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# Analyzing resilience properties in oscillatory biological systems using parametric model checking

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ABSTRACT

Automated verification of living organism models allows us to gain previously unknown knowledge about underlying biological processes. In this paper we show how parametric time model checking can be applied to define the time behavior of biological oscillatory systems more precisely. In particular, we focus on the resilience properties of such systems. This notion was introduced to understand the behavior of biological systems (e.g. the mammalian circadian rhythm) that are reactive and adaptive enough to endorse major changes in their environment (e.g. jet-lags, day-night alternating work-time). We formalize these properties through parametric TCTL and investigate the influence of environmental conditions changes on the resilience of living organisms under the uncertainty in parameters. In particular, we discuss the influence of various perturbations, e.g. artificial jet-lag or components knock-out on the parameters controlling the oscillatory behavior. This analysis is crucial when it comes to model elicitation for dynamic biological systems. We demonstrate the applicability of this technique using a simplified model of circadian clock and discuss its results with regard to other previous studies based on hybrid modeling.

## 1. Introduction

Understanding the mechanisms involved in oscillatory biological regulation is a fundamental issue to analyze living systems. Time delays play a major role in the sustainability and control of oscillations, as shown for example in phenomena related to the mammalian circadian clock (Leloup and Goldbeter, 2003). Taking account of these delays in the modeling process is therefore necessary to have a precise understanding of the chrono-biological phenomena. A major issue consists in identifying the value of (or the interval associated to) each delay. Some of them cannot be obtained through biological experiments. And most methodologies are not well-suited to capture parametric systems, e.g. simulation is adapted to assess the quality of one (or some) run(s) of the system, but it raises difficult problems when it comes to an exhaustive analysis. That is why automatic reasoning provided by model checking techniques is useful to get formal proofs about the evolution of timed systems. The idea to consider time as a discrete variable helped the representation of the sequence of events that punctuate the featuring phases of biological systems. Given that the time delays are generally difficult to determine experimentally, computational approaches to model and infer the precise delays *in silico* are fundamental. Model elicitation procedures are then to be performed with regard to a relevant class of dynamic properties, which are to be expressed through modal logics, e.g. LTL (Pnueli, 1977), CTL (Clarke et al., 1986) or their extensions.

TCTL is one of these logics, aiming at the verification of properties with quantitative timing information (Alur et al., 1990). As TCTL model checking is undecidable for the general classes of timed extensions of Petri nets or automata, the main challenge is to identify the relevant subclasses of models (or properties) where decidability can be settled and the associated complexity can be handled in an efficient way. Recently, the authors of Jovanović et al. (2013) identified a subclass of parametric timed automata that can benefit from efficient analysis of TCTL model checking.

Here, we use a modeling paradigm with three main advantages: (1) it is expressive enough to capture the timed behavior of biological systems; (2) it is easy-to-understand for biological collaborators; (3) it comes with efficient existing tools to perform parametric model checking. The framework of (bounded) parametric time Petri nets (Traonouez et al., 2009) with parametric intervals

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associated with transitions, which extends time Petri nets (Merlin and Farber, 1976) with strong semantics, meets these requirements, thus motivating our choice.

### 1.1. Petri nets to model dynamic biological systems

Concurrence between different components, either at a micro or macro scale, is central to biological systems. Petri nets are capable to concisely represent the concept of concurrence and to simulate the behavior of concurrent systems biology models (Chaouiya et al., 2008). This framework is associated with a number of extensions, including *stochastic* Petri nets that allow to represent the stochastic behavior or *time* Petri nets to include quantitative timing information.

Stochastic extensions of Petri nets are effective, especially for modeling biochemical systems (Mura and Csikász-Nagy, 2008). The main work on stochastic networks involves Markov models (Heiner et al., 2008) for which the model checking techniques are well-established. To address the oscillatory properties, the authors of Ballarini (2014) and Spieler (2013) add an observer automaton to the system such that it allows to precisely describe the noisy oscillatory trends.

During the last decade, some work (especially Koch and Heiner, 2008; Chaouiya et al., 2006) demonstrated how Petri nets could be used for both qualitative and quantitative analysis of biological systems. The unifying framework to conduct model checking using Petri nets is given in Gilbert et al. (2007) where the three different approaches are applied to cross-validate the model of MAPK cascade (Levchenko et al., 2000). The authors use qualitative, stochastic and differential equation based approach where each corresponds to the certain modification of the Petri net. In Chaouiya et al. (2006), the authors defined a systematic re-writing of Boolean models of logical regulatory networks into a standard PN formalism. In Chaouiya et al. (2008), they extended the technique to multi-level logical models, but without incorporating delays. Then further work was proposed to tackle hybrid analysis, either by considering more expressive Petri nets or by using other formalisms. Authors of Troncale et al. (2009) proposed to use Timed Hybrid Petri nets to perform quantitative timing analysis of biological systems, where they translated both the model and the properties to check into an event clock automaton. Other authors directly addressed hybrid modeling in the context of more expressive hybrid automata (Ahmad et al., 2007). Taking inspiration in these various works, we propose here to translate the multi-level logical models into Petri nets, associating time intervals to transitions to capture the quantitative delays between discrete events, and perform the model checking procedure directly through this formalism, without any other translation.

### 1.2. Resilience properties

Emerging from studies in ecological systems (Holling, 1973), *resilience* recently raised a growing interest among the research community (Grimm and Calabrese, 2011; Tavana et al., 2012; Maruyama et al., 2014). This notion is critical to design a system reactive enough to face major changes in its environment: at an organizational level, this can be the security logistics in case of an earthquake; in biology, the functionality of circadian rhythm confronted to a wide range of perturbations. Resilience encompasses a family of four core properties, which are *resistance*, *recoverability*, *functionality* and *stability* (Schwind et al., 2013, 2016). The main difference to the design of critical systems lies in the fact that resilient systems may experience changes to its very nature, adapt and maintain certain properties (Leveson et al., 2006). In this paper, we are investigating the resilience properties in oscillatory models, more specifically in a biological context. While most existing works around resilience are limited to chronological models, we focus on an analysis based on quantitative timing information.

### 1.3. Modeling of the mammalian circadian clock

Circadian rhythms control numerous biological mechanisms in various species. These endogenous oscillators are entrained by environmental factors ("Zeitgeber") such as light and temperature conditions (Golombek and Rosenstein, 2010). One of the main oscillatory mechanisms in mammals is associated with the so-called suprachiasmatic nucleus (SCN) that serves as the master clock for cellular clocks in peripheral tissues (Edery, 2000). This effect of signal propagation triggered by the oscillatory trend in the master clock is known as the coupling of oscillators. The numerical analysis of entrainment properties is conducted in Abraham et al. (2010) where the authors compare the result of simulations in the continuous framework to the experimental data on SCN tissues, where the external stimulus is given by the oscillating temperature profile. The experimental analysis of circadian clock in the mouse heart is given in Sakamoto and Ishida (2000), where the effects induced by the light conditions are studied. The investigation of the corresponding theoretical synchronization properties using the population of correlated Kuramoto oscillators is given in Brede (2010).

One of the first models of mammalian circadian clock formulated using differential equations is given in Leloup and Goldbeter (2003). We consider its simplified version presented in Comet et al. (2012), where Comet et al. applied a series of transformations to obtain the minimal discrete-state model with delays which allows to show important behavioral patterns. We converted this model into a parametric time Petri net where the state of each gene is encoded by a place, whose (safe) marking corresponds to the Boolean status of the gene.

Previous research in the literature includes the earlier hybrid Petri net representation of the circadian clock by Matsuno et al. (2006), which has been analyzed using simulation. Recently, there were also approaches to analyze the effect of coupling on post-translational oscillator (Jolley et al., in press) using continuous Pi-calculus process algebra (Banks et al., 2015) and to study the influence of circadian clock on the cell cycle (Traynard et al., 2015).

In our paper, we aim to provide a method to study the dynamic properties of the gene regulatory network behind circadian rhythm. For the sake of simplicity, we chose to stick to the model from Comet et al. (2012), but connections with the model in Matsuno et al. (2006) need to be investigated in future works.

### 1.4. Our contribution

In this paper, we propose a methodology based on parametric time Petri nets to assess the resilience of the gene regulatory network controlling the mammalian circadian clock system. Analyzing the literature, we formalize the corresponding properties in the TCTL logic and apply them on a simplified version of the circadian rhythm (Comet et al., 2012). In particular, we are able to perform model elicitation and gain the information about the delays involved in the regulations of this system. We also consider the extension of the simplified model of circadian clock where we allow for uncertainty in parameters therefore modeling the situation where only approximate information about the time behavior of the biological system is available. In this extended model we use "minutes" as time units and another definition of the parametric interval firing function thus covering the larger set of possible behaviors. The same kind of approach could be applied to larger models of the circadian clock by changing the input model