Unwinding biological systems

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Abstract

Unwinding conditions have been fruitfully exploited in Information Flow Security to define persistent security properties. In this paper we investigate on their meaning and possible uses in the analysis of biological systems. In particular, we elaborate on the notion of robustness and propose some instances of unwinding over the process algebra Bio-PEPA and over hybrid automata. We exploit such instances to analyze two case-studies: *Neurospora Crassa* circadian system and Influenza kinetics models.

Introduction

Different languages have been proposed for modeling biological systems. As noticed in [24] we can distinguish two main categories: mathematical models (e.g., differential equations) and computational models (e.g., process algebras). Mathematical models rely on denotational semantics, i.e., they describe relationships between quantities. Algorithms for finding/approximating solutions of such models are not part of the model itself. On the other hand, computational models are equipped with "natural" operational semantics which describes the evolution of the system. Simulation algorithms for such models can be easily defined.

Temporal Logics and Model Checking are standard tools for the analysis of computational models (e.g., see [18]). The former provide specification languages for formulating the properties of interest. The latter furnishes algorithms for verifying properties on models. Two models are considered behaviourally equivalent when they are indistinguishable with respect to the temporal logic formulæ.

Behavioural equivalences can then be used to both compare models and reduce their sizes. While their use as reduction criteria is well known in Model Checking, where the state explosion problem is a major concern, their importance for comparing models has been fruitfully exploited in other fields. In particular, in Information Flow Security behavioural equivalences are at the basis of unwinding conditions which allow to establish whether the system would behave correctly in hostile environments (see [61, 10]).

In this paper, we propose the use of unwinding conditions in Systems Biology. In order to achieve this goal we develop a framework, based on the notion of labelled transition system, that exploits the unwinding conditions and we suggest how to instantiate this framework to handle different computational formalisms. Unwinding conditions allow to generalize notions of robustness based on formulæ satisfaction which have been introduced in the literature, giving rise to robust notions of robustness where possibly infinite set of formulæ can be preserved over possibly infinite sets of perturbations.

The paper is organized as follows: Section 1 introduces Labelled Transition Systems and bisimulations as standard operational semantics tools. Section 2 presents the unwinding framework in both Information Flow Security and Systems Biology, showing how it can be used to formalize a robust notion of robustness. In Section 3, we briefly recall the main features of Bio-PEPA process algebra and we instantiate our unwinding framework on Bio-PEPA systems. In Section 3.1, we test it on the Bio-PEPA model of the *Neurospora crassa* circadian network. In Section 4 we present two unwinding conditions over hybrid automata and in Section 4.1 we exploit them on kinetic models of influenza virus. Conclusions are drawn in Section 5.

1 Labelled Transition Systems and Equivalences

Labelled Transition Systems (latter on referred as LTS's) are a general tool used to define the operational semantics of wide variety of models. An LTS is a directed graph with labels on edges.

Definiton 1 (Labelled Transition System). *A LTS is a tuple (V, V_I, A, E) where:*

- *V is a set of nodes and $V_I \subseteq V$ is a set of initial nodes;*

- *A is the set of edge labels (alphabet);*

- *$E \subseteq V \times A_E \times V$ is a set of edges.*

We may write $v \xrightarrow{\alpha} v'$ to indicate that (v, α, v') is an edge, i.e., $(v, \alpha, v') \in E$, and $v \to v'$ to denote that there exists some $\alpha \in A_E$ such that (v, α, v') is an edge. In some cases, also labels on nodes can be introduced.

Well-known classes of models which are given in terms of LTS's are *Discrete Time Markov Chains* (DTMCs) and *Continuous Time Markov Chains* (CTMCs). Both DTMCs and CTMCs are used for modeling stochastic memoryless processes in which the next state of the system only depends on the current state

and on a probability distribution over its out-going edges. While in DTMCs labels on the edges represent the instantaneous crossing probability, in CTMCs they are the parameters of exponential distributions which allow one to compute the crossing probability within a time interval.

Definiton 2 (Markov Chains). *A DTMC is a LTS $D = (V^D, V_I^D, A^D, E^D)$ such that $A^D = [0, 1] \subseteq \mathbb{R}$ and the sum of the labels of the edges leaving each node is equal to 1, i.e., $\left(\sum_{(v,\alpha,u) \in E^D} \alpha \right) = 1$ for each $v \in V^D$.*
A CTMC is a LTS $C = (V^C, V_I^C, A^C, E^C)$ such that $A^C = \mathbb{R}_{\geq 0}$.

As examples of the broad and heterogeneous use of LTS's in modeling, we will consider later in this paper two formalisms, having a quite different approach to the modeling problem, whose evolutions are naturally described by means of LTS's: the process algebra Bio-PEPA (see, e.g., [27]), and hybrid automata (see, e.g., [48]).

Once an LTS representing a system has been obtained, behavioural equivalences can be used to reduce the size of the LTS, prove properties over the system, and compare different systems. Behavioural equivalences are equivalences over LTS's that relate nodes having "similar behaviours". *Trace equivalence* and *bisimulation* are two of the most used behavioural equivalences in the literature.

Trace equivalence relates two nodes if and only if they generate the same sequences of node and edge labels (traces). It produces a drastic reduction of the model size, but its computation is expensive (PSPACE-complete) [51]. Moreover, in many applications, it equates models that are "different". In particular, it cannot distinguish models which differ because of "the time at which non determinism occur" (see Figure 1).

Definiton 3 (Trace Equivalence). *Given an LTS $T = (V, V_I, A, E)$, a path over T starting from $u \in V$ is a sequence ph of transitions of the form $u = u_0 \xrightarrow{\alpha_1} u_1 \xrightarrow{\alpha_2} \ldots \xrightarrow{\alpha_n} u_n$. The* trace *underlying the path ph is the sequence tr defined as $\alpha_1 \alpha_2 \ldots \alpha_n$. The set $Tr(u)$ is the set of traces underlying paths starting from u. Two nodes $u, v \in V$ are said to be* trace equivalent *if $Tr(u) = Tr(v)$.*

On the contrary, bisimulation is a finer relation which is easier to compute. In its strong version it equates models that satisfy exactly the same formulæ of modal and branching temporal logics (see, e.g., [18]) Its co-inductive characterization is at the basis of efficient polynomial time algorithms for its computation [39, 53].

Definiton 4 (Strong Bisimulation). *Given an LTS $T = (V, V_I, A, E)$, a* strong bisimulation *over T is a relation $R \subseteq V \times V$ such that for each $(u, v) \in R$ the following conditions hold:*

- *$u \in V_I$ if and only if $v \in V_I$;*

- *if $u \xrightarrow{\alpha} u'$, then $v \xrightarrow{\alpha} v'$ and $(u', v') \in R$;*

- if $v \xrightarrow{\alpha} v'$, then $u \xrightarrow{\alpha} u'$ and $(u', v') \in R$.

Two nodes $u, v \in V$ are said to be strongly bisimilar, *denoted as $u \sim v$, if there exists a strong bisimulation R such that $(u, v) \in R$.*

When also labels on nodes are considered two bisimilar nodes must share the same labels. The following proposition states some well known and appealing properties of bisimulation.

Proposition 1. *Given an LTS $T = (V, V_I, A, E)$. The relation $\sim$ is an equivalence relation, it is the largest strong bisimulation relation, and Paige-Tarjan algorithm [53] computes it in time $O(|E| \log |V|)$. Moreover, $u \sim v$ implies $Tr(u) = Tr(v)$.*
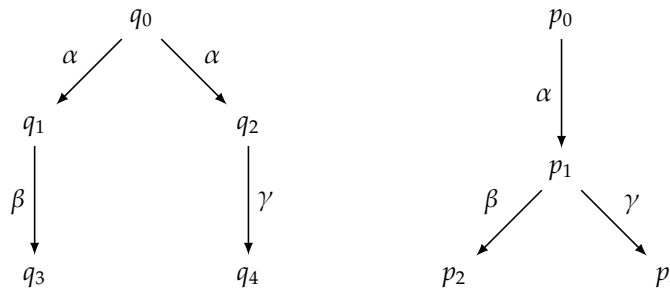

Figure 1: Trace equivalence vs strong bisimulation: the two nodes p_0 and q_0 are are trace equivalent but not strongly bisimilar.

There exist many variant of bisimulation (e.g., weak and stuttering), depending on the modal/temporal logic one wants to preserve. In the context of DTMCs and CTMCs bisimulations, which need to "preserve" probabilities, are usually called *lumpability relations* (see, e.g., [70]).

2 Robustness through Non-interference and Unwinding

2.1 From Biology . . .

As observed in [42], ". . . robustness is one of the fundamental characteristics of biological systems . . . Nevertheless, a mathematical foundation that provides a unified perspective on robustness is yet to be established". In [40], robustness is defined as a property ensuring that a system maintain its functions against internal and external perturbations. A framework for analyzing robustness should support the definition of both functions to be preserved and admissible perturbations.

Robustness has not to be confused with stability. A robust system can exploit instability or even evolve through new steady states in order to preserve its functionalities against perturbations.

In the following example we try to clarify these concepts.

Example 1 (Research on Pathogens). *Let us consider the case of a system infected by a pathogen agent. Of course the pathogen stimulates the immune system and would probably affect some organs, i.e., the system is not stable. Hence, the immune system and all the organs directly attacked by the pathogen will exhibit a behavior which could be very different from the standard one (at least in the acute phase). However, if the system is robust against the pathogen, behaviour of critical organs (e.g., heart, lung, kidney, brain) should not be dramatically affected.*

A formalization of "how much" a system is robust *with respect to a pathogen is a fundamental question in medicine both in the diagnosis process (to avoid expensive/invasive exams) and in the therapy phase.*

On the other hand, if a system is stable *with respect to a pathogen, this means that the pathogen has almost no effects on the system. Hence, probably such pathogen has low medical interest: not even the patient will notice that his immune system is interacting with the pathogen.*

It is important to notice that robustness is not a *local* property. The "functions to be preserved" could have no apparent relationship with the "admissible perturbations". This is better shown by the following example.

Example 2 (Drug Development). *The case of different drugs for mitigating inflammations is described in [41]. Cyclooxygenase 2 (COX2) is expressed in tissues with inflammation and its inhibition reduces the inflammation process. Some drugs were designed to inhibit COX2, but since they also inhibit COX1 adverse gastrointestinal effects were observed. Hence, drugs with more selective inhibition of COX2 were considered. These do not have gastrointestinal side-effects, but at high doses the risk of cardiovascular problems increased. As Kitano pointed out this example "highlights the fact that selectivity for a molecule in the target cells does not eliminate the risk of side effects, as the target molecule might have an important role in off-target cells. . . . Drug side effects can be caused by unwanted interactions with molecules that expose the fragility of cellular or organ-level functions to specific interventions in both target cells and off-target cells."*

In the next section we present an unwinding framework typical of information flow security. We will take inspiration from such framework to define a general notion of robustness which tries to answer Kitano's requirements.

2.2 . . . To Security . . .

Information flow security deals with multilevel systems in which confidential and public data coexist. Its main goal is to ensure that no information flow from a level to a lower one. Traditionally, only two security levels are considered: *high* (H) and *low* (L). High level users have access to confidential information, while low level users can only handle public data. The interaction between high level users and the system should not influence the low level behaviours. In the case of deterministic systems, this was formalized in [31] as the notion of *non-interference*. Such notion has been generalized in different non-deterministic

settings such as programming languages [62], process calculi [25, 60], probabilistic models [2], cryptographic protocols [8].

In information flow security, *unwinding conditions* have been introduced as local properties on high level actions aiming at ensuring non-interference [60, 61, 10]. We present here the notion of *unwinding* over LTS's. First we need to partition the edge labels into two sets: H, high level labels, corresponding to high level actions and L, low level labels, associated to low level actions. We dub LTS's with H and L edge labels *multilevel LTS's*. The unwinding condition is parametric with respect to two relations: a *low level behavioural equivalence*, $\sim^L$, and a *transition relation*, $\dashrightarrow$. The former establishes which nodes should be considered indistinguishable from a low level point of view. The latter identifies paths alternative to the execution of a high action.

Definiton 5 (Unwinding $\mathcal{W}(\sim^L, \dashrightarrow)$). *Let $T = (V, V_I, H \cup L, E)$ be a multilevel LTS, $\sim^L \subseteq V \times V$ be an equivalence relation, and $\dashrightarrow \subseteq V \times V$ be a transition relation. We say that T satisfies the unwinding condition $\mathcal{W}(\sim^L, \dashrightarrow)$ if for each $h \in H$ for each $u \in V$ the following condition holds:*

$$u \xrightarrow{h} u' \text{ implies } \exists u''(u \dashrightarrow u'' \wedge u' \sim^L u'')$$

In Figure 2 we give an intuitive representation of a generic unwinding condition.

Whenever the information included in the nodes is also important, generalized unwinding conditions come into play. They are parametric on a further relation $=^L$ establishing which node information is low level visible or, in other terms, when two nodes are locally low level indistinguishable, i.e., indistinguishable without considering their outgoing edges.

Definiton 6 (Generalized Unwinding $\mathcal{W}(=^L, \sim^L, \dashrightarrow)$). *Let $T = (V, V_I, H \cup L, E)$ be a multilevel LTS, $=^L, \sim^L \subseteq V \times V$ be two equivalence relations, and $\dashrightarrow \subseteq V \times V$ be a transition relation. We say that T satisfies the generalized unwinding condition $\mathcal{W}(=^L, \sim^L, \dashrightarrow)$ if for each $h \in H$ for each $u \in V$ the following condition holds:*

$$u \xrightarrow{h} u' \text{ implies } \forall v(u =^L v \text{ implies } \exists v'(v \dashrightarrow v' \wedge u' \sim^L v'))$$

Intuitively, this corresponds to say that the low level user is not even able to tell whether the system before the high level transition was in u or in v. Generalized unwinding conditions have been used in [9] to study information flows on a basic concurrent imperative language. Unwinding conditions are nothing but generalized unwinding conditions in which the relation $=^L$ is the identity relation.

Surprisingly, in [10] it has been proved that some instances of the unwinding schema were equivalent to well known security properties defined in terms of high level attacker models. Intuitively, if a system E satisfies the unwinding condition, then a high level malicious process Π interacting with any state E' reachable from E (denoted by $E'|\Pi$) cannot send down to the low level user
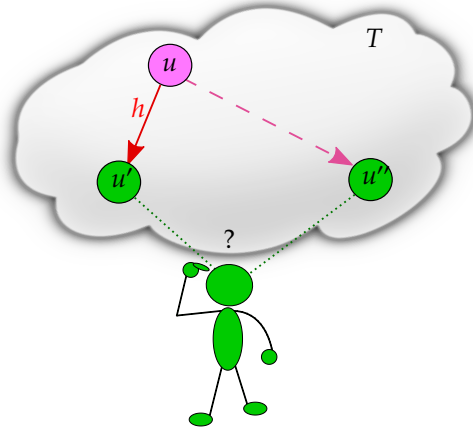
Figure 2: Intuitive representations of unwinding condition. The system T satisfies the unwinding conditions $\mathcal{W}(\sim^L, \dashrightarrow)$ if, whenever a high action can lead the state from u to u', $\dashrightarrow$ can lead T from u to u'' and u' and u' are not distinguishable by the low user.

private informations (i.e., $E'|\Pi \sim^L E'$). In such context, the (generalized) unwinding framework is appealing for many reasons. It allows a universal quantification over an infinite set of attackers. It characterizes persistent security properties, i.e., properties which hold also when the attack starts in the middle of the computation. It localizes the reasoning/computation over the system. In many cases it naturally suggests refinements and correction policies.

2.3 . . . and Back: a Robust notion of Robustness

Our proposal is to use the notion of non-interference and its formalization through unwinding conditions to define a general framework for robustness. Informally a system is robust if, when it moves to a perturbed state, the behaviors of its critical components remain almost unchanged. In other words, when a robust system perform a "high level action" which leads to a perturbed state the "low level behaviors" of its critical components are not influenced. Hence, in the biological setting high level actions play the role of perturbations and they interfere only with some specific components of the system, while low level actions represent the functions to be preserved, where "preserved" mean having a behaviour which is "equivalent" to the standard one. We try to better understand this idea on the examples presented in Section 2.1.

Example 3 (Research on Pathogens – part II). *Let us consider again the system infected by a pathogen agent. The immune system and all the organs directly attacked by the pathogen are high level components, while the organs which we do not want to be dramatically affected are the low level ones (e.g., heart, lung, kidney, brain).*

Some changes in the immune system (high actions) could slightly modify the usual behaviour of the "low level" organs. To be acceptable such changes should be low level behaviourally equivalent to the usual evolution.

Example 4 (Drug Development – part II). *In the anti-inflammatory drugs example, COX1 and COX2 inhibition is the high level expected effect of the drugs, while gastrointestinal and cardiovascular problems are the low level undesirable changes. The "unwanted interactions" mentioned by Kitano [41] are the* interference *effects we intend to measure with low level observational equivalences.*

The above examples point out that when we define a notion of robustness based on non-interference, the low level actions are the ones we want to preserve and hence, they are the ones we are interested in observing. So, in the biological setting we call them *Exposed* (*Exp*). On the other hand, high level actions are perturbations usually coming from the environment and, of course, actions of the system in response to such perturbations. Since, the system has limited (or possible none) control on external perturbations, we will call them *Imposed* (*Imp*). So we get the following definition of robustness.

Definiton 7 (Robustness through Non-Interference). *A biological system S which can both interact with the behaviour through Imposed Imp actions and perform Exposed Exp actions is said to be* robust through Non-Interference *if the Imposed behaviours do not influence the Exposed ones.*

As in the field of Information Flow Security, Non-Interference has been formalized through (generalized) unwinding conditions, in Systems Biology we can exploit them to characterize robustness.

Definiton 8 (Robustness through Unwinding). *Let $T = (V, V_I, Imp \cup Exp, E)$ be a multilevel LTS representing a biological system, $=^{Exp}, \sim^{Exp} \subseteq V \times V$ be two equivalence relations, and $\dashrightarrow \subseteq V \times V$ be a transition relation. We say that T is* unwinding robust, *denoted as $T \in W(=^{Exp}, \sim^{Exp}, \dashrightarrow)$, if for each $i \in Imp$ for each $u \in V$ the following condition holds:*

$$u \xrightarrow{i} u' \text{ implies } \forall v(u =^{Exp} v \text{ implies } \exists v'(v \dashrightarrow v' \wedge u' \sim^{Exp} v'))$$

In order to instantiate the unwinding framework in the biological context we need to choose the exposed behavioural equivalences $\sim^{Exp}$ and $=^{Exp}$ and the transition relation $\dashrightarrow$. The exposed behavioural equivalences $\sim^{Exp}$ and $=^{Exp}$ establish which variations in the behaviour of the system are considered acceptable. In the literature comparisons between systems under different conditions are performed either observing simulations of the systems or checking that some system properties are true in all cases. These are just two possible cases of exposed behavioural equivalences. As we further explain in the rest of this section unwindings generalize these approaches. The transition relation $\dashrightarrow$ has a less intuitive meaning. We can view $\dashrightarrow$ as a possible *delay* between the behaviour of the system running in normal conditions and the system after an

imposed change. In the case of biological systems this is coherent with a situation in which the imposed action speeds up a "reaction". Consider for instance the case of a model representing the behaviour of a patient with a bacterial infection. The imposed action represents the decision of taking an antibiotic and immediately reaching a recovery state, while the ⇢ relation slowly leads to the recovery state without taking any drug. In this case ⇢ clearly represents a delay. On the other hand, when the imposed action is a perturbation on a system at the steady-state, the perturbed system needs time to go back to a steady-state. In the case ⇢ is the identity relation, i.e., no action is performed, while the "delay" needed by the perturbed system has to be absorbed by the exposed observational equivalence $\sim^{Exp}$.

Going back to Kitano's requirements for a robustness framework [40], we can now say that in our general notion of robustness based on unwinding conditions, the functions of a system are mainly defined through the *exposed observational equivalences* $=^{Exp}$ *and* $\sim^{Exp}$, while the perturbations are modeled through *imposed actions*. Moreover, as far as the difference between stability and robustness is concerned, unwinding conditions allow to move inside a lattice of equivalences where, travelling from finer (qualitative) relations to coarser (quantitative) ones, stability transits to robustness.

Let us explain in which sense *our notion robustness is robust*. Recently many authors have proposed models for robustness in the biological setting (see, e.g., [43, 22, 21, 59, 20, 12, 7]). We can distinguish two main approaches: robustness as "sensitivity" and robustness as "properties preservation".

Sensitivity analysis aims at explaining the uncertainty in the output of a mathematical model by means of different sources of uncertainty in its inputs. It is based on statistical techniques and it has been successfully applied in the area of control theory to ensure robustness of engineered controllers. More recently, it has been proposed for the robustness analysis of biological systems (see e.g., [43]).

As far as properties preservation is concerned, Temporal Logics can be used as specification languages for expressing the properties of interest. Such logics have been traditionally used by the computer science community for the verification of real-time critical systems. Properties of system traces can be specified by these formalisms and Model Checking algorithms verify them on a given system (see, e.g, [18] for a general introduction). [55] suggested to model biological systems through LTS's and exploit these well-established techniques in the biological setting. In such context robustness has been defined as a way to "measure the distance" (or in other terms a "degree of satisfaction") of a set of traces from a given specification (see, e.g., [22, 21, 59, 20, 12, 7]). In particular, Fainekos and Pappas defined in [22], and Brim *et al.* extended in [12], both a distance $\delta(s, s')$ between two signals and a robustness degree **Dist**(s, φ) of the signal s on a Metric Temporal Logics formula φ. They prove that if $\delta(s, s') \leq$ **Dist**(s, φ), then the evaluations of φ on both s and s' give the same result. Hence, **Dist**(s, φ) represents the diameter of a maximal flow tube which includes s and over which the value of φ is constant. In other terms, it represents the minimum distance between s and signals over which φ has

a different truth value. If $\mathbf{Dist}\,(s, \varphi) = 0$, then s is not robust on φ. More in general the following definition applies.

Definiton 9. *[Robustness as property preservation [22]] Given a set of perturbations S, i.e., a set of signals, s is robust on φ with respect to S if $\delta(s, S) = sup_{s' \in S} \delta(s, s') \leq Dist\,(s, \varphi)$.*

We can embed such notion of robustness in our framework as follows.

Definiton 10 (Signals LTS). *A signal s is a function $s : \mathbb{R}_{\geq 0} \to \mathbb{R}^n$ which associates to any time instant $t \in \mathbb{R}_{\geq 0}$ a tuple $s(t) = (x_1, \ldots, x_n)$ of values.*

The timed LTS $LT(s) = (V, V_I, Imp \cup \mathcal{E}xp, E)$ associated to a signal s is defined as follows:

- $V \stackrel{def}{=} \{(t, s(t)) \mid t \in \mathbb{R}_{\geq 0}\}$;

- $V_I \stackrel{def}{=} \{(0, s(0))\}$;

- $Imp \stackrel{def}{=} \emptyset$ *and* $\mathcal{E}xp \stackrel{def}{=} \mathbb{R}_{\geq 0}$;

- $E \stackrel{def}{=} \left\{ \left(\left(t_1, s(t_1) \right), t_2 - t_1, \left(t_2, s(t_2) \right) \right) \mid t_1 \geq t_2 \right\}$.

The untimed LTS is obtained from $LT(s)$ by removing edge labels.

Given a signal s and a set of signals S we build a model containing s and all the signals of S in which s is interpreted as the standard behaviour, while the elements of S are the perturbed behaviours which occur after an Imposed action ι.

Definiton 11 (Set Signals LTS). *Let s and S be a signal and a set of signals, respectively. Moreover, let $(V^p, V_I^p, Imp \cup \mathcal{E}xp^p, E^p)$ be the LTS $LT(p)$ for all p in $S \cup \{s\}$. The set signals LTS $LT(s, S)$ is the tuple $(V, V_I, Imp \cup \mathcal{E}xp, E)$ where:*

- $V \stackrel{def}{=} \{init\} \cup (V^s \times \{s\}) \cup \bigcup_{s' \in S} (V^{s'} \times \{s'\})$;

- $V_I \stackrel{def}{=} \{init\}$;

- $Imp \stackrel{def}{=} \{\iota\}$ *and* $\mathcal{E}xp \stackrel{def}{=} \mathbb{R}_{\geq 0} \cup \{\epsilon\}$;

- $E \stackrel{def}{=} \{(init, \epsilon, ((0, s(0)), s))\} \cup \{(init, \iota, ((0, s'(0)), s')) \mid s' \in S\} \cup lift\,(E^s, s) \cup \bigcup_{s' \in S} lift\left(E^{s'}, s' \right)$;

and $lift\,(F, q) \stackrel{def}{=} \{((u, q), \alpha, (v, q)) \mid (u, \alpha, v) \in F\}$.

From an intuitive point of view, $LT(s, S)$ is the disjoint union of the LTS's of s and of each of $s' \in S$ together with a new distinguished node *init* which reaches all the nodes of the form $(0, r)$. From the node *init*, $LT(s, S)$ can reach the LTS $LT(s)$ by crossing an edge whose label is $\epsilon \in \mathcal{E}xp$; on the contrary, all the edges that connect *init* to one of the LTS $LT(s')$, where $s' \in S$, are labelled by $\iota \in Imp$.

We define an unwinding condition which embed the robustness notion defined in [22] in our framework.

Definiton 12. *The unwinding condition for the temporal formula φ is $\mathcal{W}^\varphi = (id, \approx^\varphi$ $, \xrightarrow{\epsilon})$ where id is the identity relation and $\approx^\varphi$ is the smallest equivalence relation such that:*

$$(0, s(0)) \approx^\varphi (0, s'(0)) \quad \textit{iff} \quad \delta(s, s') \leq \textbf{Dist}(s, \varphi)$$

Proposition 2. *Let s be a signal, S be a set of signals, and φ a temporal formula. It holds that s is robust on φ with respect to S if and only if $LT(s, S)$ satisfies $\mathcal{W}^\varphi$.*

Proof. $\Rightarrow$) In $LT(s, S)$ if $u \xrightarrow{\iota} u'$, then $u = init$ and u' is of the form $(0, s'(0)$ with $s' \in S$. Hence, $init \xrightarrow{\epsilon} (0, s(0))$. Moreover, by Definition 9, it holds that $\delta(s, s') \leq \delta(s, S) \leq \textbf{Dist}(()s, \varphi)$ and hence $(0, s'(0)) \approx^\varphi (0, s(0))$. Since id is the identity relation this is sufficient to prove that $LT(s, S)$ satisfies $\mathcal{W}^\varphi$.

$\Leftarrow$) Since $LT(s, S)$ satisfies $\mathcal{W}^\varphi$, for each $s' \in S$ we have that $(0, s'(0)) \approx^\varphi (0, s(0))$. By definition of $\approx^\varphi$ this means that for each $s' \in S$ $\delta(s, s') \leq \textbf{Dist}(s, \varphi)$. Hence, $\delta(s, S) = sup_{s' \in S} \delta(s, s') \leq \textbf{Dist}(s, \varphi)$, i.e., the thesis. □

Of course this is a trivial way to map the notion of robustness defined in [22] in our framework. However, this embedding reveals that we can immediately generalize this robustness notion and consider sets of formulæ by simply modifying $\approx^\varphi$. As a matter of fact any set Φ of formulæ over a temporal language $\mathcal{L}$ can be used to define an exposed observational equivalence $\approx^\Phi$ as follows:

$$u \approx^\Phi v \qquad \text{iff} \qquad \forall \varphi \in \Phi \, u \approx^\varphi v.$$

In temporal and modal logics, bisimulations are the natural equivalences that establish which states are indistinguishable with respect to the logic, i.e., whenever two states are bisimilar all the formulæ of the logic have the same truth values on them. So, if we consider as exposed observational equivalence a bisimulation equivalence over $\mathcal{L}$, we are sure that the perturbed and unperturbed systems are undistinguishable with respect to any formula of $\mathcal{L}$. In this sense unwinding conditions based on bisimulation relations can be seen as *robust robustness* definitions.

A strong objection against the use of bisimulation as exposed observational equivalence for unwinding conditions characterizing robustness concerns the fact that in the case of real time/space systems and even more crucially in the case of probabilistic systems (e.g., DTMCs and CTMCs), bisimulation relations could be too demanding. An answer to this objection comes from bisimulations notions which take into consideration continous domain, probability, errors (see, e.g., [11, 68, 65, 23, 64, 28]). In particular, [11] and [68] introduced a bisimulation distance $\delta B(u, v)$ between states that can be used to define exposed observational equivalences as $u \approx^k v$ iff $\delta B(u, v) \leq k$, where k is a constant. The algorithm proposed in [6] can be used to efficiently compute such relations on DTMC models.

Notice also that in our encoding we used LTS's in the simplest possible way. In general, using LTS's we can finitely represent any *regular* infinite set S of perturbed signals. Moreover, with a clever use of exposed actions we can exploit unwinding robustness also to compare perturbed signals and to analyse the effects of applying more than one perturbation.

3 Bisimulation Based Unwindings on Bio-PEPA

As an example of a computational language for modeling biological systems, we consider in this section the stochastic process algebra Bio-PEPA. A detailed description of Bio-PEPA is out of the scope of our work and we refer the reader to Bio-PEPA website[1], where complete bibliography, implementations, and case-studies can be found. The following definitions and notions mainly come from [27].

Definiton 13 (Bio-PEPA [27]). *A well-defined Bio-PEPA sequential component C is a recursive process of the form:*

$$C \stackrel{def}{=} \sum_{i=1}^{q} (\alpha_i, \kappa_i) op_i C$$

where $op_i \in \{\uparrow, \downarrow, \oplus, \ominus, \odot\}$, α_i is an action name, κ_i is a stoichiometry coefficient, and $\alpha_i \neq \alpha_j$ for $i \neq j$.
A well-defined Bio-PEPA model component *is a process of the form:*

$$P \stackrel{def}{=} C_1(x_1) \bowtie_{\mathcal{L}_1} \ldots \bowtie_{\mathcal{L}_{p-1}} C_p(x_p)$$

where the C_i's are well-defined sequential components which are pairwise different, the x_i's represent concentrations, and $\mathcal{L}_i$'s are sets of actions that appear in P.

We may interpret $\downarrow$ as a reactant, $\uparrow$ a product, $\oplus$ an activator, $\ominus$ as an inhibitor, and $\odot$ as a generic modifier. Bio-PEPA model components are used to rule the evolution of a Bio-PEPA system.

Definiton 14 (Bio-PEPA System [27]). *A Bio-PEPA system $\mathcal{P}$ is a 6-tuple $(\mathcal{V}, \mathcal{N}, \mathcal{K}, \mathcal{F}, Comp, P)$ where $\mathcal{V}$ is the set of compartments, $\mathcal{N}$ is the set of quantities describing each species, $\mathcal{K}$ is a set of parameters, $\mathcal{F}$ is the set of functional rates, Comp is the set of well-defined sequential components, and P consists of a well-defined Bio-PEPA model over Comp.*

The operational semantics of Bio-PEPA is defined through the set of rules, reported in Table 1 and 2, whose meaning is left to the specific domain. In [17] these rules are at the basis of the construction of a *Stochastic LTS* representing a Bio-PEPA model. In [16] such LTS's are mapped into CTMCs with levels. In [27] LTS over Bio-PEPA systems are defined by both discretizing concentrations through compartments and abstracting the above mentioned rules from quantitative aspects. The authors obtain LTS's whose nodes are tuples of integers and whose edges have the form $\stackrel{\alpha}{\rightarrow}$, where α is a reaction name.

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[1]`http://homepages.inf.ed.ac.uk/jeh/Bio-PEPA/biopepa.html`

$$\text{prefixProd} \qquad \frac{}{(\alpha,\kappa) \downarrow S(l) \xrightarrow{(\alpha,[S:\downarrow(l,\kappa)])}_c S(l-\kappa)} \quad N'_S + \kappa \le l \le N_S$$

$$\text{prefixReac} \qquad \frac{}{(\alpha,\kappa) \uparrow S(l) \xrightarrow{(\alpha,[S:\uparrow(l,\kappa)])}_c S(l+\kappa)} \quad N'_S \le l \le N_S - \kappa$$

$$\text{prefixMod} \qquad \frac{}{(\alpha,\kappa) \ \text{op} \ S(l) \xrightarrow{(\alpha,[S:\text{op}(l,\kappa)])}_c S(l)} \quad \begin{matrix} N'_S + \kappa \le l \le N_S \text{ if op} = \oplus \\ N'_S \le l \le N_S \text{ if op} = \{\ominus,\odot\} \end{matrix}$$

$$\text{choice1} \qquad \frac{S_1(l) \xrightarrow{(\alpha,w)}_c S'_1(l')}{(S_1+S_2)(l) \xrightarrow{(\alpha,w)}_c S'_1(l')} \qquad\qquad \text{choice2} \qquad \frac{S_1(l) \xrightarrow{(\alpha,w)}_c S'_2(l')}{(S_1+S_2)(l) \xrightarrow{(\alpha,w)}_c S'_2(l')}$$

$$\text{coop1} \qquad \frac{P_1 \xrightarrow{(\alpha,w)}_c P'_1}{P_1 \bowtie_{\mathcal{L}} P_2 \xrightarrow{(\alpha,w)}_c P'_1 \bowtie_{\mathcal{L}} P_2} \ a \notin \mathcal{L} \qquad\qquad \text{coop2} \qquad \frac{P_2 \xrightarrow{(\alpha,w)}_c P'_2}{P_1 \bowtie_{\mathcal{L}} P_2 \xrightarrow{(\alpha,w)}_c P_1 \bowtie_{\mathcal{L}} P'_2} \ a \notin \mathcal{L}$$

$$\text{coop3} \qquad \frac{P_1 \xrightarrow{(\alpha,w_1)}_c P'_1 \quad P_2 \xrightarrow{(\alpha,w_2)}_c P'_2}{P_1 \bowtie_{\mathcal{L}} P_2 \xrightarrow{(\alpha,w_1::w_2)}_c P'_1 \bowtie_{\mathcal{L}} P'_2} \ a \in \mathcal{L} \qquad\qquad \text{constant} \qquad \frac{S(l) \xrightarrow{(\alpha,[S:\text{op}(l,\kappa)])}_c S'(l')}{C(l) \xrightarrow{(\alpha,[C:\text{op}(l,\kappa)])}_c S'(l')} \ C \overset{\text{def}}{=} S$$

Table 1: The operational semantics of Bio-PEPA model components [27]

$$\text{Final} \qquad \frac{P \xrightarrow{(\alpha,w)}_c P'}{(\mathcal{V},\mathcal{N},\mathcal{K},\mathcal{F},Comp,P) \xrightarrow{(\alpha,r_\alpha[w,\mathcal{N},\mathcal{K}])}_s (\mathcal{V},\mathcal{N},\mathcal{K},\mathcal{F},Comp,P')}$$

$$\text{Qual} \qquad \frac{P \xrightarrow{(\alpha,w)}_c P'}{(\mathcal{V},\mathcal{N},\mathcal{K},\mathcal{F},Comp,P) \xrightarrow{\alpha} (\mathcal{V},\mathcal{N},\mathcal{K},\mathcal{F},Comp,P')}$$

Table 2: The operational semantics of Bio-PEPA systems [27]

Example 5 (A simple Bio-PEPA model). *Let us consider one of the examples presented in [27]. It represents a system with 3 species interacting through 3 reactions.*

$$A = (\alpha_1,1) \downarrow A + (\alpha_2,1) \uparrow A + (\alpha_3,2) \downarrow A$$
$$B = (\alpha_3,1) \uparrow B$$
$$C = (\alpha_1,1) \uparrow C + (\alpha_2,1) \downarrow C$$
$$P = A(x_A) \bowtie_* B(x_B) \bowtie_* C(x_C)$$

where $\bowtie_*$ denote the synchronization on all actions.

The possibility of associating different semantics to Bio-PEPA systems allows to exploit different behavioural equivalences on them. While lumpability

relations can be computed on the CTMCs associated to Bio-PEPA systems [16], a qualitative notion of bisimulation has been introduced in [27], where LTS's are first abstracted from level quantities, then compared using strong bisimulation.

We introduce here a simple instance of our framework over Bio-PEPA process algebra. First we extend the standard notation of Bio-PEPA systems. We partition the set of species into two sets *Imp* and *Exp*. As a consequence we partition the set $\mathcal{A}$ of action names into two sets *Imp* and *Exp* of imposed and exposed actions, respectively, as follows: each action which occurs in the definition of species of *Imp* is in *Imp*, all the remaining actions are in *Exp*.

Example 6. *Let us consider the Bio-PEPA model specified in Example 5. If Imp = {C} and Exp = {A, B}, then Imp = {α_1, α_2} and Exp = {α_3}. From now on we will use this system as illustrative example*

As observed in [27], given a system $\mathcal{P}$ we can denote the states of its LTS by using tuples of integers representing the levels of the species (see Example 7). We use the same convention, but we distinguish between species of *Imp* and *Exp*, i.e., our tuples have the form $(i_1, \ldots i_m, e_1, \ldots e_n)$, where $i_1, \ldots, i_m$ are the levels of the species of *Imp*, while $e_1, \ldots, e_n$ are the levels of the ones of *Exp*.

Definiton 15 (LTS of $\mathcal{P}$ [27]). *The LTS $L(\mathcal{P})$ of a Bio-PEPA system $\mathcal{P}$ is the LTS ($\mathbb{P}, \{\mathcal{P}\}$, Imp $\cup$ Exp, $\rightarrow$) where:*

- *$\mathbb{P}$ is the set of all the possible Bio-PEPA systems;*

- *Imp $\cup$ Exp is the set of admissible actions;*

- *$\rightarrow$ is the relation associated to the rule `Qual` of the Table 2.*

Whenever $\mathcal{P} \rightarrow \mathcal{P}'$, $\mathcal{P}'$ differs from $\mathcal{P}$ only in the levels of some components. Hence, the state of $L(\mathcal{P})$ can be represented by a vector of levels. Moreover, since all the species range over a finite set of levels, the LTS of a Bio-PEPA system is finite.

Example 7 (LTS of a Bio-PEPA system). *Let us reconsider the Bio-PEPA system described in Example 6. When the concentration level admitted for each species ranges in $[0, 4]$ and the evolution begins from the state $x_A = 4$, $x_B = 0$, and $x_C = 0$, the LTS of it is the transition system $P(4, 0, 0[4, 4, 4])$ depicted in Fig. 3. We recall that in this LTS we have Imp = {α_1, α_2} and Exp = {α_3}.*

We define the relations which equates nodes from an exposed point of view as follows. We use the notation $u \rightarrow u'$ to denote $u \xrightarrow{\alpha} u'$ for some $\alpha \in \mathcal{A}$.

Definiton 16 ($\doteq^{Exp}$ and $\approx^{Exp}$). *Let u, v be two states of $L(\mathcal{P})$. We say $u \doteq^{Exp} v$ iff u and v coincide on the species of Exp. A strong exposed bisimulation over $L(\mathcal{P})$ is a symmetric binary relation R over $L(\mathcal{P})$ such that for each $(u, v) \in R$:*

- *$u \doteq^{Exp} v$;*

- *if $u \rightarrow u'$, then $v \rightarrow v'$ and $(u', v') \in R$.*

$$\text{start} \longrightarrow (4,0,0) \underset{\alpha_2}{\overset{\alpha_1}{\rightleftarrows}} (3,0,1) \underset{\alpha_2}{\overset{\alpha_1}{\rightleftarrows}} (2,0,2) \underset{\alpha_2}{\overset{\alpha_1}{\rightleftarrows}} (1,0,3) \underset{\alpha_2}{\overset{\alpha_1}{\rightleftarrows}} (0,0,4)$$

$$\alpha_3 \downarrow \qquad \alpha_3 \downarrow \qquad \alpha_3 \downarrow$$

$$(2,1,0) \underset{\alpha_2}{\overset{\alpha_1}{\rightleftarrows}} (1,1,1) \underset{\alpha_2}{\overset{\alpha_1}{\rightleftarrows}} (0,1,2)$$

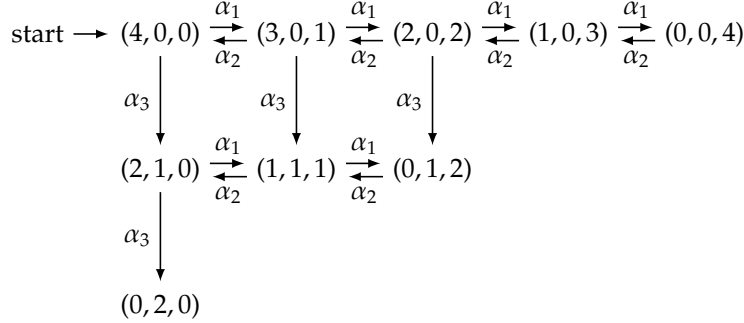$$\alpha_3 \downarrow$$

$$(0,2,0)$$

Figure 3: The transition system $P(4,0,0[4,4,4])$ of Example 5.

Two states u and v are strongly exposed bisimilar, *denoted by $u \approx^{Exp} v$, if there exists a strong exposed bisimulation R such that $(u,v) \in R$.*

It is easy to prove that $\approx^{Exp}$ is the largest exposed bisimulation and it is an equivalence relation.

We are now ready to define our instance of the generalized unwinding framework.

Definiton 17 (Quantitative Unwinding). $\mathcal{P}$ *satisfies the* quantitative unwinding *if $L(\mathcal{P}) \in \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow)$.*

Unwinding conditions can be introduced over any operational semantics based on LTS's. The advantages of considering unwinding conditions over process algebras such as Bio-PEPA are twofold. On the one hand, we can prove properties over the systems satisfying the unwinding conditions in terms of their interactions with other systems. In particular, we can prove that these systems are not critically affected by hostile environments (see Theorem 1). On the other hand, we can exploit compositionality properties of the syntactic operators to both reduce the complexity of checking the unwinding condition and to suggest *corrections* when unwinding is not satisfied.

Theorem 1. *It holds that $L(\mathcal{P}) \in \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow)$ if and only if for each $u, v \in L(\mathcal{P})$ if $u \doteq^{Exp} v$, then $u \approx^{Exp} v$.*

Proof. $\Rightarrow$) Let $\mathcal{S} = \{(u,v) \mid u, v \in L(\mathcal{P}) \text{ and } u \doteq^{Exp} v\}$. We prove that $\mathcal{S}$ is a strong exposed bisimulation up to $\approx^{Exp}$. Let $(u,v) \in \mathcal{S}$, i.e., $u \doteq^{Exp} v$. If $u \overset{\epsilon}{\rightarrow} u'$ with $\epsilon \in \mathcal{E}xp$, then since $u \doteq^{Exp} v$ and both the side-coditions and the resultant of applying an ϵ transition only depend on Exposed quantities, it holds that there exists v' such that $v \overset{\epsilon}{\rightarrow} v'$ and $u' \doteq^{Exp} v'$, i.e., $(u',v') \in \mathcal{S}$. On the other hand, if $u \overset{\iota}{\rightarrow} u'$ with $\iota \in Imp$, then, since $L(\mathcal{P}) \in \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow)$ and $u \doteq^{Exp} v$, there exists v' such that $v \rightarrow v'$ and $u' \approx^{Exp} v'$.

$\Leftarrow$) Let $u \overset{\iota}{\rightarrow} u'$ with $\iota \in Imp$ and $u \doteq^{Exp} v$. Since $u \doteq^{Exp} v$ implies $u \approx^{Exp} v$, we get that $v \rightarrow v'$ with $u' \approx^{Exp} v'$. $\qquad\square$

The above theorem states that changes in the imposed environment of a system satisfying the unwinding condition do not affect the exposed behaviour of the system itself. Moreover, it suggests an efficient algorithm for testing $L(\mathcal{P}) \in \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow)$ as explicitly stated in the following corollary.

Corollary 1. *It holds that $L(\mathcal{P}) \in \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow)$ if and only if $L(\mathcal{P})/ \approx^{Exp}$ coincides with $L(\mathcal{P})/ \doteq^{Exp}$.*

Proof. This is an immediate consequence of Theorem 1 and of the fact that $u \approx^{Exp} v$ implies $u \doteq^{Exp} v$. □

Hence, to test whether $L(\mathcal{P}) \in \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow)$ one should simply start a partitioning bisimulation algorithm, such as Paige-Tarjan algorithm [53], on $L(\mathcal{P})$ to compute $\approx^{Exp}$. If the algorithm terminates without performing any split, then $L(\mathcal{P})$ satisfies the unwinding condition. On the other hand, if it performs a split, we can immediately stop the computation and return $L(\mathcal{P}) \notin \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow)$. Since, such bisimulation algorithms can work both symbolically and on-the-fly, they allow to test the unwinding condition without fully computing $L(\mathcal{P})$ and avoid space-explosion problems.

Example 8. *If we consider the system of Example 6, we get that it satisfies the quantitative unwinding, since as shown by Figure 3 in this case both $\doteq^{Exp}$ and $\approx^{Exp}$ are the identity relation.*

Notice that if a system is in $\mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow)$, then imposed species cannot produce/consume exposed species, unless the same production/consumption can be also achieved exploiting only exposed species. In the case of closed chemical systems satisfying the conservation mass law this corresponds to imposing a complete separation between exposed and imposed species. However, this is not the case in biological systems where the presence of degradation laws make systems non-conservative.

Example 9. *Consider a simple system in which a species $E \in Exp$ can be produced from a reaction which involves a species $I \in Imp$. Moreover, both species can degrade.*

$$E = (\epsilon, 1) \downarrow E + (\iota_1, 1) \uparrow E$$
$$I = (\iota_1, 1) \downarrow I + (\iota_2, 1) \downarrow I$$
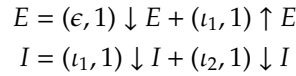
No matter which are the boundary levels defining such system, if its LTS includes one ι_1 action, then the system does not satisfy the unwinding condition. Intuitively, if I is a vaccine (or a pathogen), since it can produce a specie in Exp, it interferes with the system's observable behaviour. We obtain the same result also if ι_1 would require the presence of other species of Exp.

On the other hand, if we allow the presence of another reaction from which E can be produced, then we obtain a system which satisfies the unwinding condition.

$$E = (\epsilon, 1) \downarrow E + (\iota_1, 1) \uparrow E + (\delta, 1) \uparrow E$$
$$I = (\iota_1, 1) \downarrow I + (\iota_2, 1) \downarrow I$$
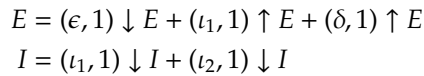
Since $\approx^{\mathcal{E}xp}$ is qualitative with respect to the rates, this second system satisfies the unwinding condition for each possible values of ι_1 and δ. Imposing a more qualitative equivalence we would obtain constraints on these values.

Let us now introduce a second unwinding condition, called *quasi-quantitative* on Bio-PEPA LTS's. At a first sight this could seem less demanding than quantitative unwinding however we will show that this is not the case.

Definiton 18 ($\doteq^{\mathcal{E}xp}$ and $\cong^{\mathcal{E}xp}$)**.** *Let u, v be two states of L($\mathcal{P}$). We say $u \doteq^{\mathcal{E}xp} v$ iff u and v have the same sets of $\mathcal{E}xp$ labels on outgoing edges. A strong quasi-exposed bisimulation over L($\mathcal{P}$) is a symmetric binary relation R over L($\mathcal{P}$) such that for each $(u, v) \in R$:*

- *$u \doteq^{\mathcal{E}xp} v$;*

- *if $u \to u'$, then $v \to v'$ and $(u', v') \in R$.*

Two states u and v are strongly quasi-exposed bisimilar*, denoted by $u \cong^{\mathcal{E}xp} v$, if there exists a strong exposed bisimulation R such that $(u, v) \in R$.*

Definiton 19 (Quasi-Quantitative Unwinding)**.** *$\mathcal{P}$ satisfies the quasi-quantitative unwinding if $L(\mathcal{P}) \in \mathcal{W}(\doteq^{\mathcal{E}xp}, \cong^{\mathcal{E}xp}, \to)$.*

Even if $\doteq^{Exp}$ implies $\doteq^{\mathcal{E}xp}$, the following examples show that $\mathcal{W}(\doteq^{\mathcal{E}xp}, \cong^{\mathcal{E}xp}, \to) \not\supseteq \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \to)$.

Example 10. *Let us consider the Bio-PEPA model defined as follows:*

$$A = (\epsilon_1, 2) \downarrow A + (\epsilon_2, 3) \downarrow A$$
$$B = (\epsilon_1, 5) \uparrow B + (\epsilon_2, 6) \uparrow B + (\iota, 6) \downarrow B$$
$$C = (\iota, 1) \uparrow C$$
$$P = A(x_A) \underset{*}{\bowtie} B(x_B) \underset{*}{\bowtie} C(x_C)$$

where A and B are exposed species, while C is an imposed species.

The LTS $P(3, 0, 0[6, 6, 6])$ that is associated to above model when the concentration level admitted for each species ranges in $[0, 6]$ and the evolution begins from the state $x_A = 3$, $x_B = 0$, and $x_C = 0$ is depicted in Figure 4. It is easy to see that $P(3, 0, 0[6, 6, 6]) \in \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \to)$ because the states in $P(3, 0, 0[6, 6, 6])$ are pairwise distinct with respect to the exposed species. On the contrary, $(1, 5, 0)$, $(0, 6, 0)$, and $(0, 0, 1)$ have the same exposed actions and, thus, $(1, 5, 0) \doteq^{\mathcal{E}xp} (0, 6, 0)$. However, $(0, 6, 0) \xrightarrow{\iota} (0, 0, 1)$, while there is no transition leaving $(1, 5, 0)$. It follows that $P(3, 0, 0[6, 6, 6]) \in \mathcal{W}(\doteq^{Exp}, \cong^{Exp}, \to)$ does not hold.

Notice that also the system of Example 6 satisfies the quantitative unwinding (as proved in Example 8), but not the quasi-quantitative unwinding. In particular, $(0, 0, 4) \doteq^{\mathcal{E}xp} (0, 2, 0)$ and $(0, 0, 4)$ reaches $(1, 0, 3)$ through an imposed action, but $(0, 2, 0)$ does not reach any state equivalent to $(1, 0, 3)$, since it does not reach any state.

$$\text{start} \longrightarrow (3,0,0) \xrightarrow{\ \epsilon_2\ } (0,6,0) \xrightarrow{\ \iota\ } (0,0,1)$$

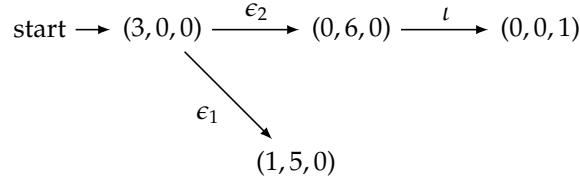with $(3,0,0) \xrightarrow{\epsilon_1} (1,5,0)$

Figure 4: The LTS $P(3,0,0[6,6,6])$ of the Bio-PEPA model described in Example 10.

Given a system $\mathcal{P}$ the LTS $L(\mathcal{P})$ can be very large. Moreover, it distinguishes too precisely on the basis of quantitative information also LTS's which represent the same qualitative behaviours. The qualitative LTS of $\mathcal{P}$ is obtained as a quotient of $L(\mathcal{P})$ in which nodes having the same capabilities (outgoing actions) are identified.

Definiton 20 (QLTS of $\mathcal{P}$). *Let $\mathcal{P}$ be a Bio-PEPA system. Given two states u, v of $L(\mathcal{P})$ we say that $u \doteq v$ iff u and v have the same sets of labels on outgoing edges.*

The qualitative LTS $QL(\mathcal{P})$ of a Bio-PEPA system is the LTS $L(\mathcal{P})/\doteq$, where $[u] \xrightarrow{\alpha} [u']$ if and only if there exist $\overline{u} \in [u]$ and $\overline{u'} \in u'$ such that $\overline{u} \xrightarrow{\alpha} \overline{u'}$ in $L(\mathcal{P})$.

Since all the states in any class of $\mathbb{P}/\doteq$ share the same admissible actions, we can denote each node of $QL(\mathcal{P})$ by using the set of these actions. The qualitative semantics defined in [27] for Bio-PEPA systems is close to our definition, with the difference that in [27] for each pair of classes $[u], [v]$ in $QL(\mathcal{P})$ there exists at most one edge from $[u]$ to $[v]$ whose label is the set of actions which allow to move from $[u]$ to $[v]$.

Example 11 (QLTS of a Bio-PEPA system). *Let us reconsider the Bio-PEPA system described by Example 5. When the concentration level admitted for each species ranges in $[0,4]$ and the evolution begins from the state $x_A = 4$, $x_B = 0$, and $x_C = 0$, the QLTS of it is the transition system depicted in Figure 5.*

In order to define a generalized unwinding condition over $QL(\mathcal{P})$ we introduce the following relations. With a slight abuse of notation we use the same symbols used for quasi-quantitative unwinding.

Definiton 21 ($\doteq^{\mathcal{E}xp}$ and $\cong^{\mathcal{E}xp}$). *Let $[u], [v]$ be two states of $QL(\mathcal{P})$. We define the relation $\doteq^{\mathcal{E}xp}$ as follows: for each $[u], [v] \in QL(\mathcal{P})$ it holds that $[u] \doteq^{\mathcal{E}xp} [v]$ iff $[u]$ and $[v]$ have the same sets of $\mathcal{E}xp$ labels on outgoing edges.*

We define the relation $\cong^{\mathcal{E}xp}$ as follows: for each $[u], [v] \in QL(\mathcal{P})$ it holds that $[u] \cong^{\mathcal{E}xp} [v]$ iff there exist $u' \in [u]$ and $v' \in [v]$ such that $u' \cong^{\mathcal{E}xp} v'$ holds in $L(\mathcal{P})$.

Notice that $[u] \doteq^{\mathcal{E}xp} [v]$ in $QL(\mathcal{P})$ if and only if $u \doteq^{\mathcal{E}xp} v$ in $L(\mathcal{P})$. Both $\doteq^{\mathcal{E}xp}$ and $\cong^{\mathcal{E}xp}$ are equivalence relations on $QL(\mathcal{P})$. Exploiting such relations we obtain the following notion of qualitative unwinding.
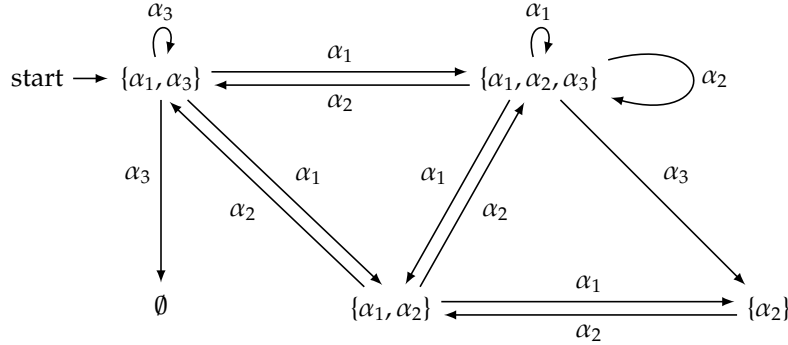
Figure 5: The qualitative transition system of the Bio-PEPA system described in Example 5.

Definiton 22 (Qualitative Unwinding). *$\mathcal{P}$ satisfies the* qualitative unwinding *if $QL(\mathcal{P}) \in \mathcal{W}(\doteqdot^{Exp}, \cong^{Exp}, \rightarrow)$.*

Example 12. *The system of Example 6 does not satisfy the qualitative unwinding since as shown in Figure 5 $\{\alpha_1, \alpha_2\} \doteqdot^{Exp} \emptyset$ and $\{\alpha_1, \alpha_2\}$ reaches other states through imposed actions, while $\emptyset$ does not reach any state.*

This third instance of unwinding is an over-approximation of the previous one in the following sense.

Theorem 2. *If $L(\mathcal{P}) \in \mathcal{W}(\doteqdot^{Exp}, \cong^{Exp}, \rightarrow)$, then $QL(\mathcal{P}) \in \mathcal{W}(\doteqdot^{Exp}, \cong^{Exp}, \rightarrow)$.*

Proof. Let $[u] \xrightarrow{\iota} [u']$, with $\iota \in Imp$, and $[u] \doteqdot^{Exp} [v]$. This means that there exists $\overline{u} \in [u]$ and $\overline{u'} \in [u']$ such that $\overline{u} \xrightarrow{\iota} \overline{u'}$ and $\overline{u} \doteqdot^{Exp} u \doteqdot^{Exp} v$. Hence, since $L(\mathcal{P}) \in \mathcal{W}(\doteqdot^{Exp}, \cong^{Exp}, \rightarrow)$ we have that $v \rightarrow v'$ and $\overline{u'} \cong^{Exp} v'$. Hence, by definition of $\cong^{Exp}$ over $QL(\mathcal{P})$ we have that $[u'] \cong^{Exp} [v']$. □

So, since $QL(\mathcal{P})$ is usually significantly smaller than $L(\mathcal{P})$ this result can be fruitfully exploited when $L(\mathcal{P})$ is too large. We will do this in the next section on a real world case-study.

3.1 *Neurospora Crassa* **circadian network in Bio-PEPA**

The *Neurospora crassa* is a fungus whose circadian network has been widely investigated and almost completely brought to light (e.g., see [47]). In total darkness this organism generates spores, a process called conidiation, every 22 hours. Both light and temperature affect the rhythm of this cycle. The alternation between day and night causes spore production in the middle of the night during all the year, independently on the length of daylight. From the metabolic point of view, the conidiation period is ruled by the *white collar-1* gene and the rhythmic gene frequency *frq*. The protein associated to the *white*

collar-1 gene, WC-1, activates the transcription of *frq*, while the protein product of *frq* both interacts with *frq*-bound WC-1 (PW) inhibiting the expression of *frq* and positively regulates the expression of WC-1. The light promotes the binding between a flavin chromophore and WC-1 (PWL) which increases the transcription of *frq*.

Akman *et al.* presented in [1] a Bio-PEPA model of the *Neurospora crassa* circadian network accounting 22 actions and 9 species. The effects of light on the system are described by a rate change in the reaction that produce the species PWL out of PW. We call this reaction *PWtoPWL*. The authors investigated the system in two different light conditions: constant darkness (D) and light and dark 12-hour alternation (LD). We considered this model to prove the effectiveness of our framework. We focused on constant darkness (D) and constant light conditions (L) and we formally proved that *Imp* = {*PWtoPWL*} is relevant for the system. While this result is not surprising, it is worth to notice that it was obtained by analyzing exclusively the (Q)LTS's of the system and it does not rely on reaction rates.

The full LTS of the investigated Bio-PEPA model may contain up to 2^{103} nodes and, thus, it is not feasible for the analysis. However, the corresponding QLTS has at most $2^{|\mathcal{A}|}$ nodes and we can to compute it directly. We wrote a Python program to assemble the QLTS of any Bio-PEPA system avoiding the LTS construction and we applied it to the system in both D and L conditions. The program computes the set of species constraints corresponding to each node of the QLTS and reduces the reachability of the region satisfying these constraints to a set of Interger Linear Programming problems having the following form:

$$\text{Objective:} \qquad \min \sum_i n_i$$

$$\begin{pmatrix} l_0 \\ \vdots \\ l_n \end{pmatrix} - \begin{pmatrix} b_0 \\ \vdots \\ b_n \end{pmatrix} \leq \sum_i n_i \begin{pmatrix} e_{i,0} \\ \vdots \\ e_{i,n} \end{pmatrix} \leq \begin{pmatrix} u_0 \\ \vdots \\ u_n \end{pmatrix} - \begin{pmatrix} b_0 \\ \vdots \\ b_n \end{pmatrix} \qquad (1)$$

where the natural variable n_i represent the number of repetitions of the action α_i, $e_{i,j}$ is the effect of the action α_i on the species s_j, and b_j and $[l_j, u_j]$ are the LTS starting value and the admissible interval for the species s_j, respectively. Whenever System (1) is not satisfiable, there is no way to reach the considered QLTS state from the starting value. On the contrary, other tests are necessary to verify that the proposed path does not cross the imposed boundaries.

By using the developed program, we built two QLTS's, QL_D and QL_L, representing the Bio-PEPA model in D and L conditions, respectively. The former has 3969 nodes and misses the *PWtoPWL* action, the latter has 11529 nodes and uses that action. We know that if the *PWtoPWL* action is not relevant for the system evolution, then, whenever a state u performs a *PWtoPWL* transition and reaches u', then each other state v such that $u \doteq^{\mathcal{E}xp} v$ should be able to perform an action and reaches a v' such that $u' \cong^{\mathcal{E}xp} v'$.

We projected the QL_L on *PWtoPWL* by joining all the nodes that differ

exclusively for that label i.e., we merged all the pairs of nodes of QL_L, v and v', such that $v \setminus \{PWtoPWL\} = v' \setminus \{PWtoPWL\}$. The obtained QLTS, named QL'_L, has 8505 nodes and we observed that some of the nodes in QL'_L are not present in QL_D. Moreover, both QL_D and QL_L are strongly connected (i.e., all the nodes are reachable from each other) and, hence, whenever a node is reachable from the considered starting conditions, it is reachable also from any other admissible value. It follows that there exist states in QL'_L, and, as a consequence, in both QL_L and QL_{LD}, that are not reachable if we avoid the action $PWtoPWL$ (i.e. the model is in D condition). Thus, the investigated system in LD conditions does not satisfy the qualitative unwinding and, by Theorem 2, does not satisfy the quasi-quantitative unwinding too. As noticed above, this means that $PWtoPWL$ is relevant for the evolution of the *Neurospora crassa* model in LD conditions or in other terms *Neurospora crassa* is not robust with respect to perturbations on *PWtoPWL* action.

4   Unwinding Conditions over Hybrid Automata

Hybrid models, such as hybrid automata, mix mathematical and computational models. The discrete part of hybrid models is computational while the continuous one is mathematical.

Many different definitions of hybrid automaton have been suggested in the literature so far [3, 34, 50, 57, 35, 15, 13]. In this paper, we rely on a definition which is close to the one given by Lynch *et al.* [48] as the unwinding framework that we are proposing fits into it in a natural way.

Definiton 23 (Hybrid Automata). *A hybrid automaton (HA) $\mathcal{A} = (Imp, Exp, Q, \Theta, Imp, Exp^-, D, \mathcal{T})$ consists of:*

- *Two disjoint sets of variables: a set Imp of* imposed variables *and a set Exp of* exposed variables. *$Var \overset{def}{=} Imp \cup Exp$;*

- *A set $Q \subseteq val(Var)$ of states, where $val(Var)$ is the set of values that Var can assume;*

- *A nonempty set $\Theta \subseteq Q$ of start states;*

- *Two disjoint sets of actions: a set Imp of* imposed discrete actions *and a set Exp^- of* exposed discrete actions. *$Act \overset{def}{=} Imp \cup Exp^-$;*

- *A set $D \subseteq Q \times Act \times Q$ of discrete transitions. The action a is* enabled *in x if there exists a x' such that $(x, a, x') \in D$;*

- *A set $\mathcal{T}$ of* trajectories *for Var. Each $\tau \in \mathcal{T}$ is a function whose domain, $dom(\tau)$, is an initial subset of $\mathbb{R}_{\geq 0}$ and whose image set is a subset of Q, i.e., $\tau(t) \in Q$, for all $\tau \in \mathcal{T}$ and all $t \in dom(\tau)$. The following axioms must hold:*

T1 *(Prefix closure)*
   If $\tau \in \mathcal{T}$, $dom(\tau') \subseteq dom(\tau)$, and $\tau'(t) = \tau(t)$ for all $t \in dom(\tau')$, then $\tau' \in \mathcal{T}$;

T2 *(Suffix closure)*
   If $\tau \in \mathcal{T}$ and $t' \in dom(\tau)$, then $\tau'(t) \stackrel{def}{=} \tau(t + t')$ belongs to $\mathcal{T}$;

T3 *(Concatenation closure)*
   Let $S = \{\tau_0, \tau_1, \tau_2, \ldots\}$ be a subset of $\mathcal{T}$ such that $dom(\tau_i)$ has the form $[0, t_{max,i}]$ and $\tau_i(t_{max,i}) = \tau_{i+1}(0)$ for all $i + 1 < |S|$. Then the trajectory:

$$(\tau_0 \frown \tau_1 \frown \tau_2 \frown \ldots)(t) \stackrel{def}{=} \begin{cases} \tau_0(t) & \text{if } t \in dom(\tau_0) \\ (\tau_1 \frown \tau_2 \frown \ldots)(t - t_{max,0}) & \text{otherwise} \end{cases}$$

   belongs to $\mathcal{T}$.

The trajectories can be given in implicit form, for instance, as a differential system: the set $\mathcal{T}$ contains all the solutions of the provided differential system. In such cases, the computation of the trajectories themselves is not always trivial and may be not even computable.

The semantics of hybrid automata are usually given in terms of LTS by associating a hybrid automaton with an infinite LTS whose nodes are the states of the automaton and edges have the form $\xrightarrow{t}_C$ or $\xrightarrow{e}_D$. As the *continuous transition relation* $\xrightarrow{t}_C$ concerns, $q \xrightarrow{t}_C q'$ holds if and only if there exists a $\tau \in \mathcal{T}$ such that $dom(\tau) = [0, t_{max}]$, $\tau(0) = q$ and $\tau(t_{max}) = q'$. Regarding the *discrete transition relation* $\xrightarrow{t}_D$, $q \xrightarrow{a}_D q'$ holds if and only if $(q, a, q') \in D$.

Definiton 24 (Hybrid Automata - Semantics)**.** *Given a hybrid automaton $H = (Imp, Exp, Q, \Theta, Imp, \mathcal{E}xp^-, D, \mathcal{T})$, the LTS associated to H is the tuple $L(H) = (Q, \Theta, Imp \cup \mathcal{E}xp, R)$, where $\mathcal{E}xp = \mathcal{E}xp^- \cup \mathbb{R}_{\geq 0}$ and $R \stackrel{def}{=} \{(q, \alpha, q') \mid q \xrightarrow{\alpha}_C q' \wedge \alpha \in \mathbb{R}_{\geq 0} \text{ or } q \xrightarrow{\alpha}_D q' \wedge \alpha \in Imp \cup \mathcal{E}xp^-\}$.*

In the above definition we consider all the actions associated to the continuous transitions, i.e., the positive real numbers labeling the transition relation $\rightarrow_C$, as exposed actions. This is an arbitrary choice. Intuitively we interpret all the continuous evolutions as internal transitions not influenced by the environment. The imposed interactions with the environment are represented only through some discrete transition labels. For those who are more familiar with the definition of hybrid automata given in [3, 34], this corresponds to say that the imposed interactions cause a change of location in the automaton and hence a possible change in the differential laws regulating the continuous evolution.

Example 13 (A Simple Thermostat Model)**.** *Let us model a simple thermostat by using a hybrid automaton. The discrete variable mode represents the state of the heater (i.e., mode = 1 means "heater on" and mode = 0 "heater off"), while the variable x_T is associated to the temperature. Whenever the temperature reaches $15\,°C$, the thermostat activates the heater (exposed action* switchOn*), while, if the temperature rises up to $20\,°C$, the heater is turned off (exposed action* switchOff*). The users can switch on and*

off the heater independently from the thermostat status by using the imposed actions forceOn *and* forceOff, *respectively. The two constants k_r and k_h are the dispersion and heating coefficients, respectively, while the variable X represents the room temperature. Initially, the heater is switched off and the temperature is* 17 °C.

Meaning	Actions
Turn off the heater	*switchOff*
Turn on the heater	*switchOn*
Force off the heater	*forceOff*
Force on the heater	*forceOn*

(a) Actions

$Imp \setminus Exp$	Preconditions	Action	Effects
Exp	$(mode = 1) \wedge (x_T \geq 20)$	*switchOff*	$mode \leftarrow 0$
Exp	$(mode = 0) \wedge (x_T \leq 15)$	*switchOn*	$mode \leftarrow 1$
Imp	$mode = 1$	*forceOff*	$mode \leftarrow 0$
Imp	$mode = 0$	*forceOn*	$mode \leftarrow 1$

(b) Transitions

Trajectories
$\dot{x}_T = k_h * mode - k_r * x_T$
$\dot{mode} = 0$

(c) Trajectories

Meaning	Variables
Temperature	$x_T : \mathbb{R} \leftarrow 17,$
Heater state	$mode : \{0, 1\} \leftarrow 0$

(d) Internal Variables

Table 3: A formal specification of the hybrid automaton representing a simple thermostat.

In the context of hybrid automata, (bi)simulation reductions [4, 44], series of abstractions [67], piecewise linear approximations [29, 30] have been proposed in the literature to abstract the infinite LTS representing the semantics of an hybrid automaton into finite ones.

We now introduce an unwinding condition over hybrid automata. Notice that the unwinding conditions defined over LTS's generated from Bio-PEPA systems can be used also on LTS's generated from hybrid automata. As a matter of fact one of the advantages of unwinding conditions is that they are defined on LTS and hence they do not depend on the modeling language from which the LTS has been inferred. However, we prefer to introduce here a further unwinding condition to give to the reader an idea of the flexibility of the framework. As done in the quantitative unwinding in the previous section, we consider two nodes of the LTS to be "equal" if the have the same values on the exposed variables . We consider them to be "equivalent" if using only exposed transitions they generate the same sequences of exposed variables values. Finally, the transition relation ⤏ is a generic exposed transition.

Definiton 25 ($\doteq^{Exp}, \asymp^{Exp}, \rightarrow^{Exp}$ and $\rightarrow^{>t}$). *Let H be a hybrid automaton and $L(H) = (Q, \Theta, Imp \cup Exp, R)$ be the LTS associated to H. The relation $\doteq^{Exp} \subseteq Q \times Q$ is defined as follows: $u \doteq^{Exp} v$ if and only if the exposed variables have the same values on u and v. A* strong bi-exposed bisimulation *over $L(H)$ is a symmetric binary relation R over $L(H)$ such that for each $(u,v) \in R$:*

- *$u \doteq^{Exp} v$;*

- *if $u \xrightarrow{\epsilon} u'$ with $\epsilon \in Exp$, then $v \xrightarrow{\epsilon} v'$ and $(u', v') \in R$.*

Two states u and v are strongly bi-exposed bisimilar, *denoted by $u \asymp^{Exp} v$ if there exists a strong exposed bisimulation R such that $(u,v) \in R$.*

The relations $\rightarrow^{Exp} \subseteq Q \times Q$ and $\rightarrow^{>t} \subseteq Q \times Q$, where $t \in \mathbb{R}_{\geq 0}$, are defined as follows: $u \rightarrow^{Exp} v$ if and only if there exists $\epsilon \in Exp$ such that $u \xrightarrow{\epsilon} v$ and $u \rightarrow^{>t} v$ if and only if there exists $t' > t$ such that $u \xrightarrow{t'} v$.

Notice that $\doteq^{Exp}$ is the same relation introduced on LTS's of Bio-PEPA systems. We are now ready to introduce two unwinding conditions. We the first one is called bi-exposed since exposed actions are involved in the definition of both the equivalence relation $\asymp^{Exp}$ and the transition relation $\rightarrow^{Exp}$. Once the system has moved from u to u' through an imposed action if $u \doteq^{Exp} v$, then v has to reach v' through an exposed action and u' and v' are compared only on the basis of exposed values and exposed transitions. The second one is called delayed unwinding since the transition relation $\dashrightarrow$ is instantiated as $\rightarrow^{>t}$ which represent a delay of time greater than t. In this second instance u' and v' are compared on the basis of all transitions through the relation $\approx^{Exp}$ introduced in the previous section.

Definiton 26 (Bi-Exposed Unwinding and Delayed Unwinding). *H satisfies the bi-exposed unwinding if $L(H) \in \mathcal{W}(\doteq^{Exp}, \asymp^{Exp}, \rightarrow^{Exp})$. H satisfies the t-delayed unwinding if $L(H) \in \mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow^{>t})$.*

In the next section we apply bi-exposed unwinding in the analysis of influenza models, proving that, as one could expect, influenza is not robust with respect to antiviral and interferons treatments. Moreover, t-delayed unwinding allows to show that also if we refer to a single type of treatment (e.g., antiviral) the result strongly depends on the time at which the treatment is imposed.

4.1 Influenza Kinetics Analysis through hybrid automata

Influenza is an infectious disease caused by a family of RNA viruses known as influenza viruses. Its symptoms include fever, weakness, and coughing and, in the most acute form, it can bring a severe threat to the respiratory system. Worldwide, 250,000 to 500,000 deaths per years are ascribed to the complications of the seasonal influenza virus [52] and the infamous *Spanish flu* of the 1918 infected 500 million people leading to the death of 20 to 100 million of them [37, 66].

Understanding the dynamics of infection plays a crucial role in avoiding or, at least, controlling possible influenza pandemics. Many models have been suggested so far to achieve this goal [46, 5, 33, 63, 54, 49].

Handel *et al.* deal with the effects of the most effective drugs against influenza, the *Neuraminidase inhibitors* (NI), and take into account the rise of virus strains resistant to this antiviral [33]. The model distinguishes the load of viruses that are NI-sensitive (V_s) from that of viruses that are NI-resistant (V_r). Uninfected cells (U) are infected by either NI-resistant virus or NI-sensitive virus at a rate proportional to the correspondent virus load and became NI-resistant infected (I_r) or NI-sensitive infected (I_s), respectively. Both NI-resistant and NI-sensitive infected cells increase virus load of the respective strain. Moreover, due to natural mutations, a fraction of the viruses released by NI-sensitive infected cells belongs to the NI-resistant strain. Whatever is the strain of the viruses produced by NI-sensitive infected cells, NI represses their release at a rate that is proportional to the efficiency of the antiviral itself. A natural immune response (X) restrains the increase in viral load in all the strains too. As it occurs also to the virus strains, both the kinds of infected cells share the same decay rate.

On the contrary, Saenz *et al.* consider the interactions between viral agents and immune system and describe the antiviral response modulated by the type I interferon (IFN-α/β) which triggered by infection [63]. In their model, uninfected cells (U) are infected at a rate proportional to the virus load (V). Newly infected cells spend some time in an eclipse phase (E_1) and then they move to a state (I), having a limited span life, in which they increase the virus load. Infective cells produce IFN (F) which is able to bring uninfected cells into a prerefractory state (W) and, possibly, in a refractory cells (R) at a rate proportional to F itself. Whenever cells in the prerefractory state are infected, they move to an eclipse phase (E_2), in which they release IFN, and, eventually, become infective (I).

IFNs have been long used as a treatment for various autoimmune, viral, and tumor diseases [58, 73, 45, 32, 56, 36]. So, one may wonder if IFNs can be used as antiviral drugs and, in particular, whether INFs and NIs are equivalent or not with respect to the virus load, i.e., if IFNs can be used in place of NIs, and viceversa, in influenza treatment. Notice that, whatever the answer is, the NI-based therapies will be still preferable to the IFN-based ones in normal condition since the latter exhibit many serious side effects in humans [69, 71, 26] and they are less cost-effective than the former. However, above questions maintain some relevance in the case of a pandemic produced by a viral strain that is resistant to the antivirals.

We developed a model that takes into account the effects of both IFN and NI on the virus load. As done in [33], we admit the existence of a NI-resistant strain and, analogously to [63], we represent the antiviral response due to IFN. The trajectories, the actions, and the transitions of our model are reported in Table 6. The values of their parameters are dependent on the virus strain and on the host species; we focused on human hosts infected with influenza A/Texas/91 (H1N1) and we fit our model on the data produced by the IR kinetic model

Meaning	Variables
Uninfected cells	$U : \mathbb{R}_{\geq 0} \leftarrow 4.0 * 10^8$
Cells infected by virus sensitive to NI in eclipse phase	$E_{1,s} : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Cells infected by virus resistent to NI in eclipse phase	$E_{1,r} : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Prerefractory cells infected by virus sensitive to NI in eclipse phase	$E_{2,s} : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Prerefractory cells infected by virus resistent to NI in eclipse phase	$E_{2,r} : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Cells in prerefractory state	$W : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Refractory cells	$R : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Cells infected by virus sensitive to NI	$I_s : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Cells infected by virus resistant to NI	$I_r : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Virus sensitive to NI	$V_s : \mathbb{R}_{\geq 0} \leftarrow 7.7 * 10^{-3}$
Virus resistant to NI	$V_r : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Interferons (IFN-α/β)	$F : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Immune response	$X : \mathbb{R}_{\geq 0} \leftarrow 3.4 * 10^{-1}$
Elapsed time	$T : \mathbb{R}_{\geq 0} \leftarrow 0.0$
Antiviral injection mode ($A = 1$ injecting NI,) $A = 0$ not injecting NI)	$A : \{0, 1\} \leftarrow 0$
Model mode ($M = 0$ treatment to be selected, $M = 1$ NI-based treatment, $M = 2$ IFN-based treatment, $M = 3$ treatment concluded)	$M : [0, 3] \leftarrow 0$

Table 4: Variables of the hybrid automaton modeling the influenza kinetics.

described in [33] by minimizing the cost function:

$$SSE \stackrel{\text{def}}{=} \sum_i \left(\frac{\log V_s(i) - \log \hat{V}_s(i)}{\log \max_i \hat{V}_s(i)} \right)^2 + \sum_i \left(\frac{\log V_r(i) - \log \hat{V}_r(i)}{\log \max_i \hat{V}_r(i)} \right)^2 + (D - \hat{D})^2, \quad (2)$$

where V_s, V_r, and D are the number of NI-sensitive virus, the number of NI-resistant virus, the total death cell estimated by our model, respectively, while $\hat{V}_s$, $\hat{V}_r$, and $\hat{D}$ are the same quantities evaluated by the IR model suggested in [33].

Notice that the IR model does not take into account IFN and, thus, the value estimated for the parameters that directly connected with IFN, i.e., q and ψ, may need some scaling to match the real kinetics of interferons in humans infected by H1N1. Nevertheless, our model exhibits the same IFN peek as the one proposed by Saenz *et al.* in the case of an A/equine/Kildare/89 (H3N8) infection [63].

Our hybrid model chooses one treatment between the IFN-based and the NI-based one by using either the action *treatIFN* or the action *treatNI*, respectively.

Symbol	Meaning	Value	Source
k_i	Eclipse phase period	$2\,\text{Days}^{-1}$	[38, 63]
a	Prefactory period	$4\,\text{Days}^{-1}$	[19, 63]
μ	Mutation rate	10^{-5}	[33]
σ	Fitness cost of resistance	0.1	[33]
n	IFN-reduced production	1	[63]
m	IFN-reduced infectivity	1	[63]
β	Virus infectivity	$1.18368 * 10^{-1}$	Fitted
p	Virus production per cell	$5.96623 * 10^{-4}$	Fitted
q	IFN induction per cell	$5.18653 * 10^{-11}$	Fitted
ψ	IFN efficiency	3.68109	Fitted
d_I	Infected cell death rate	$1.23661 * 10^{-1}$	Fitted
d_V	Virus death rate	$0.80710 * 10^{-1}$	Fitted
d_F	IFN clearance rate	1.80413	Fitted
r	Immune response growth rate	1.14835	Fitted
ϵ_{NI}	Antiviral efficacy	0.97647	Fitted
f	IFN released per injection	6	Arbitrary

Table 5: Trajectory parameters of the hybrid automaton modeling the influenza kinetics.

In the former case, the effect of the antiviral is assumed to be constant along all the treatment; in the latter case, the host is injected with an arbitrary dose f of IFN every t_i days from the begin of the treatment until the end of it. Figure 6 represents the evolution of the two variables V_s (plain line) and V_r (dotted line) under both the considered treatments.

First of all, we may want to decide whether the effectiveness of two treatments depends or not on the timing. We notice that the LTS associated to our hybrid model does not satisfy the 0.5-delayed unwinding $\mathcal{W}(\doteq^{Exp}, \approx^{Exp}, \rightarrow^{>0.5})$. As the matter of the facts, the peek of the V_r load observable by starting the NI-based treatment at the time of the infection can be obtained neither with different timing nor by using the IFN-based treatment (see Figure 6). This proves that the effectiveness of the treatments are time-dependent.

Another question that deserves attention is whether IFN and NI are equivalent or not with respect to the virus level. We consider *treatNI* being an imposed action and verify that the LTS associated to the proposed influenza model does not satisfies the bi-exposed unwinding $\mathcal{W}(\doteq^{Exp}, \preceq^{Exp}, \rightarrow^{Exp})$, i.e., it is not possible to obtain the same virus load produced by the NI-treatment, whenever it has began, in the same time.

More details about this example are given in [14].

Trajectories
$\dot{U} = -\beta(V_r + V_s)U - \phi FU$
$\dot{E}_{1,j} = \beta V_j U - k_1 E_{1,j}$
$\dot{E}_{2,j} = m\beta V_j W - k_2 E_{2,j}$
$\dot{W} = \phi FU - m\beta(V_r + V_s)W - aW$
$\dot{R} = aW$
$\dot{I}_j = k_1 E_{1,j} + k_2 E_{2,j} - d_I I_j$
$\dot{V}_s = (1 - A * \epsilon_{NI})(1 - \mu)pI_s - d_V V_s - XV_s$
$\dot{V}_r = (1 - A * \epsilon_{NI})\mu pI_s + (1 - \sigma)pI_r - d_V V_r - XV_r$
$\dot{F} = nq(E_{2,r} + E_{2,s}) + q(I_r + I_s) - d_F F$
$\dot{X} = rX$
$\dot{T} = 1$
$\dot{A} = 0$
$\dot{M} = 0$

(a) Trajectories

Meaning	Actions
Begin NI-based treatment	*treatNI*
Begin IFN-based treatment	*treatIFN*
End the treatment	*conclude*
Inject IFN	*injectIFN*

(b) Actions

Preconditions	Action	Effects
$M = 0$	*treatNI*	$A \leftarrow 1 \land M \leftarrow 1$
$M = 0$	*treatINF*	$M \leftarrow 2$
$M = 1$	*conclude*	$A \leftarrow 0 \land M \leftarrow 3$
$M = 2$	*conclude*	$M \leftarrow 3$
$M = 2 \land t_i \mid T$	*injectIFN*	$F \leftarrow F + f$

(c) Transitions

Table 6: Trajectories, actions, and transitions of the hybrid automaton modeling the influenza kinetics.
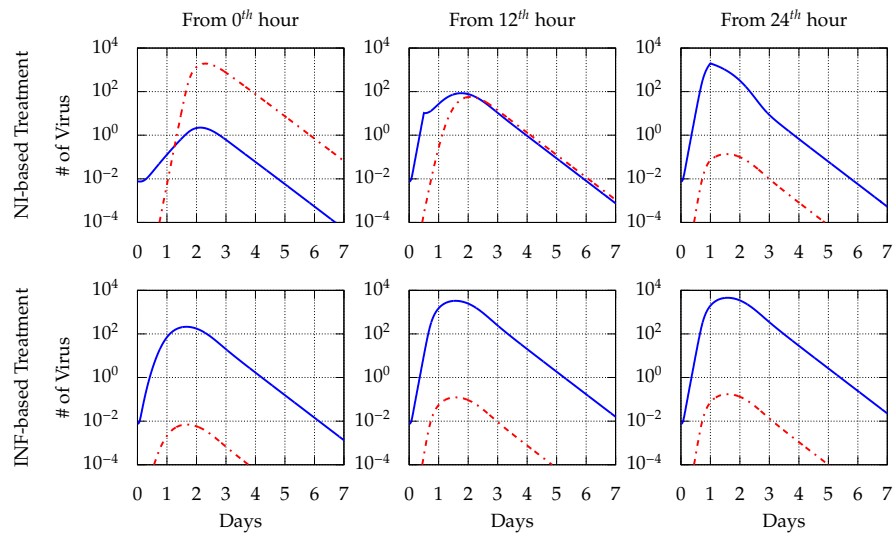
Figure 6: A comparison between the kinetics of the proposed model under NI-based treatment (first row) and IFN-based treatment (second row). The plain and the dotted lines represent the number of NI-sensible virus (V_s) and the number of NI-resistent virus (V_r), respectively. The first, the second, and the third columns depict the evolutions when the treatment, whatever it is, begins 0, 12, and 24 hours after the infection, respectively. All the treatments continue until the 7th day. Early inoculation of NI leads to a proliferation of the NI-resistant strain, while interferons can be used in early stage of the infection to control the proliferation of both the virus strains. On the contrary, NI maintains effectiveness throughout the course of the disease as opposed to IFN which seems to be useless whenever the treatment begins after the peek in the number of virus, i.e., about 1.5 day after the infection.

5 Conclusions

We proposed a framework for the analysis of biological systems based on unwinding conditions. The framework can be instantiated on all modeling languages which rely on operational semantics defined in terms of LTS's. From the modeling point of view, unwinding conditions have the advantage of clarifying which questions one has to answer in order to choose the right formalization: (1) which are the exposed (observable) actions/species and which are the imposed ones? (2) which are the relevant exposed observational equivalences? (3) which are the alternative transitions ($\dashrightarrow$)?

As observed in [72] robustness in biology is strongly related with redundancy. This is exactly in the spirit of our unwinding framework, where for each imposed transition we have to find an alternative path leading to an equivalent situation.

In this paper, we proposed the use of "approximated" behavioural equivalences (e.g., see [11, 27]) to obtain flexible instantiations. As future work we intend to explore also the use of downgrading like techniques (see [9]) to this aim.

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