Complexity of Model Checking for Reaction Systems

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Abstract

Reaction systems are a new mathematical formalism inspired by the living cell and driven by only two basic mechanisms: facilitation and inhibition. As a modeling framework, they differ from the traditional approaches based on ODEs and CTMCs in two fundamental aspects: their qualitative character and the non-permanency of resources. In this article we introduce to reaction systems several notions of central interest in biomodeling: mass conservation, invariants, steady states, stationary processes, elementary fluxes, and periodicity. We prove that the decision problems related to these properties span a number of complexity classes from P to NP- and coNP-complete to PSPACE-complete.

Keywords: Reaction systems; model checking; biomodeling; conserved sets; invariants; steady state; stationary process; elementary flux; periodicity; complexity classes.

1. Introduction

Reaction systems were introduced in [5], see also [2] for a recent survey, as a framework inspired by the functioning of living cells. Reactions are driven in this formalism by only two basic mechanisms: facilitation and inhibition. A reaction is presented through its (finite, nonempty) sets of reactants, inhibitors and products. Resources in reaction systems are subject to two fundamental principles: the threshold principle and the non-permanency principle. The former principle indicates that if present in the system, a resource is available in unlimited amounts. This effectively defines reaction systems as a qualitative, rather than quantitative framework, and eliminates any mechanism of concurrency through competition on resources. The latter principle indicates that the next state of a reaction system consists of only the products of the reactions enabled in the current state. This means that any resource that is not sustained by an enabled reaction will disappear from the system. A reaction system also

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accepts in every step an external flow of resources from the context, making it an open system.

Two main lines of research have been investigated for reaction systems so far. In one line of research, the focus has been on investigating their mathematical properties, e.g., functions defined by reaction systems, state sequences, effect of limited resources, cycles and attractors, connections to propositional logic, see, e.g., [4, 6, 7, 15, 16]. On the other main line of research, the focus has been on the capabilities of reaction systems as a modeling framework, see, e.g., [1, 3]. Within this line of research, there is growing interest in model checking for reaction systems. For example, a temporal logic is introduced in [11] for defining and checking temporal properties of reaction systems; the authors prove that model checking in this logic is PSPACE-complete.

Our paper continues the investigation of reactions systems as a modeling framework and considers the formalization of several notions of central interest in biomodeling: mass conservation, invariants, steady states, stationary processes, elementary fluxes, and periodicity. Our definitions of these notions for reaction systems aim to be a natural correspondent of their usual definitions in quantitative frameworks, see, e.g. [9]. Going from a quantitative framework to a qualitative one is reflected in our definitions; for example, whereas mass conservation in a quantitative framework is a linear relation, in the context of reactions systems it becomes a set-theoretic relation on the states of the system. We focus on the computational complexity of deciding these properties for a given reaction system and we establish their complexity classes. In contrast with the quantitative case, the problems are difficult in the case of reaction systems. We prove that the decision problems related to these properties span a number of complexity classes from P to NP- and coNP-complete to PSPACE-complete.

The paper is organized as follows. In Section 2 we introduce some basic definitions of reaction systems, as well as the running case study of our paper, on the eukaryotic heat shock response. In Section 3 we formulate for reaction system the biomodeling properties mentioned above, while in Section 4 we establish their complexity classes. We discuss our conclusions in Section 5.

2. Preliminaries

In this section, we recall the notion of a reaction system, as well as some related concepts capturing the static structure and the dynamic aspects of the model. For more details we refer to [5] and [4].

Definition 2.1 ([5])**.** *Let S be a finite set. A* reaction *a in S is a triplet of finite nonempty sets $a = (R_a, I_a, P_a)$, where $R_a, I_a, P_a \subseteq S$ and $R_a \cap I_a = \varnothing$. We say that R_a, I_a, and P_a are the sets of reactants, inhibitors, and products of a, respectively. The set of all reactions in S is denoted by* $\mathrm{rac}(S)$.

A reaction system (RS) *is an ordered pair $\mathcal{A} = (S, A)$, where S is a finite set of* symbols *(also called sometimes elements, species, or entities) and $A \subseteq \mathrm{rac}(S)$. The set S is called the* background *(set) of A.*

We use the following notations:

$$\mathcal{R} = \bigcup_{a \in A} R_a, \quad \mathcal{P} = \bigcup_{a \in A} P_a, \quad \textit{and} \quad \mathrm{supp}(\mathcal{A}) = \mathcal{R} \cup \mathcal{P}.$$

The set $\mathrm{supp}(\mathcal{A})$ *will be called the* support set *of $\mathcal{A}$.*

The following definition introduces the result of a reaction and of a reaction system.

Definition 2.2 ([5]). *Let $\mathcal{A} = (S, A)$ be a reaction system, $T \subseteq S$, and $a \in A$. We say that a is* enabled *by T, denoted by $\mathrm{en}_a(T)$, if $R_a \subseteq T$ and $I_a \cap T = \varnothing$.*

(1) The result of a on T *is defined as follows:*

$$\mathrm{res}_a(T) = \begin{cases} P_a, & \text{if } \mathrm{en}_a(T), \\ \varnothing, & \text{otherwise.} \end{cases}$$

(2) The result of $\mathcal{A}$ on T *is defined as follows:*

$$\mathrm{res}_{\mathcal{A}}(T) = \bigcup_{a \in A} \mathrm{res}_a(T).$$

(3) An interactive process *in $\mathcal{A}$ is a pair $\pi = (\gamma, \delta)$, where $\gamma = (C_0, C_1, ..., C_n)$ and $\delta = (D_1, D_2, ..., D_n)$, $n \geq 1$, are sequences of subsets of S with $D_1 = \mathrm{res}_{\mathcal{A}}(C_0)$ and, for each $1 < i \leq n$, $D_i = \mathrm{res}_{\mathcal{A}}(C_{i-1} \cup D_{i-1})$.*

Example 2.1 (Running case-study: the eukaryotic heat shock response). *We introduce in this example our running case study on the eukaryotic heat shock response. For more details we refer to [17]. The heat shock response is a defense mechanism by which the cell reacts to elevated temperatures. The key players of such a mechanism are heat shock proteins,* hsp*, which are responsible for facilitating the refolding process of misfolded proteins,* mfp*, by operating as molecular chaperons. The heat shock proteins form* hsp: mfp *complexes and help* mfp *to refold. The heat shock response is regulated by the transactivation of the* hsp*-encoding genes. The heat shock factor,* hsf*, is the protein which promotes gene transcription and, in the absence of the environmental stressors, is found abundantly bound to* hsp*'s. Heat stress results in dimerization and subsequently trimerization of* hsf*, producing* hsf_2 *and* hsf_3 *respectively. The* hsf *trimers can then bind to the promoter site of the* hsp*-encoding gene, i.e., the heat shock element* (hse)*; the transcription of the gene is thus activated and the synthesis of* hsp *enabled. The* hsp *synthesis is turned off as soon as the heat shock protein is raised to a sufficient level by breaking the bond in* hsf_3: hse*, i.e., unbinding* hsf_3 *from* hse*.*

A simple molecular model for the heat shock response was introduced in [13] and its molecular reactions are presented in Table 1. We follow in this paper the reaction system-based formulation of this model, introduced in [1]. Its reactions are listed in Table 2.

3. Biological properties in reaction system models

In this section we formulate for reaction systems several properties that are commonly looked at in biological modeling: mass conservation, steady states, elementary fluxes, periodicity [9]. We also propose a few additional properties that generalize or are strongly related to these.

We start with the principle of mass conservation.

Table 1: The molecular model for the eukaryotic heat shock response proposed in [13].

Reaction	Reaction
$2\,\mathsf{hsf} \leftrightarrows \mathsf{hsf}_2$	$\mathsf{hsp} + \mathsf{hsf}_3 \to \mathsf{hsp}\colon \mathsf{hsf} + 2\,\mathsf{hsf}$
$\mathsf{hsf} + \mathsf{hsf}_2 \leftrightarrows \mathsf{hsf}_3$	$\mathsf{hsp} + \mathsf{hsf}_3\colon \mathsf{hse} \to \mathsf{hsp}\colon \mathsf{hsf} + 2\,\mathsf{hsf} + \mathsf{hse}$
$\mathsf{hsf}_3 + \mathsf{hse} \leftrightarrows \mathsf{hsf}_3\colon \mathsf{hse}$	$\mathsf{hsp} \to \varnothing$
$\mathsf{hsf}_3\colon \mathsf{hse} \to \mathsf{hsf}_3\colon \mathsf{hse} + \mathsf{hsp}$	$\mathsf{prot} \to \mathsf{mfp}$
$\mathsf{hsp} + \mathsf{hsf} \leftrightarrows \mathsf{hsp}\colon \mathsf{hsf}$	$\mathsf{hsp} + \mathsf{mfp} \leftrightarrows \mathsf{hsp}\colon \mathsf{mfp}$
$\mathsf{hsp} + \mathsf{hsf}_2 \to \mathsf{hsp}\colon \mathsf{hsf} + \mathsf{hsf}$	$\mathsf{hsp}\colon \mathsf{mfp} \to \mathsf{hsp} + \mathsf{prot}$

Table 2: The reaction system model HSR for heat shock response introduced in [1].

Reaction		Reaction	
$(\{\mathsf{hsf}\}, \{\mathsf{hsp}\}, \{\mathsf{hsf}_3\})$	(1)	$(\{\mathsf{hsp}, \mathsf{hsf}\}, \{\mathsf{mfp}\}, \{\mathsf{hsp}\colon \mathsf{hsf}\})$	(11)
$(\{\mathsf{hsf}, \mathsf{hsp}, \mathsf{mfp}\}, \{\mathsf{d_I}\}, \{\mathsf{hsf}_3\})$	(2)	$(\{\mathsf{hsp}\colon \mathsf{hsf}, \mathsf{stress}\}, \{\mathsf{nostress}\}, \{\mathsf{hsp}, \mathsf{hsf}\})$	(12)
$(\{\mathsf{hsf}_3\}, \{\mathsf{hse}, \mathsf{hsp}\}, \{\mathsf{hsf}\})$	(3)	$(\{\mathsf{hsp}\colon \mathsf{hsf}, \mathsf{nostress}\}, \{\mathsf{stress}\}, \{\mathsf{hsp}\colon \mathsf{hsf}\})$	(13)
$(\{\mathsf{hsf}_3, \mathsf{hsp}, \mathsf{mfp}\}, \{\mathsf{hse}\}, \{\mathsf{hsf}\})$	(4)	$(\{\mathsf{hsp}, \mathsf{hsf}_3\}, \{\mathsf{mfp}\}, \{\mathsf{hsp}\colon \mathsf{hsf}\})$	(14)
$(\{\mathsf{hsf}_3, \mathsf{hse}\}, \{\mathsf{hsp}\}, \{\mathsf{hsf}_3\colon \mathsf{hse}\})$	(5)	$(\{\mathsf{hsp}, \mathsf{hsf}_3\colon \mathsf{hse}\}, \{\mathsf{mfp}\}, \{\mathsf{hsp}\colon \mathsf{hsf}, \mathsf{hse}\})$	(15)
$(\{\mathsf{hsf}_3, \mathsf{hse}, \mathsf{hsp}, \mathsf{mfp}\}, \{\mathsf{d_I}\}, \{\mathsf{hsf}_3\colon \mathsf{hse}\})$	(6)	$(\{\mathsf{prot}, \mathsf{stress}\}, \{\mathsf{nostress}\}, \{\mathsf{prot}, \mathsf{mfp}\})$	(16)
$(\{\mathsf{hse}\}, \{\mathsf{hsf}_3\}, \{\mathsf{hse}\})$	(7)	$(\{\mathsf{prot}, \mathsf{nostress}\}, \{\mathsf{stress}\}, \{\mathsf{prot}\})$	(17)
$(\{\mathsf{hse}, \mathsf{hsf}_3, \mathsf{hsp}\}, \{\mathsf{mfp}\}, \{\mathsf{hse}\})$	(8)	$(\{\mathsf{hsp}, \mathsf{mfp}\}, \{\mathsf{d_I}\}, \{\mathsf{hsp}\colon \mathsf{mfp}\})$	(18)
$(\{\mathsf{hsf}_3\colon \mathsf{hse}\}, \{\mathsf{hsp}\}, \{\mathsf{hsf}_3\colon \mathsf{hse}, \mathsf{hsp}\})$	(9)	$(\{\mathsf{mfp}\}, \{\mathsf{hsp}\}, \{\mathsf{mfp}\})$	(19)
$(\{\mathsf{hsf}_3\colon \mathsf{hse}, \mathsf{hsp}, \mathsf{mfp}\}, \{\mathsf{d_I}\}, \{\mathsf{hsf}_3\colon \mathsf{hse}, \mathsf{hsp}\})$	(10)	$(\{\mathsf{hsp}\colon \mathsf{mfp}\}, \{\mathsf{d_I}\}, \{\mathsf{hsp}, \mathsf{prot}\})$	(20)

Definition 3.1 (Conserved sets)**.** *Let $\mathcal{A} = (S, A)$ be a reaction system. We say that a set $M \subseteq \operatorname{supp}(\mathcal{A})$ is* conserved *if for any $W \subseteq \operatorname{supp}(\mathcal{A})$, $M \cap W \neq \varnothing$ if and only if $M \cap \operatorname{res}_{\mathcal{A}}(W) \neq \varnothing$.*

Note in this definition that it is crucial that $\operatorname{supp}(\mathcal{A}) \subset S$. Indeed, if $\operatorname{supp}(\mathcal{A}) = S$, then the only conserved set of $\mathcal{A}$ is the empty set; this follows from the definition by taking $W = S$ and recalling that each reaction has a non-empty set of inhibitors.

Example 3.1. *For our running example, the reaction system* HSR *in Example 2.1, a conserved set is $M = \{\mathsf{hse}, \mathsf{hsf}_3\colon \mathsf{hse}\}$. Indeed, consider an arbitrary set $W \subseteq \operatorname{supp}(\mathcal{A})$.*

First consider the case $M \cap W \neq \varnothing$. If $\mathsf{hse} \in W$, then either reaction (5)*,* (6)*,* (7)*, or* (8) *is enabled, so $\operatorname{res}_{\mathcal{A}}(W)$ contains* hse *or* $\mathsf{hsf}_3\colon \mathsf{hse}$*. If $\mathsf{hsf}_3\colon \mathsf{hse} \in W$, then at least one of the reactions* (9)*,* (10)*, or* (15) *is enabled, and therefore $M \cap \operatorname{res}_{\mathcal{A}}(W) \neq \varnothing$ again.*

Notice now that, to produce hse *or* $\mathsf{hsf}_3\colon \mathsf{hse}$*, we always need at least one of these species to be present as a reactant. Thus, if $M \cap W = \varnothing$, then $M \cap$*

$\mathrm{res}_{\mathcal{A}}(W) = \varnothing$.

The following definition introduces a stronger form of conservation, the invariant set.

Definition 3.2 (Invariant sets)**.** *Let $\mathcal{A} = (S, A)$ be a reaction system. We say that a set $M \subseteq \mathrm{supp}(\mathcal{A})$ is* invariant *if for any $W \subseteq \mathrm{supp}(\mathcal{A})$, $M \subseteq W$ if and only if $M \subseteq \mathrm{res}_{\mathcal{A}}(W)$.*

Similarly as for conserved sets, note that if $\mathrm{supp}(\mathcal{A}) = S$, then the only invariant set of $\mathcal{A}$ is the empty set. Moreover, if $M \neq \varnothing$ is an invariant set, then for any $x \in M$, there exists a reaction $a = (R, I, P)$ with $x \in P$ and $I \subseteq S \setminus \mathrm{supp}(\mathcal{A})$. Indeed, if this were not the case, then every reaction that produces x has at least one inhibitor in $\mathrm{supp}(\mathcal{A})$ and, consequently, we can augment the set M to a set $W \subseteq \mathrm{supp}(\mathcal{A})$ that completely inhibits the production of x, thus violating the invariance of M.

Example 3.2. *Consider again the reaction system* HSR*. Based on the observation above, the only elements that can be part of some invariant set for our example are* $\{\mathsf{hsf_3}, \mathsf{hsf_3{:}\,hse}, \mathsf{hsp}, \mathsf{hsp{:}\,mfp}, \mathsf{prot}\}$ *– these are the only elements produced in reactions with inhibitor set* $\{d_I\}$*.*

Assume that there is an invariant set M such that $\mathsf{prot} \in M$ *and consider a set $W \subseteq \mathrm{supp}(\mathcal{A})$ such that $M \subseteq W$. In order to ensure that* $\mathsf{prot} \in \mathrm{res}_{\mathcal{A}}(W)$*, the only reaction that produces* prot *should be enabled, i.e. it should be that* $\mathsf{hsp{:}\,mfp} \in W$*. But the only way to assure this condition is to add* $\mathsf{hsp{:}\,mfp}$ *to M. Similar reasoning for* $\mathsf{hsp{:}\,mfp}$ *indicates that M must include* hsp *and* mfp*, so* $\{\mathsf{prot}, \mathsf{hsp{:}\,mfp}, \mathsf{hsp}, \mathsf{mfp}\} \subseteq M$*. Now consider a set W such that $M \subseteq W$ and* $\mathsf{stress} \notin W$*. In this case* $\mathsf{mfp} \notin \mathrm{res}_{\mathcal{A}}(W)$*, which is a contradiction to the fact that M was assumed invariant. Note that we cannot add* stress *to M as we did with the other elements, since we already know that* stress *may not be part of any invariant set. Hence we conclude that there is no invariant set M that contains* prot*. Similarly we can consider the other elements of* $\{\mathsf{hsf_3}, \mathsf{hsf_3{:}\,hse}, \mathsf{hsp}, \mathsf{hsp{:}\,mfp}, \mathsf{prot}\}$*, with the same outcome. Thus, the empty set is the only invariant set.*

Definitions 3.1 and 3.2 of conserved and invariant sets of a reaction system $\mathcal{A} = (S, A)$ present a notable common feature: both require that certain conditions hold for $W \subseteq \mathrm{supp}(\mathcal{A})$ and $\mathrm{res}_{\mathcal{A}}(W)$. We formulate now a property that generalizes the conserved and the invariant sets.

We recall first that a *Boolean formula* φ is said to be over an alphabet S if all its variables names are from S. In the following we assume all Boolean formulae to be given in a disjunctive normal form. A subset $W \subseteq S$ is said to *satisfy* the Boolean formula φ over S if the expression for φ contains a conjunction $x_1 \wedge \ldots \wedge x_n \wedge \bar{y}_1 \wedge \ldots \wedge \bar{y}_m$ such that

(1) $\{x_i \mid 1 \leq i \leq n\} \subseteq W$, and

(2) $\{y_j \mid 1 \leq i \leq m\} \cap W = \varnothing$.

By convention, we write $\varphi(W) = 1$, or simply $\varphi(W)$, if the subset W satisfies φ, and $\varphi(W) = 0$ otherwise. For more details about the relationship between reaction systems and Boolean functions we refer to [2] and [5].

Definition 3.3 (The formula correspondence problems)**.** *Consider a reaction system $\mathcal{A} = (S, A)$. The* formula correspondence problems *for $\mathcal{A}$ are defined as follows: given two Boolean formulae φ_1 and φ_2 over S, does the following hold for every $W \subseteq \mathrm{supp}(\mathcal{A})$:*

$$\varphi_1(W) \Rightarrow \varphi_2(\mathrm{res}_{\mathcal{A}}(W)) \quad (\varphi_1(W) \Leftrightarrow \varphi_2(\mathrm{res}_{\mathcal{A}}(W)),\ \textit{resp.})?$$

Here we write $\phi \Rightarrow \psi$ to denote implication and $\phi \Leftrightarrow \psi$ to denote equivalence.

It is easy to see that the conservation of a subset $M \subseteq S$ in $\mathcal{A}$ can be expressed as a particular case of the formula correspondence problems: M is conserved if and only if for every $W \subseteq \mathrm{supp}(\mathcal{A})$,

$$\varphi_1(W) \Leftrightarrow \varphi_2(\mathrm{res}_{\mathcal{A}}(W)),\ \text{where}$$
$$\varphi_1 = \varphi_2 = \bigvee_{x \in M} x.$$

Stating that the set M is invariant can be expressed in a similar way:

$$\varphi_1(W) \Leftrightarrow \varphi_2(\mathrm{res}_{\mathcal{A}}(W)),\ \text{where}$$
$$\varphi_1 = \varphi_2 = \bigwedge_{x \in M} x.$$

Since we can freely choose φ_1 and φ_2, this general approach allows for the formulation of additional, more complex properties. In particular we can make use of negative literals to specify the conservation of some set whenever particular species are not present in the current state.

Besides mass conservation, a number of other properties inspired from biology can be formulated for reaction systems. We define next the notions of steady state, stationary process, elementary flux, and periodic interactive process.

Definition 3.4 (Steady state)**.** *Let $\mathcal{A} = (S, A)$ be a reaction system. We say that $W \subseteq S$ is a* steady state *of $\mathcal{A}$ if $\mathrm{res}_{\mathcal{A}}(W) = W$.*

Example 3.3. *For our running example* HSR*, $W = \{\mathsf{hsf}, \mathsf{hsf_3}\}$ is a steady state of $\mathcal{A}$, because only reactions* (1) *and* (3) *are enabled on W, and therefore $\mathrm{res}_{\mathcal{A}}(W) = \{\mathsf{hsf}, \mathsf{hsf_3}\} = W$.*

Definition 3.5 (Stationary process)**.** *Let $\mathcal{A} = (S, A)$ be a reaction system and let the pair $\pi = ((C_n)_{n \geq 0}, (D_n)_{n \geq 0})$ be an interactive process in $\mathcal{A}$. We say that π is a* stationary process *of $\mathcal{A}$ if, for every $n \geq 0$, $\mathrm{res}_{\mathcal{A}}(C_n \cup D_n) = C_n \cup D_n$.*

Example 3.4. *For the same case study, let $\pi = ((C_n)_{n \geq 0}, (D_n)_{n \geq 0})$ be an interactive process in $\mathcal{A}$, with $C_0 = \{\mathsf{hse}, \mathsf{prot}, \mathsf{hsp}\colon \mathsf{hsf}, \mathsf{nostress}\}$ and $C_n = \{\mathsf{nostress}\}$ for every natural $n > 0$. Since only reactions* (7)*,* (13)*, and* (17) *are enabled in every state of π, we conclude that $C_n \cup D_n = \{\mathsf{hse}, \mathsf{prot}, \mathsf{hsp}\colon \mathsf{hsf}, \mathsf{nostress}\}$ for every $n \geq 0$ and hence π is a stationary process.*

Definition 3.6 (Elementary flux)**.** *Let $\mathcal{A} = (S, A)$ be a reaction system and $W \subseteq S$ a steady state of $\mathcal{A}$. We say that a subset of reactions $E \subseteq A$ is an* elementary flux *for W, if $\mathrm{res}_{\mathcal{A}_E}(W) = W$, where $\mathcal{A}_E = (S, E)$.*

Example 3.5. *Consider the steady state* $W = \{\mathsf{hsf}, \mathsf{hsf_3}\}$ *of the reaction system* $\mathcal{A}$ *modelling the heat shock response. Then the set of reactions*

$$E = \{(\{\mathsf{hsf}\}, \{\mathsf{hsp}\}, \{\mathsf{hsf_3}\}), (\{\mathsf{hsf_3}\}, \{\mathsf{hse}, \mathsf{hsp}\}, \{\mathsf{hsf}\})\}$$

forms an elementary flux for the steady state W*, since* $\mathrm{res}_{\mathcal{A}_E}(W) = W$*.*

Definition 3.7 (Periodic interactive processes)**.** *Let* $\mathcal{A} = (S, A)$ *be a reaction system and let the pair* $\pi = ((C_n)_{n\geq 0}, (D_n)_{n\geq 0})$ *be an interactive process in* $\mathcal{A}$*. We say that* π *is* periodic *if there exists* $m > 1$ *(the period) such that, for every* $n \geq 0$*, it holds that* $C_{n+m} \cup D_{n+m} = C_n \cup D_n$*.*

Example 3.6. *Again, consider the reaction system* HSR *modelling the heat shock response and an interactive process* $\pi = ((C_n)_{n\geq 0}, (D_n)_{n\geq 0})$ *of it, where* $C_0 = \{\mathsf{hsp}\colon \mathsf{hsf}, \mathsf{stress}\}$*,* $C_{2n+1} = \varnothing$*, and* $C_{2n+2} = \{\mathsf{stress}\}$*, for every* $n \geq 0$*. Since reaction* (12) *is enabled in all even states and reaction* (11) *is enabled in all odd states,* $C_{2n} \cup D_{2n} = \{\mathsf{hsp}\colon \mathsf{hsf}, \mathsf{stress}\}$ *and* $C_{2n+1} \cup D_{2n+1} = \{\mathsf{hsp}, \mathsf{hsf}\}$*, for* $n \geq 0$*. This means that* π *is a periodic interactive process.*

4. Computational Complexity of Checking the Properties

In this section we describe the computational complexity of deciding the properties defined above for a set (sequence of sets), as well as those of deciding whether sets (sequences of sets) satisfying these properties exist at all. We will use the standard notations for complexity classes. FO is the class of problems which can be described with a first-order logic formula. NP is the set of all those decision problems for which a certificate of polynomial size exits and can be efficiently used to verify that the instance of the problem has a positive answer. Dually, coNP comprises the problems for which the negative instances have a polynomial size certificate. The class $\Sigma_2^{\mathsf{P}} = \mathsf{NP}^{\mathsf{coNP}}$ contains all those decision problems which can be solved in polynomial time by a Turing machine equipped with an oracle for some coNP-complete problem. PSPACE is the class of decision problems which can be solved by a Turing machine using a polynomial amount of space (relative to the size of its input). Finally, #P is the class of *function* problems of the form "compute $f(x)$", where f is the number of accepting paths of a non-deterministic Turing machine running in polynomial time. Note that, as different from the classes we have mentioned above, #P is a class of *counting* problems rather than decision problems.

For further details on complexity classes the reader is referred to [12] and [8].

4.1. Mass Conservation, Invariant Sets, and Formula Correspondence

The following proposition is an adapted version of [6, Theorem 7].

Theorem 4.1. *Given a reaction system* $\mathcal{A} = (S, A)$ *and a subset* $M \subseteq \mathrm{supp}(S)$*, deciding if* M *is conserved in* $\mathcal{A}$ *is a* coNP*-complete problem.*

Proof. First we prove that the problem is contained in coNP. If M is not conserved in $\mathcal{A}$, a non-deterministic Turing machine working in polynomial time can guess a set $W \subseteq \mathrm{supp}(\mathcal{A})$ such that $\neg\big(M \cap W \neq \varnothing \Leftrightarrow M \cap \mathrm{res}_{\mathcal{A}}(W) \cap M \neq \varnothing\big)$. Finding the set W requires only a polynomial number of non-deterministic

choices and verifying that M is not conserved in W is a task that can be performed in polynomial time. Hence the problem is in coNP.

We now show that the property is hard for coNP. Let $\varphi = \varphi_1 \vee \ldots \vee \varphi_m$ be a DNF formula over the variables $V = \{x_1, \ldots, x_n\}$. We show that it is possible to construct a reaction system $\mathcal{A}$ and a set M such that M is conserved in $\mathcal{A}$ iff φ is a valid formula. Denote by $\mathrm{pos}(\varphi_i)$ the set of variables appearing as positive literals in φ_i and by $\mathrm{neg}(\varphi_i)$ the set of variables appearing negated in φ_i.

The reaction system $\mathcal{A} = (S, A)$ has as background set $S = V \cup \{\heartsuit, \spadesuit\}$. Given a set $W \subseteq S$, we associate an assignment of φ to W in the following way: a variable x_i is assigned the value *true* if $x_i \in W$, and it is assigned the value *false* otherwise. We also assign to set $W \subseteq S$ a number $k \in \{0, \ldots, 2^{n-1}\}$ defined as $\sum_{i=0}^{n-1} [x_{i+1} \in W] 2^i$, where $[P]$ has value 1 if P is a true predicate and 0 otherwise. That is, we consider the entities $x_1, \ldots, x_n$ as the digits of an n-bit number, where the $(i-1)$-th digit is 1 if $x_i \in W$ and 0 otherwise. We will denote by $B_k = \{x_0, \ldots, x_{n-1}\} \subseteq V$ the set of entities representing the number $k \in \{0, \ldots, 2^n - 1\}$.

The following reactions of A implement an n-bit counter incremented at every step and overflowing at $2^n - 1$:

$$(\{x_{i-1}, \ldots, x_1, \heartsuit\}, \{x_i, \spadesuit\}, \{x_i\}) \qquad \text{for } 1 \leq i \leq n, \tag{21}$$

$$(\{x_i, \heartsuit\}, \{x_j, \spadesuit\}, \{x_i\}) \qquad \text{for } 1 \leq j < i \leq n. \tag{22}$$

A reaction of type (21) sets a previously zero bit to one when all the bits with lower indices are one. A reaction of type (22) preserves a bit with value one if there is at least one zero bit with a lower index.

The following reactions of A preserve $\heartsuit$ if $\varphi(W)$:

$$(\mathrm{pos}(\varphi_i) \cup \{\heartsuit\}, \mathrm{neg}(\varphi_i) \cup \{\spadesuit\}, \{\heartsuit\}) \qquad \text{for } 1 \leq i \leq m. \tag{23}$$

Reactions of type (23) produce $\heartsuit$ if $\spadesuit$ is not present and if there exists a term φ_i that is satisfied by W (and hence, $\varphi(W)$). Notice that $\spadesuit \notin \mathrm{supp}(\mathcal{A}) = V \cup \{\heartsuit\}$; its existence is due to the necessity of having non-empty inhibitors on reactions of type (23) if some $\mathrm{neg}(\varphi_i)$ is empty.

The result function defined by reactions of types (21)–(23) is the following one:

$$\mathrm{res}_{\mathcal{A}}(W) = \begin{cases} \varnothing & \text{if } \heartsuit \notin W \text{ or } \spadesuit \in W, \\ B_{(k+1) \bmod 2^n} \cup \{\heartsuit\} & \text{if } W \subseteq V \cup \{\heartsuit\},\ W \cap V = B_k,\ \varphi(W) = 1, \\ B_{(k+1) \bmod 2^n} & \text{if } W \subseteq V \cup \{\heartsuit\},\ W \cap V = B_k,\ \varphi(W) = 0. \end{cases}$$

We now show that, for each $W \subseteq \mathrm{supp}(\mathcal{A})$, it is true that $\{\heartsuit\} \cap W \neq \varnothing \Leftrightarrow \{\heartsuit\} \cap \mathrm{res}_{\mathcal{A}}(W) \neq \varnothing$ (i.e., the set $\{\heartsuit\} \subseteq \mathrm{supp}(\mathcal{A})$ is conserved in $\mathcal{A}$) if and only if φ is a valid formula. Given a set W it is possible to recognise the following cases:

1. If $\{\heartsuit\} \cap W = \varnothing$, no reaction of types (21)–(23) is enabled since they all have $\heartsuit$ as a reactant, so $res_{\mathcal{A}}(W) = \varnothing$.

2. If $W = B_k \cup \{\heartsuit\}$ for some k, and furthermore $\varphi(W)$, the next state will be $B_{(k+1) \bmod 2^n} \cup \{\heartsuit\}$, $\mathcal{A}$ preserves $\heartsuit$, and we end up in this case again. If however B_k does not entail φ the next state will be $B_{(k+1) \bmod 2^n}$, $\heartsuit$ is not preserved, and we end up in the former case.

Since the binary counter defined by reactions of types (21) and (22) iterates across all the possible subsets of V (i.e., all the possible assignments of φ), the set $\{\heartsuit\}$ is preserved if and only if there is no W such that $\varphi(W) = 0$, that is, if and only if φ is a valid formula. Since that problem of establishing if a formula φ is valid is coNP-complete [12] and the mapping $\varphi \mapsto (\mathcal{A}, \{\heartsuit\})$ is computable in polynomial time, the statement of the theorem holds. □

The observation that conserved singleton sets are also invariant sets allows to directly derive the following result.

Corollary 4.1. *Given a reaction system $\mathcal{A} = (S, A)$ and a set $M \subseteq \mathrm{supp}(\mathcal{A})$, deciding whether M is an invariant set of $\mathcal{A}$ is a* coNP*-complete problem.*

Proof. When M is a singleton, M is an invariant set of $\mathcal{A}$ if and only if M is conserved in $\mathcal{A}$. Therefore the proof of Theorem 4.1 also proves the coNP-hardness of this problem. It is only necessary to show that the problem is in coNP. Notice that it is possible to non-deterministically guess a state of $\mathcal{A}$ and, by computing the next state function, to verify in polynomial time that a given set M is not an invariant set. This shows that the problem lies in coNP. □

The previous two results admit the following straightforward generalization to the formula correspondence problems.

Corollary 4.2. *Given a reaction system $\mathcal{A} = (S, A)$ and two Boolean formulae φ_1 and φ_2, deciding whether*

$$\forall W \subseteq \mathrm{supp}(\mathcal{A}) \,.\, \varphi_1(W) \Rightarrow \varphi_2(\mathrm{res}_{\mathcal{A}}(W))$$

is coNP*-complete. The same is true also for deciding whether*

$$\forall W \subseteq \mathrm{supp}(\mathcal{A}) \,.\, \varphi_1(W) \Leftrightarrow \varphi_2(\mathrm{res}_{\mathcal{A}}(W))$$

Proof. Both problems are in coNP because verifying that $\varphi_1(W) \,\#\, \varphi_2(\mathrm{res}_{\mathcal{A}}(W))$ does *not* hold for a candidate set $W \subseteq S$ can be done in polynomial time, where $\# \in \{\Rightarrow, \Leftrightarrow\}$.

Let $\mathcal{A}$ be a reaction system as in the proof of Theorem 4.1 and let

$$\varphi_1 = \varphi_2 = \heartsuit.$$

Then, the formula $\forall W \subseteq \mathrm{supp}(\mathcal{A}) \,.\, \varphi_1(W) \Rightarrow \varphi_2(\mathrm{res}_{\mathcal{A}}(W))$ means that, for all $W \subseteq \mathrm{supp}(\mathcal{A})$, if $\heartsuit \in W$ then $\heartsuit \in \mathrm{res}_{\mathcal{A}}(W)$, which is true if and only if the formula φ encoded by $\mathcal{A}$ is valid.

On the other hand, the formula $\forall W \subseteq \mathrm{supp}(\mathcal{A}) \,.\, \varphi_1(W) \Leftrightarrow \varphi_2(\mathrm{res}_{\mathcal{A}}(W))$ is simply the conservation of $\{\heartsuit\}$, which once again holds if and only if φ is valid.

Since the mapping $\varphi \mapsto (\mathcal{A}, \varphi_1, \varphi_2)$ can be computed in polynomial time, this establishes the coNP-hardness of the problem. □

Note that, by definition, the empty set is always conserved and invariant. Thus, in the existence problems we will focus on finding non-empty sets.

Theorem 4.2. *Given a reaction system $\mathcal{A} = (S, A)$, deciding if there exists a non-empty conserved set $M \subseteq \mathrm{supp}(\mathcal{A})$ is a* coNP*-hard problem contained in Σ_2^{P}.*

Proof. To show the coNP-hardness of the problem it is only necessary to notice that in the proof of Theorem 4.1 the only possible non-empty conserved set is $\{\heartsuit\}$, since for each "candidate" non-empty conserved set M different from $\{\heartsuit\}$ there are two cases:

- $M = \{\heartsuit\} \cup M'$, with $\heartsuit \notin M'$. In this case $M \cap M' \neq \varnothing$ but $\mathrm{res}_{\mathcal{A}}(M') = \varnothing$, thus $M \cap \varnothing = \varnothing$ and M is not conserved.
- $\heartsuit \notin M$. In this case $M \cap M \neq \varnothing$ but $\mathrm{res}_{\mathcal{A}}(M) = \varnothing$, thus M is not conserved.

Therefore, determining if there exists a non-empty conserved set is as difficult as deciding if $\{\heartsuit\}$ is conserved.

To show that the problem is contained in Σ_2^{P} it is only necessary to notice that a non-deterministic machine with an oracle for coNP can non-deterministically guess a state and then determine if it is a conserved state using the coNP oracle (Theorem 4.1). Therefore the problem is contained in $\mathsf{NP}^{\mathsf{coNP}} = \Sigma_2^{\mathsf{P}}$. □

By following the same steps of Theorem 4.2 and observing that $\{\heartsuit\}$ is the only possible invariant set, we also obtain the following result:

Corollary 4.3. *Given a reaction system $\mathcal{A} = (S, A)$, deciding if there exists a non-empty invariant set $M \subseteq S$ is a* coNP*-hard problem contained in Σ_2^{P}.*

4.2. Steady States and Elementary Fluxes

As shown in [7, Corollary 1], deciding whether a state M is a steady state of a reaction system $\mathcal{A}$ is in FO and therefore can be done in polynomial time. The following theorem is an adaptation of [7, Theorem 2], and shows that deciding whether there exists a non-trivial steady state in a reaction system is an NP-complete problem.

Theorem 4.3. *Given a reaction system $\mathcal{A} = (S, A)$, deciding if there exists a non-empty steady state $M \subseteq S$ of $\mathcal{A}$ is an* NP*-complete problem.*

Proof. First of all we show that the problem lies in NP. Notice that it is possible to non-deterministically guess a set M and verify, in polynomial time, that $\mathrm{res}_{\mathcal{A}}(M) = M$, thus showing that the problem is contained in NP.

To prove the hardness of the problem, we reduce SAT [12] to it. Let $\varphi = \varphi_1 \wedge \ldots \wedge \varphi_m$ be a CNF formula over the variables $V = \{x_1, \ldots, x_n\}$. We define a reaction system $\mathcal{A} = (S, A)$ where $S = V \cup \{\heartsuit, \spadesuit\}$ and where A contains the following reactions (for the notations see the proof of Theorem 4.1):

$$(\mathrm{neg}(\varphi_i) \cup \{\heartsuit\}, \mathrm{pos}(\varphi_i) \cup \{\spadesuit\}, \{\spadesuit\}) \qquad \text{for } 1 \leq i \leq m \qquad (24)$$

$$(\{x_i, \heartsuit\}, \{\spadesuit\}, \{x_i\}) \qquad \text{for } 1 \leq i \leq n \qquad (25)$$

$$(\{\heartsuit\}, \{\spadesuit\}, \{\heartsuit\}). \qquad (26)$$

Reactions of type (24) produce $\spadesuit$ for state T when there is a non-satisfied clause in φ when considering the assignment given by T. Reactions of type (25) and (26) preserve the current state if it does not contain $\spadesuit$. The result function represented by $\mathcal{A}$ is then:

$$\mathrm{res}_{\mathcal{A}}(T) = \begin{cases} T & \text{if } \heartsuit \in T,\ \spadesuit \notin T, \text{ and } \varphi(T) = 1 \\ T \cup \{\spadesuit\} & \text{if } \heartsuit \in T,\ \spadesuit \notin T, \text{ and } \varphi(T) = 0 \\ \varnothing & \text{otherwise.} \end{cases}$$

From $\mathrm{res}_{\mathcal{A}}$ it is possible to observe that only sets in the form $U \cup \{\heartsuit\}$ for some $U \subseteq V$ do not necessarily end up in $\varnothing$. In particular, a set T of such form is a steady state of $\mathcal{A}$ iff $\varphi(T)$, thus showing the NP-completeness of the problem of determining the existence of a non-empty steady state for $\mathcal{A}$. □

As far as elementary fluxes are concerned, note first that the decision problem can be solved in polynomial time. Indeed, consider a reaction system $\mathcal{A} = (S, A)$ and W a steady state of $\mathcal{A}$. If we are given a set of reactions E, we only need to test that W is a stead state of the reduced system (S, E). Moreover, note that, by definition, $E = A$ is an elementary flux. To find non-trivial elementary fluxes, we constrain the cardinality of E, i.e. we look for sets of at most k reactions that preserve the steady state W. The following theorem describes the complexity of deciding their existence and counting them.

Theorem 4.4. *Given a reaction system $\mathcal{A} = (S, A)$, a steady state W of $\mathcal{A}$, and an integer $k < |A|$:*

- *it is NP-complete to decide if there exists an elementary flux for W with at most k reactions;*
- *it is #P-complete to count such elementary fluxes.*

Proof. Let $(U, \mathcal{F}, k)$ be an instance of the set covering problem [12], where U is a finite set, $\mathcal{F} = \{F_1, \ldots, F_n\}$ is a set cover of U (i.e., a family of subsets of U having union U), and k is an integer. The problem consists in deciding whether there exists a set cover $\mathcal{E} \subseteq \mathcal{F}$ of U of size at most k.

Consider the reaction system $\mathcal{A} = (S, A)$, where $S = U \cup \{\spadesuit\}$ and A contains the reaction $a_F = (U, \{\spadesuit\}, F)$ for each $F \in \mathcal{F}$. Then, we have

$$\mathrm{res}_{\mathcal{A}}(U) = \bigcup_{F \in \mathcal{F}} \mathrm{res}_{a_F}(U) = \bigcup \mathcal{F} = U.$$

If we let $\mathcal{E} \subseteq \mathcal{F}$ and $E = \{a_F \in A : F \in \mathcal{E}\}$, then we have $\mathrm{res}_{\mathcal{A}_E}(U) = U$ with $|E| \leq k$ if and only if $\bigcup \mathcal{E} = U$ with $|\mathcal{E}| \leq k$. Furthermore, the mapping $\mathcal{E} \mapsto E$ is a bijection between the set covers $\mathcal{E} \subseteq \mathcal{F}$ of U and the elementary fluxes of U, hence reductions between $(U, \mathcal{F}, k)$ and $(\mathcal{A}, U, k)$ are computable in polynomial time. The statement of the theorem follows from the NP-completeness of set covering and the #P-completeness of its counting version [14]. □

4.3. Periodicity and Stationarity

Even if a sequence of contexts $(C_n)_{n \geq 0}$ for an RS $\mathcal{A} = (S, A)$ is computable by means of a function $n \mapsto C_n$, certain properties of the resulting interactive process, such as periodicity or stationarity, are undecidable. Indeed, given a Turing machine M, we can build the sequence of contexts $n \mapsto C_n$ where $C_n = S$ if M has halted on empty input after n steps and $C_n = \varnothing$ otherwise; this context sequence is computable, but stationarity and periodicity are undecidable properties even for an RS with no reactions. In fact, even with a formalism as weak as FO logic it is possible to simulate the behaviour of a Turing machine, as shown by the proof of Trakhtenbrot's theorem [10].

When we restrict the sequences of contexts to periodic ones, they become redundant, as the reaction systems themselves are able to generate such contexts internally.

Theorem 4.5. *Given a reaction system $\mathcal{A} = (S, A)$ and a periodic sequence of contexts $(C_0, \ldots, C_{p-1})$ with $p > 1$, it is* PSPACE*-complete to decide whether the the interactive process $\pi = \big((C_n)_{n\geq 0}, (D_n)_{n\geq 0}\big)$, with $C_n = C_{n \bmod p}$ and $D_{n+1} = \mathrm{res}_{\mathcal{A}}(C_n \cup D_n)$, is periodic.*

Proof. The problem is already PSPACE-hard for context-free reaction systems [6], which can be seen as having $(\varnothing)$ as their periodic sequence of contexts.

We can also perform the converse reduction. Let $\mathcal{A}' = (S', A')$ be the reaction system having $S' = S \cup \{c_0, \ldots, c_{p-1}, \spadesuit\}$ (i.e., we augment S with one new element per each possible context and an entity $\spadesuit$ to be used as a "dummy" inhibitor) and with A' containing all reactions in A together with

$$(\{c_i\}, \{\spadesuit\}, C_{(i+1) \bmod p} \cup \{c_{(i+1) \bmod p}\}) \qquad \text{for } 0 \leq i \leq p-1.$$

The result function $\mathrm{res}_{\mathcal{A}'}$ has, for each $T \subseteq S$, the following property:

$$\mathrm{res}_{\mathcal{A}'}(T \cup \{c_i\}) = \mathrm{res}_{\mathcal{A}}(T) \cup C_{(i+1) \bmod p} \cup \{c_{(i+1) \bmod p}\},$$

that is, the reaction system $\mathcal{A}'$ behaves like $\mathcal{A}$ when considering only the elements of S without needing an external context sequence. The extra elements c_i define a counter with period p; therefore, the interactive process π is periodic with period m if and only if the (context-free) state sequence of $\mathcal{A}'$ starting at $C_0 \cup \{c_0\}$ is periodic with period at most pm.

The statement of the theorem follows from the fact that the reduction $(\mathcal{A}, C_0, \ldots, C_{p-1}) \mapsto \mathcal{A}'$ can be performed in polynomial time, and that PSPACE is closed under such reductions. □

On the other hand, the *stationarity* of the interactive process generated by a periodic (resp., ultimately periodic) sequence of contexts can be efficiently detected by simply checking whether the first n states of the system are identical, where n is the length of the period of the sequence of contexts (resp., the length of the period plus the length of the pre-period).

We summarize the results we obtained in this paper in the following general theorem.

Theorem 4.6. *The following statements are true for a reaction system $\mathcal{A}$:*

1. *deciding if $M \subseteq \mathrm{supp}(\mathcal{A})$ is conserved in $\mathcal{A}$ is* coNP*-complete;*
2. *deciding if $M \subseteq \mathrm{supp}(\mathcal{A})$ is an invariant set of $\mathcal{A}$ is* coNP*-complete;*
3. *deciding the formula correspondence problem for two Boolean formulae is* coNP*-complete;*
4. *deciding if $\mathcal{A}$ conserves a non-empty set is a* coNP*-hard problem contained in Σ_2^{P};*
5. *deciding if $\mathcal{A}$ has a non-empty invariant set is a* coNP*-hard problem contained in Σ_2^{P};*
6. *deciding if $\mathcal{A}$ has a non-empty steady state is* NP*-complete;*
7. *for a steady state W of $\mathcal{A}$, deciding the existence of an elementary flux of size at most k is* NP*-complete;*

8. *for a steady state W of $\mathcal{A}$, counting the elementary fluxes of size at most k is* #P*-complete;*

9. *deciding the periodicity of an interactive process of $\mathcal{A}$ with a periodic context sequence is* PSPACE*-complete.*

10. *deciding the stationarity of an interactive process of $\mathcal{A}$ with a periodic context sequence is in* P*.*

5. Conclusion

We considered in this paper some of the properties of central interest in biomodeling: mass conservation, invariants, steady states, stationary processes, elementary fluxes, and periodicity. We defined them in the case of reaction systems so that they extend in a natural way from the usual quantitative case to the qualitative framework of reaction systems. We then established the complexity class of checking these properties for reaction systems models.

The results of our paper, summarized in Theorem 4.6, show that checking for these properties is difficult in the sense of computational complexity. On one hand, this is surprising because checking them in quantitative frameworks such as ODE-based reaction models or Petri-net models is in general easy and reduces to problems of linear algebra. On the other hand, our results are in line with [6, 7] who show that checking properties such as fixed points, cycles, and attractors are also difficult problems for reaction systems. Moreover, our conclusions are in line with the recent results of [11] that introduce a temporal logic for reaction systems and show that model checking in this logic is PSPACE-complete. The properties we introduce in this paper can be formulated in the temporal logic of [11] and we prove that even in these special cases the problems remain intractable, albeit on lower complexity classes than PSPACE-complete. This gives an interesting insight into the complex dynamics of reaction systems, a framework in which the quantitative competition on resources is replaced by Boolean logic-based facilitation and inhibition.

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