

Hybrid Modeling of Gene Regulatory Networks: Mixing Temporal and Qualitative Biological Properties

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Abstract. Modeling gene regulatory network is a difficult task. Experiments often fail at giving enough information for parameterizing existing models. We propose herein a dedicated hybrid modeling. To overcome the lack of quantitative information, our modeling focuses on (i) the biological compound product signs and (ii) the temporal properties associated with the biological effects of the interactions. Both these constraints are easy to extract from experimental data. They aims at reasoning on a hybrid system adapted to large gene regulatory networks, which is suitable for emphasizing biological properties.

1 Introduction

Experimental approaches that studies living systems behaviors, focus on various and complementary biological components: e.g. a set of genes that encodes a set of proteins. These components interact together. These interactions can be abstracted in the so-called gene regulatory network (GRN), that is the current biological framework for many studies (see Fig.1 for illustration). For long, due to the large number of unknown biological parameters (i.e. numerical value of dynamical parameters related to biochemical reactions), modeling the gene regulatory network behavior was a difficult task. Several approaches overcome the lack of parameters values by proposing qualitative modeling approaches (see [1, 2] for overview and [3] for review). They consider the gene interaction as the corner stone to represent a biological behavior. A gene regulatory reaction indeed summarizes a protein production that activates or represses the target gene. From a computational viewpoint, these modeling approaches exploit the structure of the network (e.g. interlocked feedback loops) rather than the numerical values of biological compound concentration. Among the qualitative modeling techniques, approaches based on Piecewise-Afine Differential Equations (PADEs) [4] or the René Thomas's formalism [5] showed astonishing results when applied on concrete biological systems. As shown in [6, 7], these techniques corresponds to a

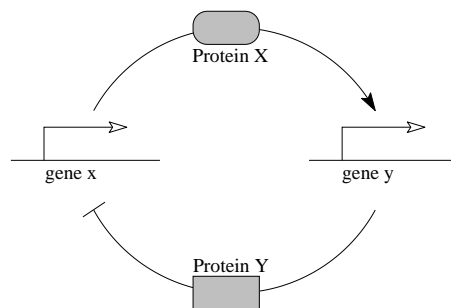


Fig. 1. Description of a two genes interaction network that resumes a system composed of genes x and y . The gene x produces the protein X that activates the transcription of genes y . It implies a production of the protein Y that represses the transcription of the gene x .

class of hybrid systems [8], for which we can apply powerful techniques for the verification and control of hybrid-systems. It particularly allows the automatic investigation of qualitative properties of genetic regulatory networks [9] .

In addition, the last decade saw the emergence of novel experimental techniques like micro-arrays. They permit the monitoring of gene behaviors over times. Therefore, a novel class of hybrid system, dedicated to the biological system modeling, must take into account a novel parameter: the time delay. Note that such a parameter was neglected before, despite its estimation from the variation of specific protein products over times. The time delay represents an opportunity to refine existing qualitative models by showing qualitative properties that verifies experimental temporal constraints. It emphasizes a need for techniques that include both qualitative properties, that comes from the biological network structure, and delays associated with the dynamics of genes. In this context, we propose herein a novel hybrid modeling technique. It abstracts the structure of the network, i.e. positive and negative feedback loops, by focusing on the variation of signs associated with genes when following qualitative behaviors. In this qualitative abstraction, we add the constraints on delays for a natural refinement of the qualitative behavior.

This paper introduces such a hybrid modeling. Section 2 presents the principle of the modeling whereas section 3 highlights connections between our modeling technique and other state of the art modeling approaches. Section 4 gives a formal description of the hybrid modeling approach, with a special emphasis on qualitative and temporal constraints. For guidance, the theoretical framework is illustrated on a simplistic system composed of two genes (Fig. 1). Finally, section 5 proposes an application of the hybrid modeling on a reference biological system: the circadian cycle in mammalian cells. This system that is particularly well-studied for its temporal properties, represents a suitable benchmark for testing our modeling approach and showing biological insights that is made possible owing to hybrid systems analysis.

2 Principle of the Hybrid Modeling

Modeling large gene regulatory networks implies the use of a large number of parameters or rough assumptions to simplify biological process. From the computational viewpoint, the major difficulty is linked with parameters valuations. Indeed, an estimation of them remains difficult despite recent experimental progresses. In this context, the hybrid modeling technique offers great advantages to overcome these difficulties.

As a major assumption, we consider the *biological qualitative behavior* as the cornerstone of our modeling. By qualitative behavior, we mean that we are interested in the succession of concentration peaks rather than the concentration values. These peaks follow as well temporal properties. These temporal pieces of information are currently available and observed by experiments. They have to be combined with the qualitative properties for a better understanding of the system (see Fig.2).

Since we are only interested in the concentration peaks, the discrete states of the system can be represented by tuples of boolean variables. Each boolean variable – named derivative sign – depicts the behavior of a gene by showing its increase or decrease of its product. For illustration, from Fig. 1, we get $(x, y) = (+, -)$. It is the discrete state that represents an increase of the concentration of the x product (i.e. corresponding to protein X) and a decrease of the concentration of the y product (i.e. protein Y).

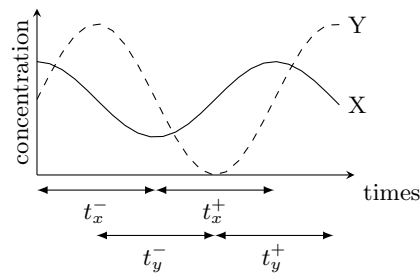


Fig. 2. Concentration variation of the system depicted in Fig.1. t_x^- (resp. t_y^-) represents the decreasing time of x (resp. of y). t_x^+ (resp. t_y^+) represents the increasing time of x (resp. of y).

Since we are taking into account discrete states for which we are not using the concentration levels, our hybrid modeling do not use the notion of threshold. Without strong hypotheses on the system's interaction, we assume that any interaction can potentially change the derivative sign of the target. Since our model abstracts the actual biological system, it encompasses some behaviors which do not actually take place.

As a complement to the qualitative behavior based on the derivative signs, our hybrid modeling approach takes into account the temporal scheduling, which

introduces a notion of time between two successive peaks, since this scheduling relies upon the respective durations of the increase or decrease phases. Thus, it gives the opportunity to estimate the time to increase or to decrease for each variables.

The parameters of our hybrid modeling technique correspond to temporal knowledge in the form of delays. They are not function of discrete states but they depend on interactions that modifies the qualitative behaviors at a given time, named active interaction. Note that, in each discrete state, active interactions are not always the same. For this reason, the transitions between discrete states are non deterministic. Thus, each transition is defined over a range of delays, that we are considering as an interval of the possible actual delays. For illustration, in Fig. 2, the delay t^+x is included in an specific interval. The boundaries of these intervals form the set of the temporal parameters of the hybrid modeling. Therefore, the number of parameters is a linear function of the number of variables in the modelled system. Thus, we use a restrictive number of parameters, which makes it possible to investigate large gene regulatory networks.

3 Context and Related Works

Qualitative modeling approaches, like those using PADEs [4] or discrete abstractions (boolean [10] and multivalued [11]), show similar characteristics but came from different theoretical backgrounds. The discrete abstractions focus exclusively on qualitative information (interlocked feedback loops), allowing easily to determine instances of parameters. At the opposite, PADEs systems qualitatively summarize quantitative informations to overcome the estimation of parameters difficult to obtain. Recently, [12–14] demonstrate the promising properties of modeling approaches that incorporate temporal notions. Their theoretical framework basically uses a qualitative modeling that is extended into a hybrid modeling. Siebert and Bockmayr [14] resume the Thomas’s modeling [11] and add temporal notions when discrete qualitative parameters are known. It allows them a delicate refinement of the discrete dynamics based on the temporal parameters. Batt et.al.[13] adapt a timed automata [15] and extend it from boolean to multivalued discrete states. Both hybrid modeling approaches use time intervals in their system of transitions, but failed at investigating large networks. Moreover, they both consider the border of the discrete states as constant over time.

Our hybrid modeling does not arise from an existing modeling framework. Nevertheless, the analysis of our model gets close to the qualitative analysis of continuous system, like the study of derivative signs of ODE systems [16] or the constraints analysis of large gene regulatory networks proposed by [17].

4 Hybrid Modeling

4.1 Interaction System

We describe a nonlinear dynamical system as being an interaction system which is defined such that:

Definition 1 (Interaction System). *An interaction system is a couple (V, I) where*

- V is a finite set of biological components.
- $I \subset V \times \alpha \times V$ is a finite set of interactions labelled with $\alpha \in \{+, -\}$ which is a sign of the interaction. $(v, \alpha, v') \in I$ is therefore the interaction of v on v' , called activation if $\alpha = +$ and inhibition otherwise.

Notice that the positive auto-regulations (i.e. interactions in the form $(v, +, v)$) have not impact on the hybrid model since such an interaction does not allow a change of derivative signs. For example, in Fig. 1, the interaction system is $\mathcal{P} = (V, I)$ such that $V = \{x, y\}$ and $I = \{(x, +, y), (y, -, x)\}$.

4.2 Hybrid Model Design

Based on the previous Interaction System, we build a Temporal Evolution Model (TEM) which is a subclass of Linear Hybrid Automaton (LHA). Given a set of variables X , let $C(X)$ be the set of conjunctions of constraints in the form of $x \diamond c$ with $x \in X$, $c \in \mathbb{Q}$ and $\diamond \in \{\leq, \geq\}$.

Definition 2 (TEM). *A Temporal Evolution Model (TEM) is a tuple $\mathcal{D} = (L, l_0, H, E, Inv, Dif)$ where*

- $L = \{(s_1, \dots, s_n)\}$ is a finite set of discrete states with n the number of variables and $s_i \in \{+, -\}$.
- l_0 is the initial discrete state.
- H is a finite set of real-valued variables (i.e. the clock of the system).
- $E \subset L \times C(H) \times 2^H \times L$ is a finite set of edges. $(l, \mu, R, l') \in E$ is therefore the transition from the discrete state l to the discrete state l' , with the guard μ and R the set of clocks to be reset.
- $Inv \in C(H)^L$ maps an invariant to each discrete state.
- $Dif \in \mathbb{Z}^{H^L}$ maps an evolution rate to each clock in each discrete state, $\frac{dH}{dt} = Dif(l, h)_{h \in H}$ being the set of derivatives of the clock wrt. time.

For the running example, we get the following TEM, as represented in Fig 3.

- $L = \{(+, +), (-, +), (-, -), (+, -)\}$,
- $H = \{h_x, h_y\}$,
- $E = \{((+, +), \{h_x \geq d_x^+\}, (-, +)), ((-, +), \{h_y \geq d_y^+\}, (-, -)), ((-, -), \{h_x \geq d_x^-\}, (+, -)), ((+, -), \{h_y \geq d_y^-\}, (+, +))\}$,
- $Inv = \{((+, +), \{h_x \leq D_x^+\}), ((-, +), \{h_y \leq D_y^+\}), ((-, -), \{h_x \leq D_x^-\}), ((+, -), \{h_y \leq D_y^-\})\}$ and

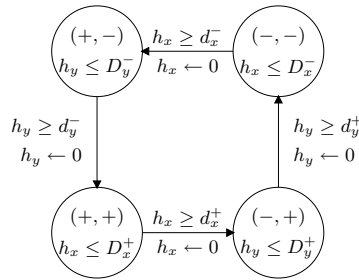


Fig. 3. TEM of the network shown in Fig. 1.

$$- Dif = \{((+, +), \frac{dh_x}{dt} = 1, \frac{dh_y}{dt} = 1), ((-, +), \frac{dh_x}{dt} = 1, \frac{dh_y}{dt} = 1), ((-, -), \frac{dh_x}{dt} = 1, \frac{dh_y}{dt} = 1), ((+, -), \frac{dh_x}{dt} = 1, \frac{dh_y}{dt} = 1)\}.$$

The dynamics of the hybrid system are depicted according to the two following features:

Qualitative (discrete) description of the derivative signs. It is represented by the finite set of discrete states. Let $l = (s_1, \dots, s_n)$ be a discrete state with n the number of variables and $s_i \in \{+, -\}$ the derivative sign of x_i . Thus, for each variable x , there exists two possible sign values that may be: $+$ (which means that x is currently increasing) and $-$ (which means that x is currently decreasing). For example in Fig. 1, the discrete state $(+, -)$ shows that x increases while y decreases. Therefore, 2^n discrete states is the cardinal of the set of all the possible qualitative behaviors.

The transition from one discrete state to another, is a discrete transition labelled with a guard μ such as $h \geq p$ where h is a clock and p a parameter of the hybrid system. A discrete transition corresponds to a concentration peak of a variable production. For each x_i , if there exists an interaction $(x_i, +, x_{i'}) \in I$ such that $s_i \neq s_{i'}$ or if there exists an interaction $(x_i, -, x_{i'}) \in I$ such that $s_i = s_{i'}$ then there exists a discrete transition $(l, h_i \geq d_{x_i}^\alpha, h_i \leftarrow 0, l')$ with $\alpha \in \{+, -\}$, $l = (s_1, \dots, s_n)$ and $l' = (s'_1, \dots, s'_n)$ such that $s_i \neq s'_i$ and $\forall i' \neq i, s_{i'} = s'_{i'}$. The guard of the discrete transition restricts the possible dynamics since it forbids specific discrete transitions. Thus, the finite set of discrete transitions describes the qualitative dynamics of the system.

Quantitative (temporal) description. It is represented by a set of continuous states. A continuous state is defined as a discrete state l together with a tuple of real-valued variables $\nu = (h_1, \dots, h_n)$ that are called clocks. Such a clock evolves over time and its evolution is defined by $\frac{dh_i}{dt} = 1$ and constrained by invariants. The clock of a specific state must always verify the invariants of its own discrete state. The invariants are constraints such as $h \leq p$ where h is a clock and p a parameter of the hybrid modeling. If there exists a discrete transition $(l, h_i \geq d_{x_i}^\alpha, h_i \leftarrow 0, l') \in E$ with $\alpha \in \{+, -\}$, then $h_i \leq D_{x_i}^\alpha$ is an invariant in the discrete state l .

The guards and invariants are the constraints on the clocks. For example, if the invariant of the discrete state l is $h_i \leq D_{x_i}^\alpha$ and the guard from l to l' is $h_i \geq d_{x_i}^\alpha$, then the system stays in l during a delay that belongs to the interval $[d_{x_i}^\alpha, D_{x_i}^\alpha]$ before it reaches l' . Each variable x is associated with 4 parameters that are the boundaries of two delay intervals: $[d_x^+, D_x^+]$ corresponding to a delay interval where x increases and respectively $[d_x^-, D_x^-]$ where x decreases. Fig. 4 shows these parameters with the gene product concentration variations. According to the TEM building, we have for each variable x the following structural constraint: $0 \leq d_x^\alpha \leq D_x^\alpha$ with $\alpha \in \{+, -\}$.

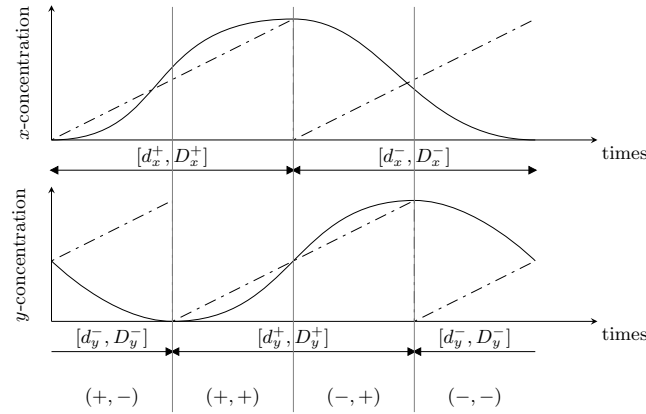


Fig. 4. Gene product concentration variations for the TEM shown in Fig. 3. The behavior corresponds to the qualitative cycle $(+, -) \rightarrow (+, +) \rightarrow (-, +) \rightarrow (-, -) \rightarrow (+, -)$. The dashed curves represent the clock evolution of x and y .

The semantics of a TEM is defined as a Timed Transition System.

Definition 3 (Semantics of a TEM). Let (S, s_0, \rightarrow) be a Timed Transition System where $S = \{(l, \nu) \mid l \in L \text{ and } \nu \models \text{Inv}(l)\}$ is the set of continuous states; $s_0 \in L$ is the initial discrete state; and \rightarrow is the transition relation which is defined for $t \in \mathbb{R}_{\geq 0}$ as:

- discrete transitions: $(l, \nu) \rightarrow (l', \nu')$ iff $\exists (l, \mu, R, l') \in E$ such that the guard μ must be true for the value ν ($\gamma(\nu) = \text{true}$); we keep the value ν of x , except after a reset ($\nu'(x) = \nu(x)$ if $x \notin R$ and 0 otherwise); and the invariant must be true in the target discrete state ($\text{Inv}(l')(\nu') = \text{true}$).
- timed transitions: $(l, \nu) \xrightarrow{t} (l, \nu')$ iff $\nu' = \nu + \frac{dH}{dt} \times t$, and $\forall t' \in [0, t]$, $\text{Inv}(l)(\nu + \frac{dH}{dt} \times t') = \text{true}$.

For example, Let 7 and 12 be initial values of x and y clocks. $((+, +), (7, 12))$ is the initial continuous state of a TEM in Fig. 3. After a delay of $d_x^+ - 7$, it will be possible to pass in the discrete state $(-, +)$ because the guard of

the discrete transition $((+, +)(d_x^+, d_x^+ - 5)) \rightarrow ((-, +)(0, d_x^+ - 5))$ evaluates to true. From this initial continuous state, it is also possible to stay in the discrete state $(+, +)$ during a maximal delay of D_x^+ . Whenever, the continuous state $((+, +)(D_x^+, D_x^+ - 5))$ is reachable. The discrete transition $((+, +)(D_x^+, D_x^+ - 5)) \rightarrow ((-, +)(0, D_x^+ - 5))$ becomes mandatory so that the invariant $h_x \leq D_x^+$ must not be violated.

5 Biological Example: the Circadian Cycle

5.1 Circadian Cycle

The originality of our hybrid modeling approach mainly lies in the use of temporal constraints. From the biological viewpoint, the most-studied system for its temporal properties is the circadian clock. A circadian rhythm (or circadian cycle) is an oscillation with a period of approximately 24 hours. The complex biological processes underlying this natural rhythm, that takes place for a wide range of organisms. It can be summarized by a set of interactions between specific genes. Several models describe the circadian clock of mammalian cells using Ordinary Differential Equations [18, 19]. They are robust and accurate with experimental knowledge (i.e. amplitude of oscillations, time series of mRNA and protein concentrations). However, they implies the use of a large number of parameter values. Based on a refinement of the interdependency between the positive and negative feedback loops [20], Sriram et al. [21] propose a discrete model for the circadian clock that sums up interactions of three biological components: BMAL1 and REV-ERB α proteins and the PER-CRY complex. Their interactions produce a dynamical oscillation of their concentrations. Fig.5(a) highlights the corresponding gene regulatory network.

5.2 TEM of the Circadian Cycle

Based on the network, and following above descriptions, we build the TEM that corresponds to the circadian clock model. The resulting qualitative graph is depicted in Fig 5(a). At this stage, we propose to analyze this model using verification tools dedicated to hybrid systems such as *HyTech* [22] or *PHAVer* [23]. They allow parametric verifications. It is particularly accurate for emphasizing results based on little information available about the parameters of the TEM.

By nature, the circadian clock system provides oscillations over a period of 24 hours. Therefore, we are investigating cycles within the TEM that provide such qualitative behaviors. However, the number of cycles is infinite. As a primary analysis, we hence analyzed cycles that (i) never passe twice by the same qualitative state and (ii) such that each variable describes only one high peak and one low peak. This class of cycles is summarized in Table 1. In a TEM, each cycle represents a qualitative variation (i.e. succession of peaks) of a biological product. Furthermore, each transition is related to a set of temporal properties. In particular, a circadian cycle shows oscillations of 24 hours. This assumption

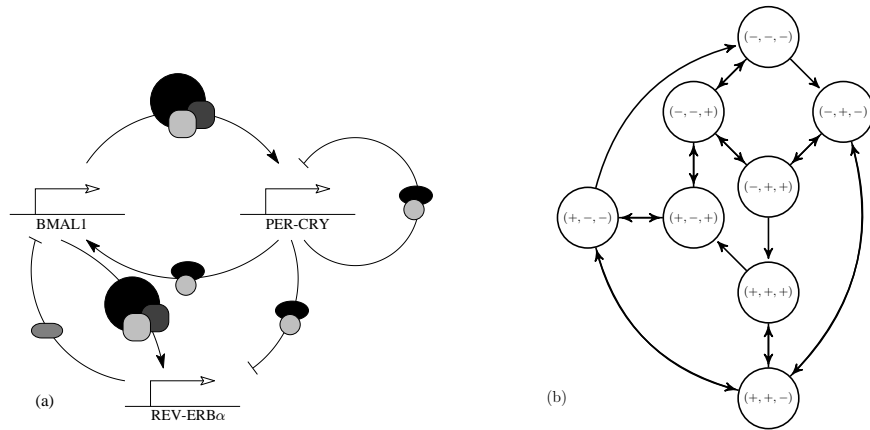


Fig. 5. Gene regulatory network and TEM of the circadian clock system. (a) resumes the system using three biological components: PER-CRY and BMAL1 protein complexes and the REV-ERB α protein. We consider the set of genes that product such components and resume their interactions by either activation (\rightarrow) or repression (\dashv). (b) show the corresponding TEM. Each discrete state is in the form of $(s_{BMAL1}, s_{REV-ERB\alpha}, s_{PER-CRY})$. For reading convenience, sake, guards, resets and invariants have been omitted and transitions from a to b and from b to a have been gathered in a single bi-direction edge.

implies a period of 24 hours for the cycles of interest as depicted in Table 1. It can be translate by the following constraints. For each variable x and for $\alpha \in \{+, -\}$:

- $d_x^\alpha = D_x^\alpha$ (the interval $[d_x^\alpha, D_x^\alpha]$ is reduced to one) and,
- $d_x^\alpha + d_x^\beta = 24$ with $\beta \neq \alpha$ (the period of the cycle is 24 hours).

For illustration, we propose to describe here the temporal properties that are associated with the first qualitative cycle labelled in Table 1. In this case $(-, -, -)$ is the initial discrete state and $0 \leq h_x \leq D_x^-$ the initial temporal constraint for each variable x . It results the constraint

$$d_{BMAL1}^+ \leq d_{PER-CRY}^-$$

that have to be satisfied by the system to qualitatively behave as mentioned by the qualitative cycle. From the biological viewpoint, it implies that, in a circadian cycle, the consumption of the PER-CRY complex cannot be smaller than the production of the BMAL1 complex (see Fig.6 for illustration).

6 Conclusion

We presented here a subclass of a linear hybrid automaton, named Temporal Evolution Model (TEM). Despite its simplicity, this approach is particularly accurate for modeling living systems. It takes into account (i) a qualitative description of derivative signs, and (ii) the quantitative temporal properties associated

Cycle ID	Qualitative Cycle
1	$(-, -, -) \rightarrow (-, -, +) \rightarrow (-, +, +) \rightarrow (-, +, -) \rightarrow (+, +, -) \rightarrow (+, -, -) \rightarrow$
2	$(-, -, -) \rightarrow (-, +, -) \rightarrow (+, +, -) \rightarrow (+, +, +) \rightarrow (+, -, +) \rightarrow (+, -, -) \rightarrow$
3	$(-, -, -) \rightarrow (-, +, -) \rightarrow (-, +, +) \rightarrow (+, +, +) \rightarrow (+, +, -) \rightarrow (+, -, -) \rightarrow$
4	$(-, -, -) \rightarrow (-, +, -) \rightarrow (-, +, +) \rightarrow (+, +, +) \rightarrow (+, -, +) \rightarrow (+, -, -) \rightarrow$
5	$(-, -, -) \rightarrow (-, +, -) \rightarrow (-, +, +) \rightarrow (-, -, +) \rightarrow (+, -, +) \rightarrow (+, -, -) \rightarrow$
6	$(-, -, -) \rightarrow (-, +, -) \rightarrow (+, +, -) \rightarrow (+, -, -) \rightarrow (+, -, +) \rightarrow (-, -, +) \rightarrow$
7	$(-, +, -) \rightarrow (-, +, +) \rightarrow (+, +, +) \rightarrow (+, -, +) \rightarrow (+, -, -) \rightarrow (+, +, -) \rightarrow$
8	$(-, +, -) \rightarrow (+, +, -) \rightarrow (+, +, +) \rightarrow (+, -, +) \rightarrow (-, -, +) \rightarrow (-, +, +) \rightarrow$
9	$(-, +, -) \rightarrow (-, +, +) \rightarrow (-, -, +) \rightarrow (+, -, +) \rightarrow (+, -, -) \rightarrow (+, +, -) \rightarrow$
10	$(-, +, -) \rightarrow (+, +, -) \rightarrow (+, -, -) \rightarrow (+, -, +) \rightarrow (-, -, +) \rightarrow (-, +, +) \rightarrow$
11	$(-, -, -) \rightarrow (-, -, +) \rightarrow (-, +, +) \rightarrow (+, +, +) \rightarrow (+, -, +) \rightarrow (+, -, -) \rightarrow$
12	$(-, -, -) \rightarrow (-, -, +) \rightarrow (-, +, +) \rightarrow (+, +, +) \rightarrow (+, +, -) \rightarrow (+, -, -) \rightarrow$
13	$(-, +, +) \rightarrow (+, +, +) \rightarrow (+, +, -) \rightarrow (+, -, -) \rightarrow (+, -, +) \rightarrow (-, -, +) \rightarrow$

Table 1. Qualitative cycles of interest for the TEM depicted in Fig. 5(b), where for a discrete state in the form of $(s_{BMAL1}, s_{REV-ERB\alpha}, s_{PER-CRY})$.

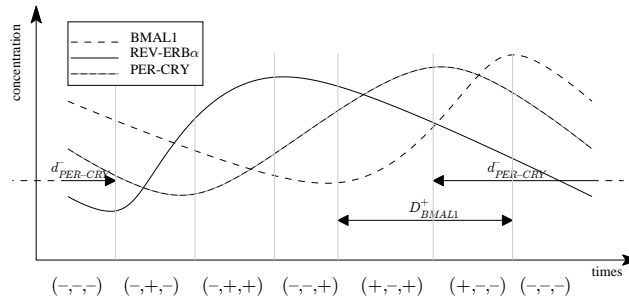


Fig. 6. Gene product concentration variations for the TEM of the circadian cycle shown in Fig. 5(b), in accord with the first qualitative cycle shown in Table 1.

to biological productions. These two particular information are notably essential to describe biological behaviors over time, as observed with recent experimental approaches. Like this, based on our hybrid modeling, a qualitative validation of a model consists in finding a succession of peaks that are consistent with experiments. In addition, TEM provides the opportunity to reason automatically on the temporal properties that are associated with the succession of peaks. It thus gives a natural refinement of the qualitative validation by showing necessary constraints on delays to achieve a specific qualitative transition, like a cycle.

In comparison with other biological hybrid modeling, TEM need less parameters for describing qualitative behaviors. They are represented only using an interaction system that focus on the derivative sign variation. This abstraction implies the lost of precise quantitative description (as provided by qualitative thresholds in PADEs), but it allows us its use for modeling larger systems. Therefore, modeling (and validating) concrete gene regulatory networks appears as a natural perspective. From the hybrid system viewpoint, future works will focus on improving the automatic verification of TEM based on the frameworks already provided by *HyTech* or *PHAVer*.

References

1. Tomlin, C.J., Axelrod, J.D.: Biology by numbers: mathematical modelling in developmental biology. *Nat Rev Genet* **8**(5) (May 2007) 331–40
2. Karlebach, G., Shamir, R.: Modelling and analysis of gene regulatory networks. *Nat Rev Mol Cell Biol* (Sep 2008)
3. de Jong, H.: Modeling and simulation of genetic regulatory systems: a literature review. *J Comput Biol* **9**(1) (Jan 2002) 67–103
4. de Jong, H., Gouzé, J.L., Hernandez, C., Page, M., Sari, T., Geiselman, J.: Qualitative simulation of genetic regulatory networks using piecewise-linear models. *Bull Math Biol* **66**(2) (Mar 2004) 301–40
5. Thomas, R., Thieffry, D., Kaufman, M.: Dynamical behaviour of biological regulatory networks—i. biological role of feedback loops and practical use of the concept of the loop-characteristic state. *Bull Math Biol* **57**(2) (Mar 1995) 247–76
6. Batt, G., Ropers, D., de Jong, H., Geiselman, J., Page, M., et al.: Qualitative analysis and verification of hybrid models of genetic regulatory networks. *International Workshop on Hybrid Systems: Computation and Control (HSCC) LNCS* **3414** (2005) 134–150
7. Richard, A., Comet, J.: Necessary conditions for multistationarity in discrete dynamical systems. *Discrete Applied Mathematics* **155**(18) (Nov 2007) 2403–2413
8. Ghosh, R., Tomlin, C.: Symbolic reachable set computation of piecewise affine hybrid automata and its application to biological modelling: Delta-notch protein signalling. *Syst Biol (Stevenage)* **1**(1) (2004 Jun) 170–183
9. Batt, G., Ropers, D., de Jong, H., Geiselman, J., Mateescu, R., Page, M., Schneider, D.: Validation of qualitative models of genetic regulatory networks by model checking: analysis of the nutritional stress response in escherichia coli. *Bioinformatics* **21 Suppl 1** (Jun 2005) i19–28
10. Thomas, R.: Regulatory networks seen as asynchronous automata : A logical description. *Journal of Theoretical Biology* **153** (1991) 1–23

11. Thomas, R.: Boolean formalization of genetic control circuits. *Journal of Theoretical Biology* **42** (1973) 563–585
12. Ahmad, J., Bernot, G., Comet, J.P., Lime, D., Roux, O.: Hybrid modelling and dynamical analysis of gene regulatory networks with delays. *ComPlexUs* **3**(4) (October 2007) 231–251
13. Batt, G., Salah, R.B., Maler, O.: On timed models of gene networks. In: *FORMATS*. (2007) 38–52
14. Siebert, H., Bockmayr, A.: Temporal constraints in the logical analysis of regulatory networks. *Theor. Comput. Sci.* **391**(3) (2008) 258–275
15. Maler, O., Pnueli, A.: Timing analysis of asynchronous circuits using timed automata. In: in P.E. Camurati, H. Eveking (Eds.), *Proc. CHARME'95*, LNCS 987, Springer (1995) 189–205
16. Bernard, O., Gouz, J.: Global qualitative description of a class of nonlinear dynamical systems. *Artificial Intelligence* **136** (2002) 29–59
17. Siegel, A., Radulescu, O., Borgne, M.L., Veber, P., Ouy, J., Lagarrigue, S.: Qualitative analysis of the relation between dna microarray data and behavioral models of regulation networks. *Biosystems* **84**(2) (May 2006) 153–174
18. Forger, D.B., Peskin, C.S.: A detailed predictive model of the mammalian circadian clock. *Proc Natl Acad Sci U S A* **100**(25) (2003 Dec 9) 14806–14811
19. Leloup, J.C., Goldbeter, A.: Toward a detailed computational model for the mammalian circadian clock. *Proc Natl Acad Sci U S A* **100**(12) (2003 Jun 10) 7051–7056
20. Becker-Weimann, S., Wolf, J., Herzog, H., Kramer, A.: Modeling feedback loops of the mammalian circadian oscillator. *Biophys J* **87**(5) (2004 Nov) 3023–3034
21. Sriram, K., Bernot, G., Képès, F.: Discrete delay model for the mammalian circadian clock. *ComPlexUs* **3** (August 2006) 185–199
22. Henzinger, T.A., Ho, P.H., Wong-Toi, H.: HYTECH: A model checker for hybrid systems. *International Journal on Software Tools for Technology Transfer* **1**(1–2)
23. Frehse, G.: PHAVer: Algorithmic verification of hybrid systems past HyTech. In: *Proceedings of the Fifth International Workshop on Hybrid Systems: Computation and Control (HSCC)*. Volume LNCS 3414. (2005) 258–273