could be used to extend our results to more complex systems, such as bimolecular networks, including signaling networks such as the so-called MESSI systems [57].

Another future direction is to extend our results to allow for more ways of joining networks. For instance, our results on identifiability pertained only to joining networks by a one-way flow, while our results on steady-state invariants focused on gluing over complexes or reactions. It would be interesting, therefore, to prove identifiability results for networks obtained by gluing over complexes or reactions, and also steady-state invariants results for networks joined by a one-way flow. Indeed, this work forms a starting point for understanding fundamental questions about joining and decomposing networks, and opens new avenues for tackling more complicated networks.

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Appendix A. Proof of Proposition 3.27

We prove Proposition 3.27, which we restate here in the language of compartmental models:

Proposition A.1. *Consider a linear compartmental model $\mathcal{M} = (\mathfrak{G}, In, Out, Leak)$, with $\mathfrak{G} = (V, E)$. Assume that there exists a compartment i such that the output-reachable subgraph to i is $\mathfrak{G}$. Then for every $j \in V \setminus \{i\}$, there exists an equation of the form $x_j = g$ that holds (for generic values of the parameters a_{kl}) along all solutions of $\mathcal{M}$, where g is a $\mathbb{Q}(a_{kl})$-linear combination of the variable x_i and the input variables u_p (for $p \in In$) and their derivatives $x_i^{(q)}$ and $u_p^{(q)}$, and the coefficient of at least one of the $x_i^{(q)}$'s is nonzero.*

We first need Lemma A.2 below, which requires several definitions. A *directed 0-tree* T on vertices $\{0, 1, \ldots, n-1\}$ is a directed graph such that the underlying undirected graph is cycle-free and for every $j = 1, \ldots, n-1$ there is a directed path $j \to \cdots \to 0$ in T from j to 0. A *walk* in a directed graph is a sequence of edges $i_1 \to i_2 \to \cdots \to i_k$ (repeated edges allowed). If W is a walk in an edge-labeled directed graph, then a^W denotes the product of the edge labels of W.

Lemma A.2. *Let $n \geq 2$. Let T be a directed 0-tree on vertices $\{0, 1, \ldots, n-1\}$ with edges $i \to j$ labeled by a_{ji}. Let $\widetilde{T}$ be the directed graph obtained from T by adding, for each edge $i \to j$, a self-loop at vertex i labeled by $-a_{ji}$. Let $\mathfrak{B}$ denote the $(n-1) \times (n-1)$ matrix where*

$$\mathfrak{B}_{ij} \;=\; \sum_{\{\textit{length-}i\textit{ walks }W\textit{ in }\widetilde{T}\textit{ from }j\textit{ to }0\}} a^W \;. \tag{25}$$

Then $\det \mathfrak{B}$*, which is a polynomial in $\mathbb{Q}[a_{ji} \mid i \to j$ is an edge of $T]$, is nonzero.*

Proof. By construction, the determinant of $\mathfrak{B}$ is as follows:

$$\det \mathfrak{B} \;=\; \sum_{\sigma \in S_{n-1}} \operatorname{sign}(\sigma) \prod_{i=1}^{n-1} \left(\sum_{\{\text{length-}\sigma(i)\text{ walks }W\text{ in }\widetilde{T}\text{ from }i\text{ to }0\}} a^W \right) . \tag{26}$$

Reordering vertices of T reorders the columns of B, which only multiplies $\det \mathfrak{B}$ by 1 or -1. So, we now reorder the vertices $1, \ldots, n-1$ of T, so that they are in an order obtained from a breadth-first search (in the underlying undirected graph of T) from vertex 0. In other words, vertices at distance 1 from 0 come first, then those at distance 2, and so on. Hence, letting $d(i)$ denote the distance of vertex i from 0, it follows by construction that $d(i) \leq i$.

For $i = 1, \ldots, n-1$, let $P(i) = (i \to j_1 \to \cdots \to j_{d(i)-1} \to 0)$ denote the unique path in T from i to 0. Let $W(i)$ denote the length-i walk in $\widetilde{T}$ obtained by prepending $i - d(i)$ self-loops at i to the path $P(i)$. The corresponding monomial $a^{W(i)}$ is as follows:

$$a^{W(i)} \;=\; (-a_{j_1 i})^{i-d(i)} a_{j_1 i} a_{j_2 j_1} \ldots a_{j_{d(i)-i} j_{d(i)-i-1}} a_{0, j_{d(i)-i}} \;.$$

It follows that the following monomial is in the expansion of $\det \mathfrak{B}$:

$$M \;=\; a^{W(1)} a^{W(2)} \ldots a^{W(n-1)} \;.$$

Specifically, this monomial is part of the summand in (26) where σ is the identity permutation.

Hence, to show that $\det \mathfrak{B}$ is nonzero, it suffices to show the following:
Claim: There is no other set of walks $\{Q(1), \ldots, Q(n-1)\}$, such that there exists a permutation $\tau \in S_{n-1}$ such that (for $i = 1, \ldots, n-1$) $Q(i)$ is a length-$\tau(i)$ walk in $\widetilde{T}$ from i to 0, and for which $M = \pm a^{Q(1)} a^{Q(2)} \ldots a^{Q(n-1)}$.

We prove this claim by induction on n, the number of vertices in T. In the base case, when $n = 2$, there is a unique walk (namely, $1 \to 0$) of length 1 from vertex 1 to 0.

For the inductive step, assume that for directed 0-trees on $(n-1)$ vertices that are "breadth-first-search ordered" (as explained above), the claim is true. Let T, as above, be a 0-tree on vertices $\{0, 1, \ldots, n-1\}$, and also let $\widetilde{T}$ and M be as above. Assume that $M = \pm a^{Q(1)} a^{Q(2)} \ldots a^{Q(n-1)}$, as in the claim. We must show that $W(i) = Q(i)$ for all $i = 1, \ldots, n-1$.

Consider the vertex $n-1$, and denote the unique path in T from $n-1$ to 0 by $n-1 \to j_1 \to j_2 \cdots \to j_{d(n-1)-1} \to 0$. By the choice of ordering, $n-1$ is a leaf of $\widetilde{T}$. So, $a_{j,n-1}$ divides $a^{W(n-1)}$ and $a^{Q(n-1)}$ but none of the other $a^{W(i)}$'s or $a^{Q(i)}$'s. In fact, $a_{j_1,n-1}^{n-d(n-1)}$ divides $a^{W(n-1)}$, by construction of $W(n-1)$ and so $a_{j_1,n-1}^{n-d(n-1)}$ also divides $a^{Q(n-1)}$ (here we use the fact that $M = \pm a^{Q(1)} a^{Q(2)} \ldots a^{Q(n-1)}$). However, $W(n-1)$ is the only walk W in $\widetilde{T}$ that (1) ends at 0, (2) has length at most $n-1$, and (3) involves enough self-loops at $n-1$ in order for $a_{j_1,n-1}^{n-d(n-1)}$ to divide the corresponding monomial a^W. Thus, $Q(n-1) = W(n-1)$.

Hence, $a^{W(1)} a^{W(2)} \ldots a^{W(n-2)} = \pm a^{Q(1)} a^{W(2)} \ldots a^{Q(n-2)}$, and the corresponding walks $W(i)$ and $Q(i)$ arise from the tree $\widetilde{T}'$ obtained from $\widetilde{T}$ by deleting the leaf $n-1$. Notice that the vertices of $\widetilde{T}'$ are "breadth-first search ordered". So, by the inductive hypothesis, $W(1) = Q(1)$, …, $W(n-2) = Q(n-2)$. Hence, the claim holds, and this completes the proof. □

Proof of Proposition A.1. Let n denote the number of compartments. We may assume $n \geq 2$, as otherwise there is nothing to prove. By relabeling the compartments, if necessary, we may assume that $i = n$ and the remaining compartments are labeled by $1, 2, \ldots, n-1$.

Our proof and notation follow the proof of [54, Lemma 3]. We write $x' = Ax + u$, where A is the $n \times n$ compartmental matrix, with entries given by:

$$A_{\ell j} := \begin{cases} -a_{0\ell} - \sum_{k:\ell \to k \in E} a_{k\ell} & \text{if } \ell = j \text{ and } \ell \in \mathit{Leak} \\ -\sum_{k:\ell \to k \in E} a_{k\ell} & \text{if } \ell = j \text{ and } \ell \notin \mathit{Leak} \\ a_{\ell j} & \text{if } j \to \ell \text{ is an edge of } \mathfrak{G} \\ 0 & \text{otherwise.} \end{cases}$$

Let $\widetilde{A}$ denote the matrix obtained from A by removing row-n and column-n. Let $\mathbf{a}$ (respectively, $\mathbf{b}$) be the row (respectively, column) vector obtained by removing the n-th entry from row-n (respectively, column-n) of A. Finally, let $\widetilde{x} := (x_1, x_2, \ldots, x_{n-1})^T$ and $\widetilde{u} := (u_1, u_2, \ldots, u_{n-1})^T$, where $u_j := 0$ if $j \notin \mathit{In}$.

Let B denote the following $(n-1) \times (n-1)$ matrix: the first row is $\mathbf{a}$, the second row is $\mathbf{a}\widetilde{A}$, the third row is $\mathbf{a}\widetilde{A}^2$, …, and the last row is $\mathbf{a}\widetilde{A}^{n-2}$. Consider the following claim:
Claim A: For generic values of the $a_{k\ell}$'s, the matrix B is invertible.

To prove this claim, we must show that $\det B$, which is a polynomial in the $a_{k\ell}$'s, is nonzero. Relabel the vertex n in $\mathfrak{G}$ by 0, and call this graph $\mathfrak{G}'$. Let T denote a subgraph of $\mathfrak{G}'$ that is a directed 0-tree (such a subgraph exists by the hypothesis of being output-reachable). Let $\widetilde{T}$ be the graph arising from T as defined in Lemma A.2, and let $\mathfrak{B}$ be the matrix (25).

We claim that $\mathfrak{B} = B|_{\{a_{j\ell}=0 | \ell \to j \text{ is not an edge of } T\}}$. To see this, note that $\widetilde{A}|_{\{a_{j\ell}=0 | \ell \to j \text{ is not an edge of } T\}}$ is the adjacency matrix for the graph $\widetilde{T}_0$ obtained by deleting vertex 0 from $\widetilde{T}$. Hence, the (i_1, i_2) entry in $(\widetilde{A})^k|_{\{a_{j\ell}=0 | \ell \to j \text{ is not an edge of } T\}}$ is a sum of monomials a^W, where the sum is over walks W in $\widetilde{T}_0$ of length k from i_1 to i_2. The vector $\mathbf{a}$ encodes the directed edges $\ell \to 0$, and so it is straightforward to check that the $\mathbf{a}(\widetilde{A}^k)|_{\{a_{j\ell}=0 | \ell \to j \text{ is not an edge of } T\}}$'s, i.e., the rows of $B|_{\{a_{j\ell}=0 | \ell \to j \text{ is not an edge of } T\}}$, form the matrix $\mathfrak{B}$ as in (25).

Hence, using Lemma A.2, we obtain:

$$\det B|_{\{a_{j\ell}=0 | \ell \to j \text{ is not an edge of } T\}} \;=\; \det \mathfrak{B} \;\neq\; 0 \;.$$

Hence, $\det B \neq 0$, and so Claim A holds.

As explained in the proof of [54, Lemma 3], solutions to the model $\mathcal{M}$ satisfy $B\widetilde{x} = c$, where c is the vector of length $n-1$ that decomposes as follows:

$$\begin{aligned} c \;&=\; \begin{pmatrix} x_n' - A_{nn}x_n - u_n \\ x_n^{(2)} - A_{nn}x_n' - u_n' - (\mathbf{ab}x_n + \mathbf{a}\widetilde{u}) \\ \vdots \\ x_n^{(k)} - A_{nn}x_n^{(k-1)} - u_n^{(k-1)} - \sum_{j=0}^{k-2}\left(\mathbf{a}\widetilde{A}^{k-2-j}\mathbf{b}x_n^{(j)} + \mathbf{a}\widetilde{A}^{k-2-j}\widetilde{u}^{(j)}\right) \\ \vdots \end{pmatrix} \\ &=\; \begin{pmatrix} x_n' - A_{nn}x_n \\ x_n^{(2)} - A_{nn}x_n' - \mathbf{ab}x_n \\ \vdots \\ x_n^{(k)} - A_{nn}x_n^{(k-1)} - \sum_{j=0}^{k-2}\mathbf{a}\widetilde{A}^{k-2-j}\mathbf{b}x_n^{(j)} \\ \vdots \end{pmatrix} - \begin{pmatrix} u_n \\ u_n' + \mathbf{a}\widetilde{u} \\ \vdots \\ u_n^{(k-1)} + \sum_{j=0}^{k-2}\mathbf{a}\widetilde{A}^{k-2-j}\widetilde{u}^{(j)} \\ \vdots \end{pmatrix} \;=:\; c^{(x)} + c^{(u)} \;, \end{aligned}$$

where $u_n := 0$ if $n \notin In$. Each coordinate of c is a $\mathbb{Q}(a_{kl})$-linear combination of the variable x_n and the input variables u_p (for $p \in In$) and their derivatives $x_n^{(q)}$ and $u_p^{(q)}$. Therefore, as B is invertible (for generic values of the a_{kl}'s), then we obtain the desired equations g_j:

$$\widetilde{x} \;=\; B^{-1}c \;=:\; (g_1, g_2, \ldots, g_{n-1})^T \;,$$

once we verify the following claim:

Claim B: In each g_ℓ, the coefficient of at least one of the $x_n^{(q)}$'s is nonzero.

To show this claim, assume for contradiction that, in some g_ℓ, the coefficient of every $x_n^{(q)}$ is zero. Then, by the above decomposition, we obtain $(B^{-1}c^{(x)})_\ell = 0$ (the zero polynomial). In other words, letting $\mathbf{d}$ denote row-l of B^{-1}, we have $\langle \mathbf{d}, c^{(x)} \rangle = 0$.

We will show that $\mathbf{d}$ is the zero vector. Among the coordinates $c_j^{(x)}$ (for $j = 1, \ldots, n-1$) of $c^{(x)}$, only the last coordinate, namely, $c_{n-1}^{(x)}$, contains as a summand $x_n^{(n-1)}$. So, in order for $\langle \mathbf{d}, c^{(x)} \rangle = 0$, we must have that $\mathbf{d}_{n-1} = 0$ (here we use the fact that the coordinates of $\mathbf{d}$ are in $\mathbb{Q}(a_{kl})$). Next, let $\widetilde{\mathbf{d}}$ and $\widetilde{c}^{(x)}$ be the vectors obtained by removing the last coordinate

from, respectively, $\mathbf{d}$ and $c^{(x)}$. We have $\langle \widetilde{\mathbf{d}}, \widetilde{c}^{(x)} \rangle = 0$, and so we can apply the same argument as above to obtain that $\mathbf{d}_{n-2} = 0$. Continuing, we obtain that every coordinate of $\mathbf{d}$ is zero. We have reached a contradiction, and so Claim B holds. This completes the proof. □

Corollary A.3. *Every output connectable linear compartmental model is algebraically observable.*

Proof. Consider a linear compartmental model $\mathcal{M} = (\mathfrak{G}, In, Out, Leak)$ that is output connectable. Let ℓ be any compartment. If $\ell \in Out$, then the state variable x_ℓ is itself an output variable, and so is already written in terms of output variables.

So, assume that $\ell \notin Out$. As the model is output connectable, there exists $i \in Out$ such that there is a path from ℓ to i. Let $\mathfrak{G}'$ denote the output-reachable subgraph to y_i.

It is straightforward to check that the restriction of $\mathcal{M}$ to $\mathfrak{G}'$ (Definition 3.10) satisfies the hypotheses of Proposition A.1 with respect to i. Also, the ODEs of $\mathcal{M}$ are obtained from those of the restriction by appending the ODEs for state variables $x_j(t)$ with j not in the vertex set of $\mathfrak{G}'$ (see the proof of [33, Lemma 3.7]). Thus, the equation $x_\ell = g$ obtained from Proposition A.1 expresses x_ℓ as a function of x_i, the inputs, their derivatives, and the parameters. Thus, by [16], $\mathcal{M}$ is algebraically observable. □

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