

Integrating time-series data in large-scale discrete cell-based models

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Abstract. In this work we propose an automatic way of generating and verifying formal hybrid models of signaling and transcriptional events, gathered in large-scale regulatory networks. This is done by integrating temporal and stochastic aspects of the expression of some biological components. The hybrid approach lies in the fact that measurements take into account both times of lengthening phases and discrete switches between them. The model proposed is based on a real case study of keratinocytes differentiation, in which gene time-series data was generated upon Calcium stimulation.

To achieve this we rely on the Process Hitting (PH) formalism that was designed to consider large-scale system analysis. We first propose an automatic way of detecting and translating biological motifs from the PID database to the PH formalism. Then, we propose a way of estimating temporal and stochastic parameters from time-series expression data of action on the PH. Simulations emphasize the interest of synchronizing concurrent events.

Keywords: time-series data, large-scale network, hybrid models, compositional approach, stochastic simulation

1 Introduction

The comprehension of the mechanisms involved in the regulation of a cell-based biological system is a fundamental issue. These mechanisms can be modeled as biological regulatory networks, which analysis requires to preliminary build a mathematical or computational model. By just considering qualitative regulatory effects between components, biologic regulatory networks depict fairly well biological systems, and can be built upon public repositories such as the Pathways Interaction Database [1] and hiPathDB [2] for human regulatory knowledge.

In this work we built an hybrid model of signaling and transcriptional events, gathered in large-scale regulatory networks, which stochastic simulation parameters were inferred from gene expression time-series data. The integration of time-series data in

dynamical models have been addressed separately by approaches that either: (a) focus first on modeling at small-scale the system and then on refining or improving it through the fitting with some data points, such as methods based on differential equations [3,4,5], (b) integrate in an efficient and complete fashion large-scale models and high-throughput data regardless from the system dynamics [6,7], or (c) fit dynamical data to middle-scale networks using an stochastic sampling of the space of behaviors and therefore without guarantee on finding global optima [8]. With this work we aim to fill the gaps between the aforementioned methodologies.

In the context of modeling and analyzing stochastic and concurrent biological systems various formalisms have been introduced such as Stochastic Petri Nets which is suitable for the representation of parallel systems [9]. They have been successfully applied in many areas; in particular, the specification of Petri Nets allows an accurate modeling of a wide range of systems including biological systems [10]. The major problem of Stochastic Petri Nets is that, generally, they do not lead to compact models. In addition, they do not provide results to deal with the state space explosion and are thus computationally expensive when modeling large-scale biological networks.

The Stochastic pi-calculus formalism was introduced by [11] and used in [12] for the modeling of biological systems. Stochastic pi-calculus has a rich expressivity and is well adapted for the use of **compositional approach [reformulate the link with compositionnal approach]** In this work we rely on this formalism through the Process Hitting (PH) framework [13], since it is especially useful for studying systems composed of biochemical interactions, and provides stochastic simulation as well as efficient static methods to analyse dynamical properties of the system. The PH framework uses qualitative and discrete information of the system without requiring enormous parameter estimation tasks for its stochastic simulation. This framework has been previously used to verify dynamical properties on biological systems without integrating high-throughput experimental data.

In this work we provide a method to build a time-series data integrated PH model and we evaluate the prediction power of this model concerning the simulatenously predicted traces of 12 gene components of the system upon system stimulation. The main results of this work are: (1) automatic generation of PH models integrating gene transcription and signaling events, with and without synchronisation of concurrent events, from the Pathways Interaction Database, (2) parameter estimation from time-series data and parameter integration in the PH model, and (3) comparison of the PH model predictions and experimental results. To illustrate our approach, we used a time-series dataset of keratinocytes cells, which shows the fluctuations of gene expression across time upon Calcium stimulation. This dataset was built to study keratinocytes differentiation, a time-dependent process in which the sequence of activation of signaling proteins is not yet completely understood. The method proposed in this paper remains general and can be applied to other case-studies.

2 Data and Methods

The general workflow for integrating time-series data in a PH model is depicted in Fig. 1, in the following sections we detail some of the workflow steps.

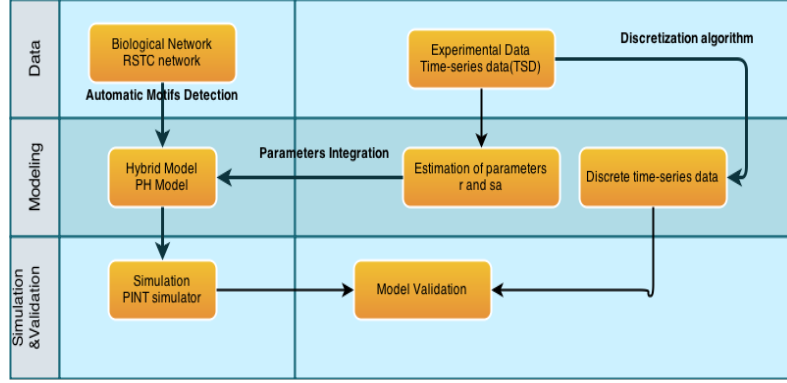


Fig. 1. Integrating stochastic and temporal information in a large-scale discrete biological model.

2.1 Data

Interaction graph. The interactions of the biological system under study were represented in an RSTC network, which stands for multi-layer receptor-signaling-transcription-cell state network and that was generated from the Pathway Interaction Database (PID). In order to build this network one needs to select a set of seed nodes related to the biological process studied. For our case study, the seed nodes were: (1) *E-cadherin*, which is a protein having Calcium binding domains and which plays an important role in cell adhesion; (2) the 12 significantly differentially expressed genes across the 10 time-points; and (3) the cell states of keratinocytes-differentiation and cell-cycle-arrest. The network was extracted automatically from the whole content of the NCI-PID database by using a subgraph algorithm to link the seed nodes [14]. In Fig. 2 we show the RSTC network obtained.

[comment] I think definition 1 is not necessary

Definition 1 (RSTC Network). A RSTC Network N is a couple (V, E) , where:

- $V = V_T \cup V_I$ is the finite set of nodes; with $V_T = \{v_{1t}, v_{2t}, \dots, v_{n1t}\}$ the set of terminal nodes; $V_I = \{v_{1i}, v_{2i}, \dots, v_{n2i}\}$ the set of transient nodes.
- $E = \{e_1, e_2, \dots, e_m\}$ is the set of edges. $E \subseteq (V_T \times V_T) \cup (V_T \times V_I) \cup (V_I \times V_T)$

In this definition, terminal nodes can be genes, proteins, complexes, cellular states, biological processes and positive conditions. On the other side, transient nodes can be transcriptions, translocations, modifications and compounds. Edges are of different types: activation (agent), inhibition, output, input and protein-family-member.

Time-series microarray dataset. We use the time-series microarray data from Calcium stimulated keratinocyte cells measured at 10 time-points. 200 transcripts were selected for their dynamic patterns, that is, their fold expression with respect to the non-stimulated cell was significant in at least one time point. We included in our model

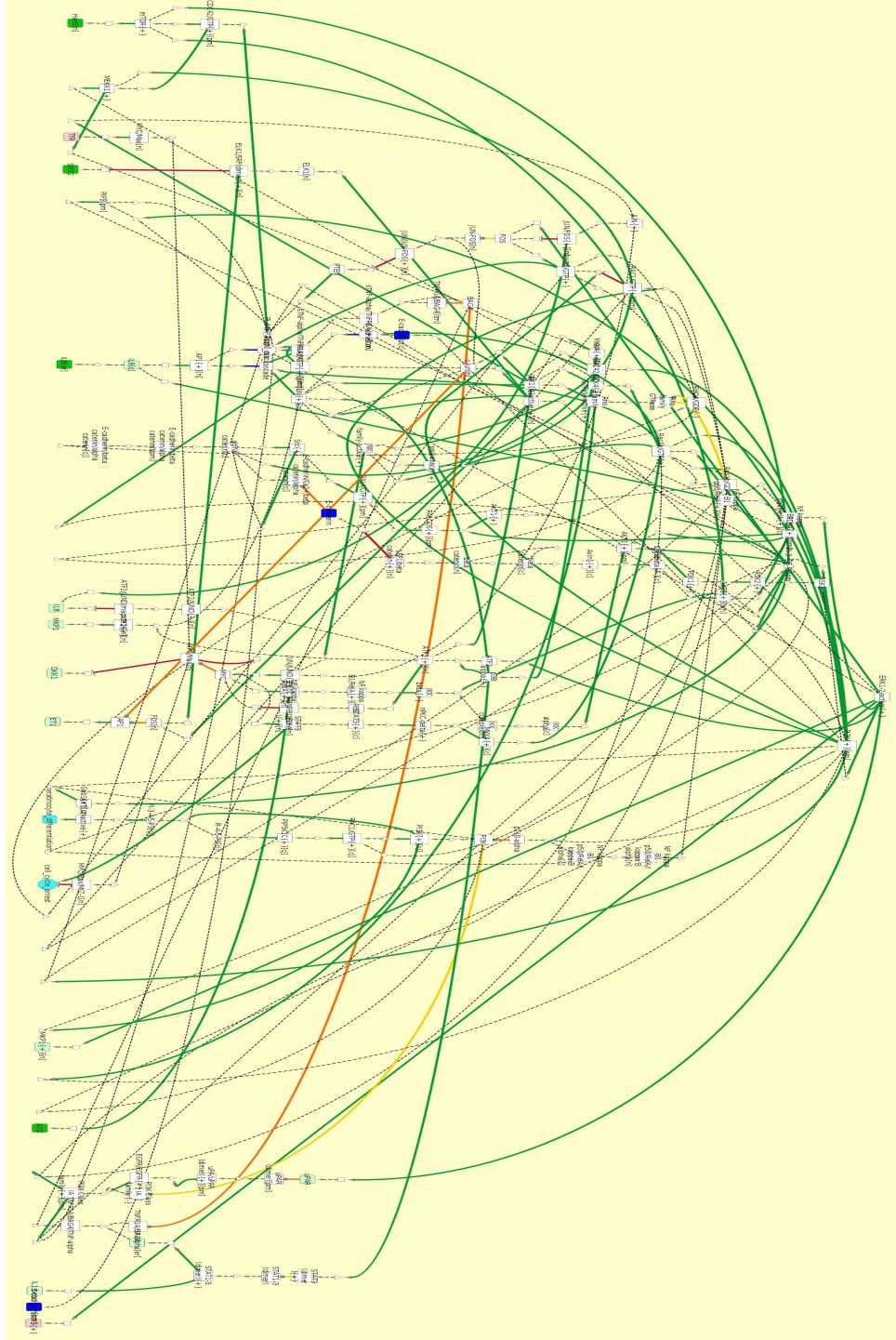


Fig. 2. Interaction graph linking E-cadherin with 12 genes of the time-series dataset. Blue nodes correspond to E-cadherin entities, red or green, to time-series genes, and cyan nodes to cellular processes. The graph is composed of 293 nodes and 375 edges (interactions). The set of nodes are composed of terminal nodes (proteins, complexes, genes, cellular state, biological processes and positive conditions) and of transient nodes (transcriptions, translocations, modifications and compounds). The set of edges are composed of interactions of type activation, inhibition, output, input and protein-family-member.

a subset of 12 of the 200 selected (see Fig. 2.1). These 12 genes had upstream regulatory mechanisms when querying the PID-NCI database and therefore could be connected in the interaction graph.

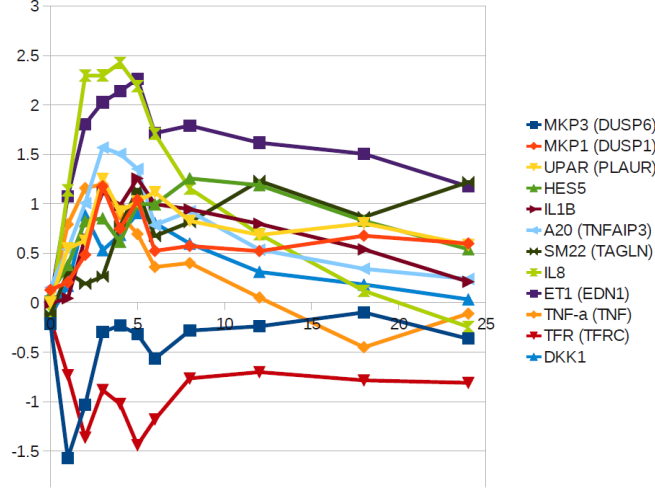


Fig. 3. 12 gene expressions upon Calcium stimulation.

The X axis represents time duration of the experiment measured in hours. The Y axis represents the \log_2 expression level of genes with respect to control.

2.2 The Process Hitting Framework

Process Hitting (PH) gathers a finite number of concurrent processes grouped into a finite set of sorts. A sort stands for a component of a biological system while a process, which belongs to a unique sort, stands for one of its expression levels. At any time exactly one process of each sort is present. A state of the PH corresponds to such a set of processes. We denote here a process by a_i where a is the sort and i is the process identifier within the sort a . The concurrent interactions between processes are defined by a set of *actions*. Actions describe the replacement of a process by another of the same sort conditioned by the presence of at most one other process in the current state. An action is denoted by $a_i \rightarrow b_j \uparrow b_k$, which is read as “ a_i hits b_j to make it bounce to b_k ”, where a_i, b_j, b_k are processes of sorts a and b , called respectively *hitter*, *target* and *bounce* of the action.

Definition 2 (Process Hitting). A Process Hitting is a triple (Σ, L, \mathcal{H}) , where:

- $\Sigma = \{a, b, \dots\}$ is the finite set of sorts;
- $L = \prod_{a \in \Sigma} L_a$ is the set of states with $L_a = \{a_0, \dots, a_{l_a}\}$ the finite set of processes of sort $a \in \Sigma$ and l_a a positive integer, with $a \neq b \Rightarrow L_a \cap L_b = \emptyset$;

- $\mathcal{H} = \{a_i \rightarrow b_j \uparrow b_k \in L_a \times L_b \times L_b \mid (a, b) \in \Sigma^2 \wedge b_j \neq b_k \wedge a = b \Rightarrow a_i = b_j\}$
is the finite set of actions.

Given a state $s \in L$, the process of sort $a \in \Sigma$ present in s is denoted by $s[a]$. An action $h = a_i \rightarrow b_j \uparrow b_k \in \mathcal{H}$ is *playable* in $s \in L$ if and only if $s[a] = a_i$ and $s[b] = b_j$. In such a case, $(s \cdot h)$ stands for the state resulting from playing the action h in s , with $(s \cdot h)[b] = b_k$ and $\forall c \in \Sigma, c \neq b, (s \cdot h)[c] = s[c]$. In order to model the fact that a molecule in the interaction graph is influenced by various molecules, two types of modeling-scenarios can be proposed: cooperation and synchronization.

Modeling cooperation The cooperation between processes to make another process bounce can be expressed in PH by building a *cooperative sort* [13]. Fig. 4 shows an example of a cooperative sort ab between sorts a and b , which is composed of 4 processes (one for each sub-state of the presence of processes in a and b). For the sake of clarity, processes of ab are indexed using the sub-state they represent. Hence, ab_{01} represents the sub-state $\langle a_0, b_1 \rangle$, and so on. Each process of sort a and b hits ab , which makes it bounce to the process reflecting the status of the sorts a and b (e.g., $a_1 \rightarrow ab_{00} \uparrow ab_{10}$ and $a_1 \rightarrow ab_{01} \uparrow ab_{11}$). Then, to represent the cooperation between processes a_1 and b_1 , the process ab_{11} hits c_1 to make it bounce to c_2 instead of independent hits from a_1 and b_1 . The same cooperative sort is used to make a_0 and b_0 cooperate to hit c_1 and make it bounce to c_0 . Cooperation sort allows to model the fact that two components cooperate to hit another component.

Modeling synchronization The synchronization sort implements another type of cooperation. If we refer to the example of Fig. 4 left, we can similarly construct a *synchronization sort* ab between sorts a and b , defined with also 4 processes. Then, component c is activated (c_1 bounces to c_2 or c_0 bounces to c_1) if either a or b are activated. Therefore, each one of these processes ab_{01} , ab_{10} , ab_{11} can activate c . In order to inhibit c , both sorts, a and b , need to be in the sub-state 0, i.e. ab_{00} . Notice that this rule is a combination of OR logical gates for activation and AND logical gates for inhibition. Imposing the synchronization sort to model a target component regulated independently by multiple predecessors avoids oscillations in the behavior of the target component over time. These oscillations appear because each predecessor can independantly activate the target component when it is active, but when one predecessor is inhibited, it will inhibit the target component. This competition between the predecessors generates oscillations on the target component.

Example 1. Fig. 4 represents a PH (Σ, L, \mathcal{H}) with $\Sigma = \{a, b, c, ab\}$, and:

$$\begin{aligned} L_a &= \{a_0, a_1\}, \\ L_b &= \{b_0, b_1\}, \\ L_{ab} &= \{ab_{00}, ab_{01}, ab_{10}, ab_{11}\}, \\ L_c &= \{c_0, c_1, c_2\}. \end{aligned}$$

This example models a Biological Regulatory Network (BRN) where the component c has three qualitative levels, components a and b are Boolean and ab is a cooperative

sort. In this BRN, ab inhibits c at level 2 through the cooperative sort ab (e.g. $ab_{00} \rightarrow c_2 \uparrow c_1$, $ab_{00} \rightarrow c_1 \uparrow c_0$) while a and b activate c through the cooperative sort ab (e.g. $ab_{11} \rightarrow c_0 \uparrow c_1$, $ab_{11} \rightarrow c_1 \uparrow c_2$). Indeed, the reachability of c_2 and c_0 is conditioned by a cooperation of a and b as explained above.

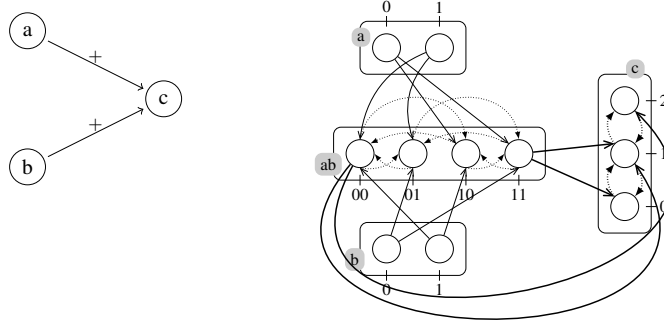


Fig. 4. (Left) biological pattern example. Nodes represent molecules (components) and edges, interactions. In this pattern components a and b cooperate to activate c . **(Right) equivalent PH model** with four sorts: three components (a , b and c) and a cooperative sort (ab). Actions targeting processes of c are drawn as thick lines.

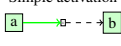
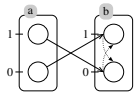
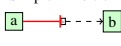
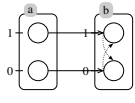
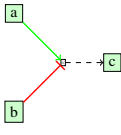
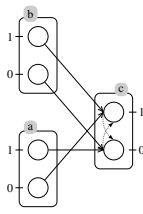
2.3 Model construction (from RSTC to PH)

Modeling the RSTC network as a PH model. In order to model the RSTC network as a PH model we select known biological regulatory patterns (atomic set of biological components and their interacting roles), represented as biochemical reactions in the RSTC network and propose their PH representation. Table 1 shows some examples of this transformation.

Automatic pattern selection and PH model generation algorithm uses two procedures. The first that take in parameter argument the graph and automatically browses it node by node and detects all patterns in the graph. For each node (output node of the pattern) we call a recursive procedure, that allows to detect a minimal set of nodes (input node of the pattern) that has a direct influence over that node. This set of nodes plus the output node and the way input and output are linked form a pattern. The type of a pattern is determined by the type of the output node, the type of regulations that arrive to that node and the type of input nodes of the pattern. Consequently, Algorithm of patterns detection returns the pattern and its type to another procedure which translates the pattern into the PH formalism. This transformation takes care of different cases (cooperation, synchronization, simple activation, simple inhibition, etc.)

For example a molecule a cooperating with a molecule b to activate a molecule c (Fig. 4, left), is a regulatory pattern because it is a protein-complex biochemical reaction that appears recurrent times. We model this pattern by four sorts (Fig. 4, right) a , b , c and ab . Sorts a , b and c stand for components a , b and c . The cooperative sort ab is

Table 1. Examples of patterns

Biological Patterns	PH Transformations	Descriptions
<p>Simple activation</p> 		<p>This pattern model the activation of the component b by the component a.</p>
<p>Simple inhibition</p> 		<p>This pattern model the inhibition of the component b by the component a</p>
<p>activation or inhibition</p> 		<p>This pattern model either the activation of the component c by the component a or the inhibition of the component c by the component b</p>

introduced in order to characterize constraints on the components a and b . In the RSTC network, we find 25 regulatory patterns. We show some examples in Table 1.

Estimating the parameters for the PH-simulation from time-series gene expression data. Since the simulation of the execution of the PH actions is done stochastically, we need to relate each action with temporal and stochastic parameters introduced into the PH framework to achieve dynamic refinement [13]. To fire an action in the PH framework we need to provide two parameters: (1) the rate $r = t^{-1}$, where t is the mean time of firing an action, and (2) the stochasticity absorption factor sa , which is introduced to control the variance of firing an action.

[comment] It will be good if in Fig 5 we have 0, 1, 2 instead of 1,2, 3

For the model components which have a measurement in the time-series data we estimate and integrate r and sa parameters in the PH model. The rest of the components will be assigned default parameters. In order to estimate r_i and sa_i for each action $h_i \in \mathcal{H}$, we need to know the different times t_i when the action could be fired as illustrated in Fig. 2.3. Each t_i represents the time in which we assume that a component moves from one process to another. Therefore the action that leads this change must be played with rate $r_i = \frac{1}{t_i - t_{i-1}}$. The integer sa represents the window of firing the action at rate r : the larger the sa , the smaller the variance around r is.

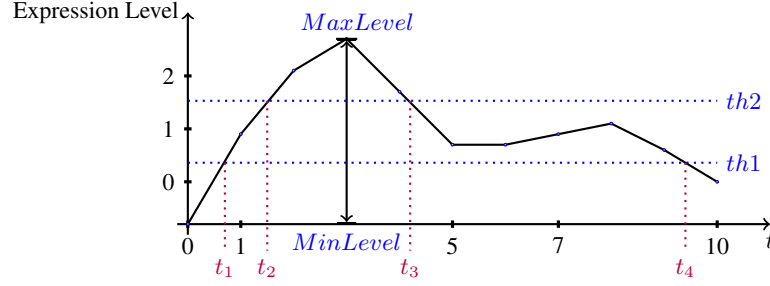


Fig. 5. Estimation of temporal parameters from time series data:

The mean firing time of an action that makes a component (gene) change of sub-state is estimated as $r_i = \frac{1}{t_i - t_{i-1}}$. $MaxLevel$ represents the maximum expression of a gene, while $MinLevel$, its minimum expression. The thresholds $th1$ and $th2$ define the PH discrete sub-states (e.g. 1,2,3) of a component according to its gene expression data.

Discretization of times-series data Because the output of a PH simulation are discrete traces of PH components, we discretized continuous experimental data to facilitate the comparison with simulation outputs. When looking at the time-series data (see Fig. 2.1) one can distinguish a high level of activity in early hours [0h-5h] and a low level in late hours [5h-10h]. We implemented a discretization method to capture these two activation times. For each time-series, we introduced two thresholds $th1$ and $th2$ (see Fig. 2.3): $th1 = \frac{1}{3}(MaxLevel - MinLevel)$ and $th2 = \frac{2}{3}(MaxLevel - MinLevel)$. In this way, the expression level in the range $[0 - th1]$ is at level 0, the one in the range $[th1 - th2]$ is at level 1, and the one in the last range is at level 2.

2.4 Simulation

We set the same initial conditions to PH components belonging to the same network layer, chosen from the RSTC structure. These initial conditions are detailed below and summarized in Fig. 6.

- **Receptor layer: E-cadherin.** We choose the pulse signal for the input node E-cadherin to be active for a duration of 5 units times in average. We made this choice to take into account the average time of the Calcium stimuli effect.
- **Signaling layer: signaling proteins.** The components in this layer are activated and inhibited with the same rate and the same stochasticity absorption factor. The actions between a controller component A and a controlled component B are constrained so that B is first activated by A and then inhibited. That is, the time interval in which an inhibition action from A to B fires is greater than the time interval in which an activation action from A to B fires. Additionally, these two time intervals must not overlap. These constraints can be seen as reachability constraints from the entry node (E-cadherin) to the output nodes (genes). The values of these parameters are selected by considering the delay of signal transduction from the entry node to the output nodes.

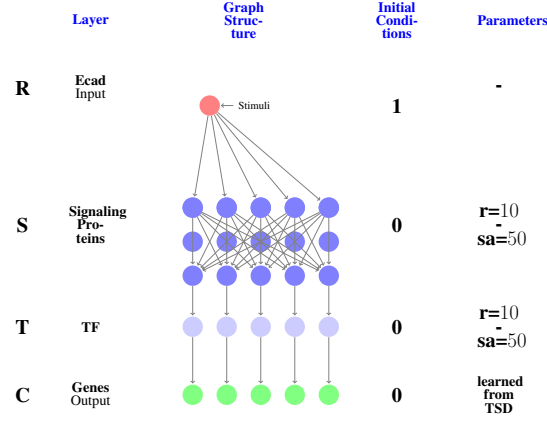


Fig. 6. RSTC network structure and initial conditions assigned to each node in the layer

- **Transcription layer: transcription factors.** In this layer, the activation/inhibition over a transcription factor (TF) comes from signaling proteins; however, for all TFs we introduced an auto-inhibition action that represents their degradation over time.
- **Genes.** The genes are activated or inhibited according to the estimated values from time-series data.

3 Results

3.1 Automatic generation of PH model from the PID network

PH models are written using the PINT³ format. PINT implements stochastic simulations and static analyses for computing dynamical properties on very large-scale PH models. For the PINT code generation we used two procedures. The first procedure detects motifs (controller and controlled components) in graphs from the Pathway Interaction Database; the second, generates the PINT code by choosing an adequate concurrency rule, based on synchronization sorts, to represent the motif dynamic in PH. With this method it is possible to convert the whole content of the PID-NCI database into a PH model, as well as individual pre-selected pathways, as is the case for the system under study. It is implemented in Java and available upon request.

3.2 Simulation of Calcium stimulated biological system

We simulated the model with and without the inclusion of the synchronization sort. In the following, we present the results of the simulation.

³ <http://process.hitting.free.fr>

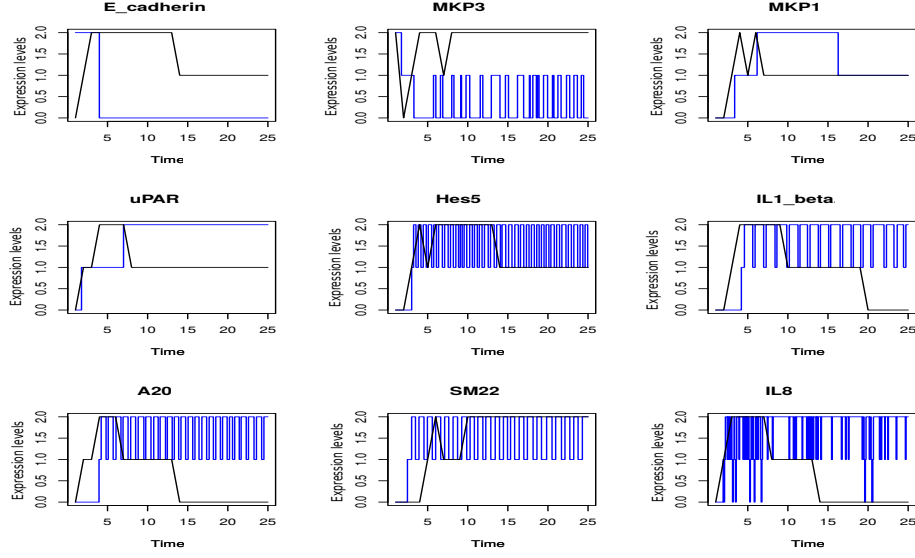


Fig. 7. Results of system simulations without introducing the synchronization sort for 9 genes. In black line we draw the traces representing the discretized time-series data. In blue lines we show the simulated traces.

Without the introduction of synchronization sort One can notice in Fig. 7 the occurrence of oscillations. This is not the expected behavior from the biological system but it is coherent with the choice of the modeling and the way the simulator works as explained in Section 2.2. In this simulation cooperation sorts were used to model multiple controllers having a common controlled (target) component. It is important to notice that the intensity of the oscillation is linked with the size of the concurrence, i.e. the number of controllers a controlled component has. Despite the presence of the oscillations, the model reproduces expected dynamical behaviours namely the dynamic of components, the signal transduction and takes into account the stochastic and time aspect of the model.

With the introduction of synchronization sort From Fig. 8 we can see that the introduction of the synchronization sort significantly reduces the impact of concurrence by the introduction of the synchronization. The result shows a clear elimination of the previously observed oscillation in Fig. 7. One can see that the expression levels of genes *uPAR*, *IL8*, *TFR* are well reproduced. Those of *IL1 beta*, *MKP3*, *MKP1*, *Hes5*, *SM22*, *ET1*, *DKK1* are also well reproduced except that they do not go down at the end while *A20* are not activated. This result can be observed when the activation signal has not been able to propagate through the network due to the non determinism and concurrency.

4 Conclusion

This work describes the steps towards the integration of time-series data in large-scale cell-based models. We proposed an automatic method to build a timed/stochastic PH model from a biological system composed of biochemical reactions, extracted automatically from public databases, relevant to keratinocyte differentiation induced by Calcium. We then proposed a method to discretize time-series gene expression data, so they can be integrated to the PH simulations and logically explained by the PH stochastic analyses. Finally we described a method to automatically estimate the temporal and stochastic parameters for the PH simulation, so this estimation process will not be biased by over fitting. Ours results show that: (1) we reproduce accurately 9 out of 12 of the genes, (2) we can model large-scale networks in which signal goes from an input node to the output nodes, (3) we can reproduce tendencies of the dynamic of components and take into account the stochastic and time aspect of the behaviors of the biological system. As concrete perspectives of this work we intend to (i) validate the RSTC network topology by confronting its *in-silico* simulation with real measurements of its components; (ii) compare the stochastic simulation results with reachability static analyses over the same PH components mapped to the 12 measured genes; and finally (iii) search for key-regulators up-stream the 12 genes which will control the dynamics of the system, to provide concrete hypotheses to test experimentally.

5 Acknowledgements

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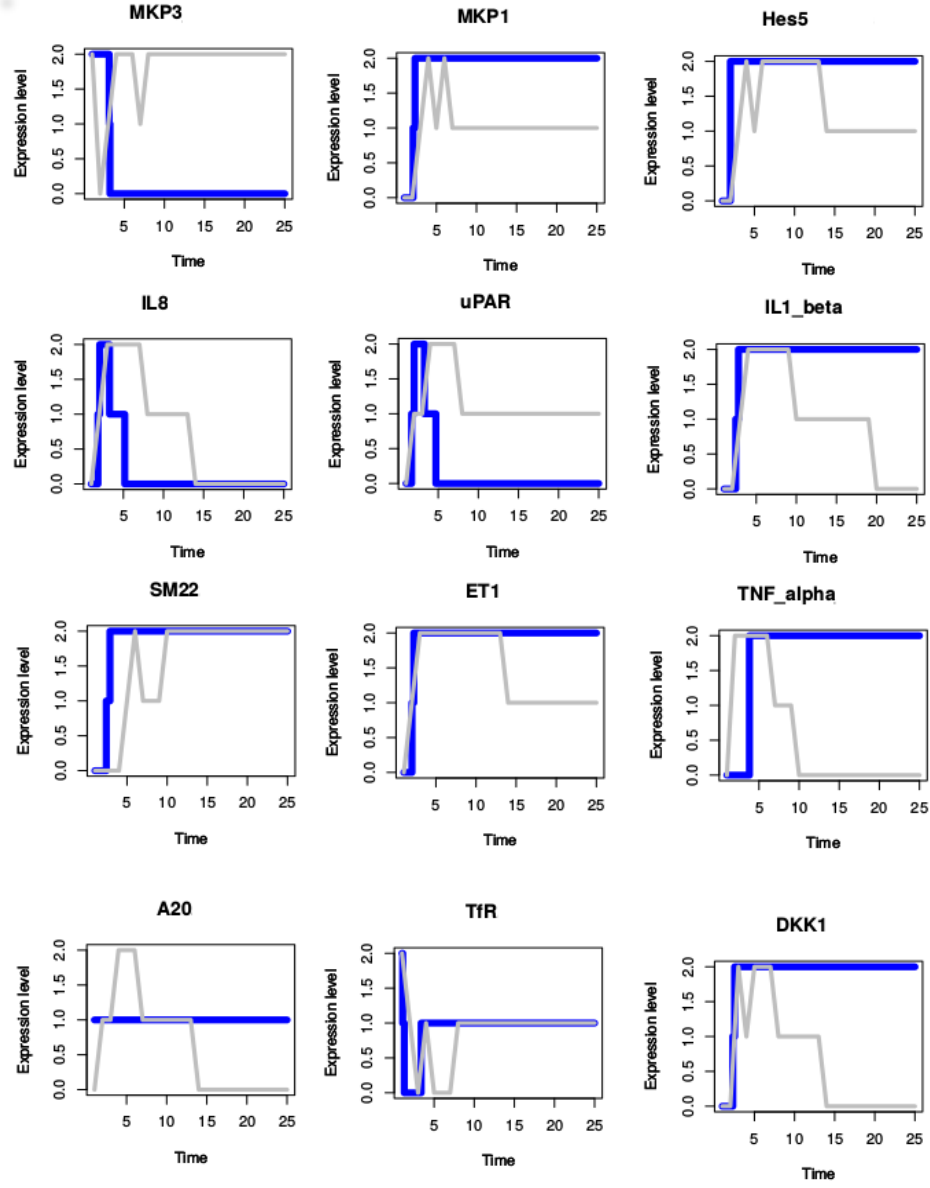


Fig. 8. Results of simulations by introducing the synchronization sort. In gray line the expected behaviors come from the discretization of time-series data. In Blue line the simulation behavior.

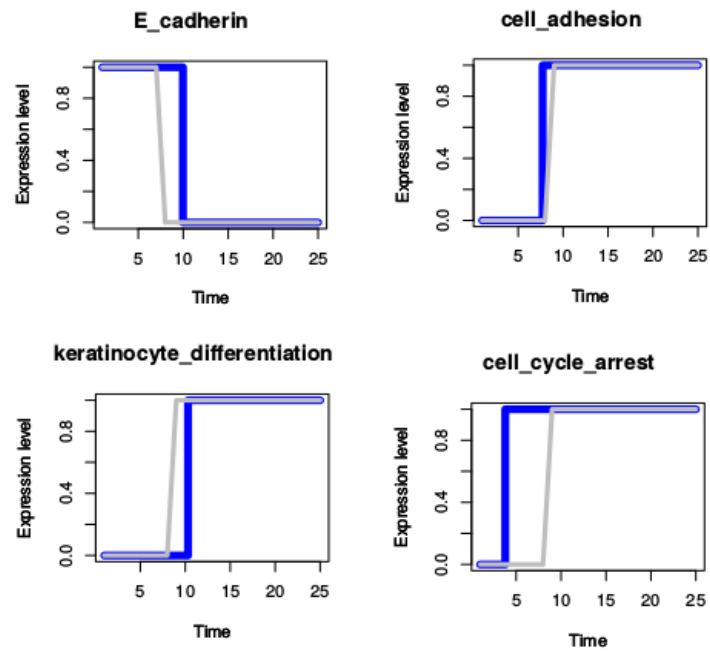


Fig. 9. Particular nodes of the biological model. In Blue line the simulation behavior