

Integrating time-series data on large-scale cell-based models: application to skin differentiation

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Abstract—In this work we propose an automatic way of generating and verifying formal models of signaling and transcriptional events, gathered in large-scale regulatory networks, by integrating temporal and stochastic aspects of the expression of some biological components, measured during a real experiment. To achieve this we rely on the Process Hitting (PH) formalism and an stochastic simulation of the built PH hybrid model. On one hand, this choice limits us to discrete data and on the other hand, allows us to handle models built upon large-scale interaction graphs, to express different levels of logic rules between a node and its direct predecessors, and to reproduce dynamic behaviors of the components with minimal stochastic parameter estimation. The model proposed is based on a real case study of keratinocyte differentiation, in which gene time-series data was generated upon Calcium stimulation.

This work has the following contributions. First, we built an interaction graph linking a signaling molecule, E-cadherin (Calcium sensitive protein), to genes present in our time-series data and to key cellular processes for our case study, such as keratinocyte-differentiation and cellular-proliferation. This graph was automatically extracted from the Pathway Interaction Database (PID) using a previously proposed method. Second, we propose an automatic transformation of selected known biological patterns present in PID in order to generate PH modules; adding necessary constraints to the PH model to avoid oscillations. Additionally, we added necessary constraints to the PH model to avoid biologically incoherent dynamical behaviors. Third, we proposed a way of estimating temporal and stochastic parameters from time-series expression data to model the measured genes. These parameters are used for the stochastic simulation of the model. Finally, we discretized the experimental data to allow the comparison with simulation results for the above mentioned case study analysis.

We show that our approach allows us to reproduce different dynamic behaviors of the components in such a large network, by stochastically simulating this hybrid model. We are able to generate, for some cases, a close match to the experimental results with very few parameter calibration.

Keywords—*Times-series data integration, large-scale biological network, stochastic and concurrent models.*

I. 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.

This work aims to propose a dynamical model of large-scale systems based on the formal integration (complete validation/invalidation) of high-throughput experimental time-series data. So far this idea has 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 sampling of the space of behaviors stochastic approaches, and therefore without guarantee on finding global optima [8]. Therefore, with this work we intend to fill the gaps between the previously cited methodologies and converge to a more realistic model of biological behavior.

For modeling and analyzing stochastic and concurrent biological systems, many formalisms have been introduced, such as stochastic Petri Nets which is a suitable approach suitable for the representation of parallel systems [9]. They have been successfully and systematically applied in many areas, and the specification 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 they do not generally lead to compact models. In addition, they do not provide results to deal with the state space explosion and are thus generally computationally expensive. Another approach is Stochastic pi-calculus introduced by [11] and used in [12] for the modeling of biological systems. Stochastic pi-calculus have a good expressivity and are well adapted for the use of compositionnal approach.

For modeling and analyzing the biological system we rely on a stochastic pi-calculus formalism which is 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 model 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. So far, this method has been successfully demonstrated only on very well-known systems and without exploiting high-throughput measures. We believe, however, that the use of high-throughput data has become unavoidable with the advent of massive, publicly available data sets in the form of well-standardized DNA microarray data and, more recently, in the form of phospho-proteomics data.

The main results of this work are: (i) automatic PH generation from a biological system composed of biochemical reactions, and extracted from public databases; (ii) discretization approach of time-series expression data, so we can reproduce these traces by using in a first attempt the PH stochastic simulation, and afterwards perform static analysis methods for reachability analyses to satisfy this data; and (iii) estimation of the temporal and stochastic parameters of the simulation, based on statistical analyses of the full-compendium of time-series expression data. The biological system used as a case-study for this work is a cell-based model of skin differentiation, which is of key importance in wound healing.

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II. DATA AND METHODS

The general workflow for integrating time-series data and model is depicted in Fig. 1 and comprises the following steps:

- **Formalization of biological model.** Building a computational hybrid model from biological network by automatic detection of known biological patterns.
- **Temporal parameters estimation.** Estimation of temporal parameters from times-series data to calibrate the model.
- **Integration of temporal parameters.**
- **Discretization of times-series data.** Discretization of time series data to compare with discrete simulation results.
- **Simulation and validation.** Compare the results of simulation with the discretized times-series data.

A. Data

1) *Interaction network:* 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, generated from the Pathway Interaction Database (PID). In order to build this network, we selected a set of seed nodes related to the biological process studied. The seed nodes for our case study were: (1) *E-cadherin*, which is a protein having *Ca* 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.

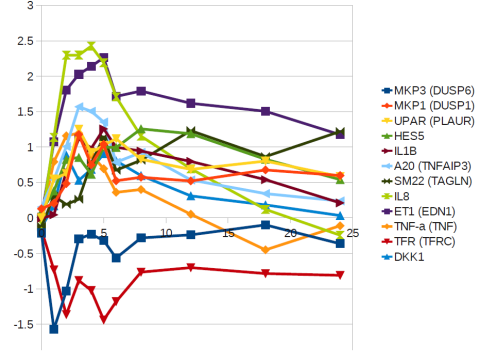


Fig. 3. Plot of 12 selected genes

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 state, biological processes and positive condition. On the other side, transient nodes can be transcriptions, translocations, modifications and compounds. Edges are of different types. We have activation (agent), inhibition, output, input and familyMemberOf.

2) *Time-series microarray data description:* To illustrate our approach, we used the time-series microarray data from Calcium stimulated keratinocyte cells measured at 10 time-points. A 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 a set of 12 of the 200 selected (see Fig. 3), because we were able to retrieve the regulatory mechanisms upstream these 12 genes from public repositories of biochemical reactions. The full dataset (data not shown) was produced by the German Cancer Research Center (DKFZ) and it's currently in the process of getting published.

B. 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.

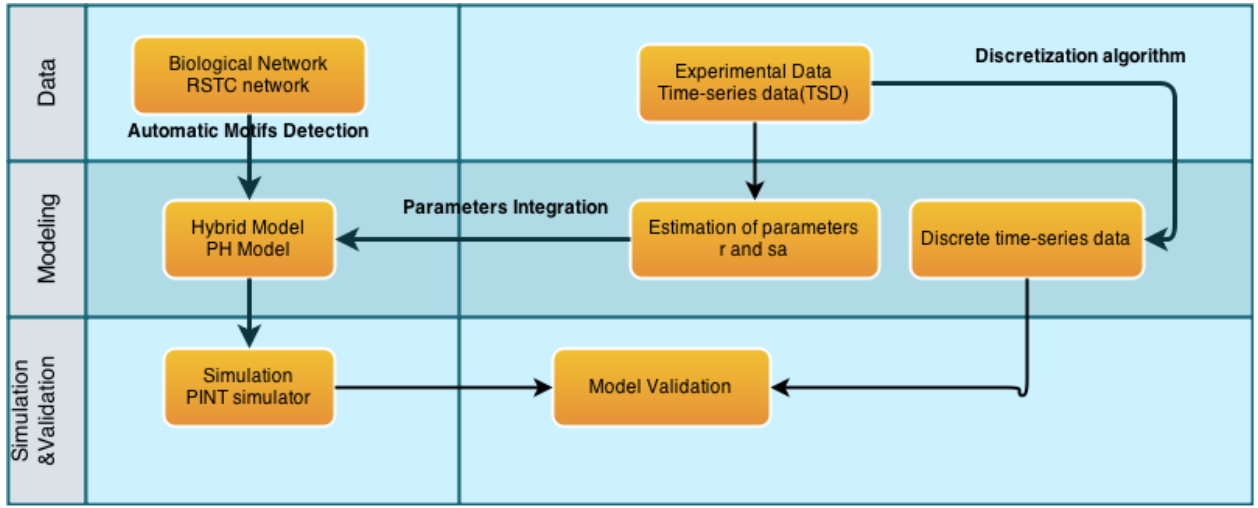


Fig. 1. Workflow for integrating stochastic and temporal informations in large scale biological networks

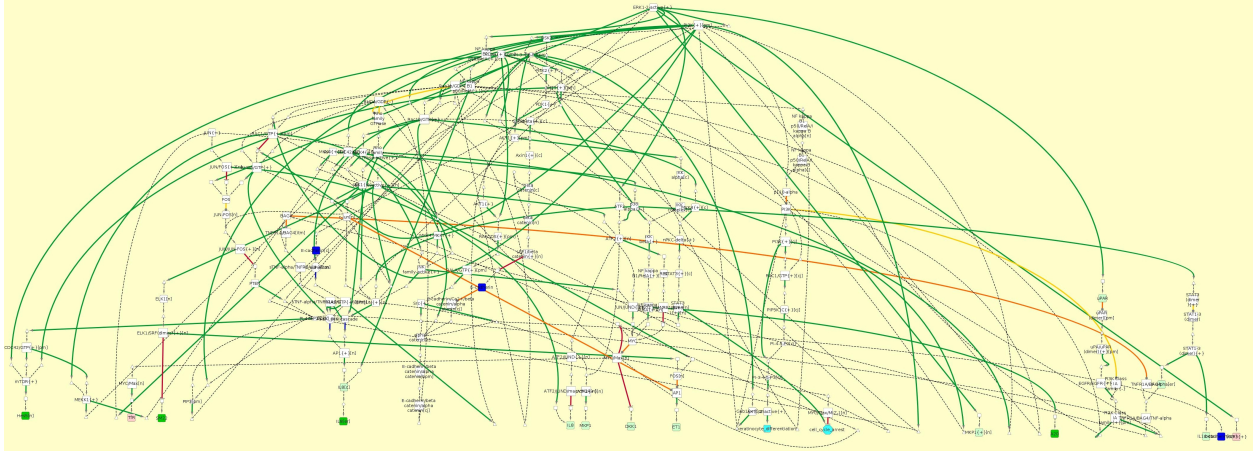


Fig. 2. RSTC network. It is the interaction graph that describe the biological case study. It is composed of 293 nodes and 375 edges (interactions). The set of nodes are composed of terminal nodes (proteins, complexes, genes, cellular state, biological processes and positive conditions) and of transient nodes (transcriptions, compound, translocations, modifications and compounds). The set of edges are composed of interactions of type activation (agent), inhibition, output, input and familyMemberOf

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 the play of the action h in s , with $(s \cdot h)[b] = b_k$ and $\forall c \in \Sigma, c \neq b, (s \cdot h)[c] = s[c]$.

1) *Modeling cooperation*: As described in [13], the cooperation between processes to make another process bounce can be expressed in PH by building a *cooperative sort*. Fig. 4

shows an example of a cooperative sort ab between sorts a and b , defined with 4 processes (one for each sub-state of the presence of processes a_1 and b_1). 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 hit 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 .

2) *Modeling synchronization*: Unlike cooperation sort which allow us to model the fact that two components cooperate to hit another component, we introduce the notion of synchronization sort, which will implement another type of cooperation. If we refer to our example Fig. 4, and assume that ab is our *synchronization sort* between sorts a and b , defined with also 4 processes. Then, component c will be activated (c_1 bounce to c_2) if each one of a and b are also activated. So

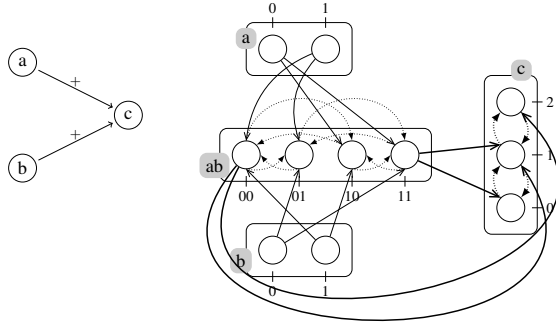


Fig. 4. (left) Biological pattern example. Nodes are components and edges are interactions. For instance, components a and b cooperate to activate c . (right) equivalent PH model. A PH example with four sorts: three components (a , b and c) and a cooperative sort (ab). Actions targeting processes of c are in thick lines.

each one of this processes ab_1 , ab_2 , ab_3 can activate c_1 . But to be deactivated, we need both a and b to be deactivated. We will see later why it is important to introduce this new rule for the cooperation between components in our real case study.

Remember that PH was designed in order to take very large system analysis into account. Indeed, algorithm were developed on such a process??? system so that it prevent for building the whole state graph which would be intractable when there are more than 10 or 20 components.

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.

C. Model construction(From RSTC to PH)

In this work, we aim to model a biological system according to his dynamic. For that, we choose to build a PH model from the biological model represented as an RSTC network.

In the following we present our automatic approach to generate a PH model from an RSTC network.

1) *Modeling the RSTC network as a PH model:* In order to model the RSTC network as a PH model we selected known biological regulatory patterns (atomic set of biological components and their interacting roles), represented as biochemical reactions in the RSTC network, and proposed their PH representation.

We build an algorithm that will automatically browse the graph node by node and detect all patterns in the graph. More precisely, for each node(output node of the pattern) we will call a recursive procedure, that will allow us to detect a minimal set

Require: Net

Ensure: return all the patterns associated to the given network Net

```

1: for all Node  $n$  in  $Net$  do
2:    $Pat = \text{detectPattern}(Net, n)$ 
3:    $\text{patternInPHModel}(out, Pat)$ 
4: end for

```

Fig. 5. Pattern detection in an RSTC Network

TABLE I. EXAMPLES OF PATTERNS

| Biological Patterns | PH Transformations |
|------------------------------|--------------------|
| Simple activation | |
| Simple inhibition | |
| activation or inhibition | |

of node(input node of the pattern) that has a direct influence on that node. This set of node plus the output node and the way there are linked are a pattern for us. The type of a pattern is determined by the type of the output node, the type of regulations come on that node and the type of input nodes of the pattern. So the algorithm that detect patterns return the pattern and its type to another procedure which one will traduce the pattern into the process hitting formalism. This transformation will take care of different case(cooperation, synchronization, simple activation, simple inhibition,...)

For example a molecule a that cooperates with a molecule b to activate a molecule c Fig. 4 (top), 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 (bottom) a , b , c and ab . Sorts a , b and c represent components a , b and c . We introduce the cooperative sort ab to characterize constraints on components a and b . In our RSTC network, we found 11(to be precise...) regulatory patterns (see Appendix I).

2) *Estimating the parameters for the PH-simulation from time-series gene expression data:* The simulation of the execution of the PH actions is done stochastically. Therefore, we need to relate each action with temporal and stochastic parameters, introduced into the PH framework to achieve dynamic refinement [13]. This is an important aspect of the modeling when taking into account the temporal and stochastic dimensions of biological reactions by performing simulations. On the one hand, we consider the probability of a reaction to occur, and on the other hand, we consider stochastic parameters

Require: Net, n

Ensure: Detect a pattern associated to a given node (n in this case)

```

1:
2:
3: switch ( $n$ )
4: case TerminalNode:
5:   added node to the pattern
6:   numberPredecessor=  $n$ .getNumberOfPredecessor() {To
   get the number of predecessor of node  $n$ }
7:   switch (numberPredecessor)
8:   case 1:
9:     for all in coming edge ( $n-m$ ) do
10:      get the node  $m$ 
11:      switch ( $m$ )
12:      case TerminalNode:
13:        added node to the pattern  $Pat$ 
14:      case TransientNode:
15:        detectPattern( $Net, m$ );
16:      end switch
17:    end for
18:    Set the code of pattern  $Pat$ 
19:    return  $Pat$ 
20:  end switch
21: case TransientNode:
22:   numberPredecessor=  $n$ .getNumberOfPredecessor() {To
   get the number of predecessor of node  $n$ }
23:   switch (numberPredecessor)
24:   case 1:
25:     for all in coming edge ( $n-m$ ) do
26:      get the node  $m$ 
27:      switch ( $m$ )
28:      case TerminalNode:
29:        added node to the pattern  $Pat$ 
30:      case TransientNode:
31:        detectPattern( $Net, m$ );
32:      end switch
33:    end for
34:    Set the code of pattern  $Pat$ 
35:    return  $Pat$ 
36:  end switch
37: end switch

```

Fig. 6. Detection of Pattern associated to the node n

in the aim at observing an expected behavior. In the PH framework, to play an action we need two essential parameters: the rate r or the temporal parameter because $t = r^{-1}$ if we assume that t is the average time of playing an action and the stochasticity absorption sa . These two parameters will be estimated according to the expression profile of time-series data of the experiment described in Section II-A2.

3) *From data to action parameters:* 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. For others, we assign default parameters. In order to estimate r and sa we ...

4) *Discretization of times-series data:* Because PH simulation is discrete we need to discretize continuous experimental data so we can compare our simulation outputs. The goal of

Require: out, Pat { Pat is The pattern to be translate into the PH Model, out is the output file }

Ensure: The correspondent PH Model of the given pattern Pat will write into the file out

```

1:  $nocp = Pat$ .getNumberOfComponents() {Number of the
   components of the pattern  $Pat$ }
2:
3: switch ( $nocp$ )
4: case 2:
5:   switch ( $type$ )
6:   case A:
7:     out.write("activation")
8:     out.write("")
9:   case I:
10:    out.write("inhibition")
11:
12:  default:
13:    out.write("unknown pattern")
14:  end switch
15: case 3:
16:  switch ( $type$ )
17:  case C:
18:    out.write("cooperation")
19:  case S:
20:    out.write("synchronization")
21:  default:
22:    out.write("unknown pattern")
23:  end switch
24: end switch

```

Fig. 7. Pattern in PH Model

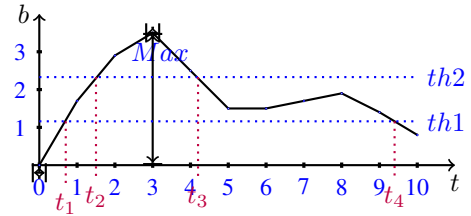


Fig. 8. Illustration of estimation of temporal parameters: $r_i = \frac{1}{t_i - t_{i-1}}$. Max represents the maximum expression. Min represents the minimum expression. In this example is 0 the minimum level. $th1$ and $th2$ represent respectively the first and the second threshold.

this method was to better determine, according to the gene expression level, when a given molecule is activated or inhibited. To do this, we introduced the new analog concept of Significant Increase or Decrease to characterize the fact that a level of a molecule increases or decreases when crossing a threshold of significance; we limited the possible expression levels for a molecule to $\{0, 1, 2\}$. We made this choice because when we look our time-series data (see Fig. 3), we can clearly observe a high level of activity between $0h$ and $5h$ and a slow level of activity between $5h$ and $10h$ on the other hand. The goal of the discretization method is to capture these two activation times. For each time-series, we introduce two thresholds $th1$ and $th2$ as in Fig. 8. The value of this two thresholds is compute as follows $th1 = \frac{1}{3}(MaxLevel - MinLevel)$ and $th2 = \frac{2}{3}(MaxLevel - MinLevel)$. Therefore, all of the expression level in the range $[0 - th1]$ will have level 0, all

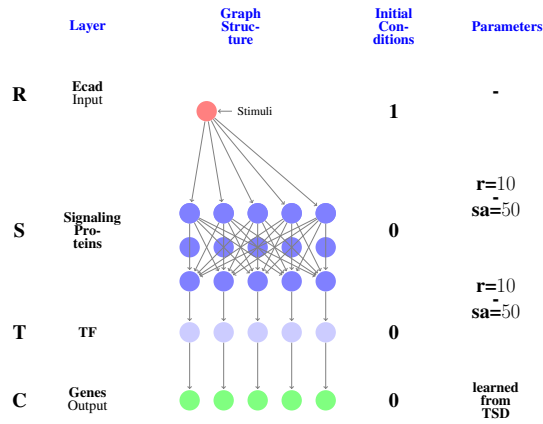


Fig. 10. RSTC network structure and initial conditions assigned to each node in the layer

those in the range $[th1 - th2]$ will have level 1 and the last group will have level 2. In this way we can automatically determine the different levels of expression of the TSD. To illustrate the result of our discretization algorithm we plot in Fig. 9 the expression of 9 selected genes from the times-series data with their respective discrete plots.

D. Simulation: Initials conditions

To run the simulation we need to set initial conditions for the components of our network. Because the components of the network are grouped into layers, the initial conditions will be the same in the different layers. In the following we will present the initial conditions that we have chosen for the different components.

- **Receptor layer: E-cadherin.** We choose the pulse signal for the input node E-cadherin, that will be active for a duration of average x units times. We made this choice to take into account the average time of calcium stimuli effect.
- **Signaling layer: signaling proteins.** At this layer, the components will be activated and inhibited according to the same rate and the same stochasticity absorption factor. The values of these parameters were selected by considering the time of signal transduction from the entry node (E-cadherin) to the output node (genes). [imposing reachability constraints from entry node Ecad].
- **Transcription layer: transcription factors.** At this stage, the signal will come from signaling proteins for activation. But for the inhibition, in addition of the signal come from signaling proteins, we introduce an auto-inhibition which will be represented a degradation of the transcription factor.
- **Cell-state: genes and cellular process.** The genes will be activated or inhibited according to the estimated values from time-series data.

We summarize the initial conditions of our simulation in Fig. 10 where we can clearly see the initial values chosen at each layer for our model.

III. RESULTS

A. From PID to PH: On automatic generation of PH code of the network

To simulate of the model, we generated a PINT code to be simulated by the PINT simulator¹. For the PINT code generation we use two procedures as describe in the method section. The first procedure that detect the patterns and his code. Then the second procedure that generate the PINT code by taking in consideration the type of the pattern to better refine the dynamic of the system on a structural point of view. This refinement is done by introducing the synchronization sort which is a generalization of the cooperation sort. This allow to avoid artificial oscillation in the dynamic of the components of the system.

B. Simulation

We simulated the model with and without the inclusion of the synchronization sort.

- We can modeled large scale network in which signal goes from an input node to the output nodes.
- We can reproduce tendencies of the dynamic of components as illustrate in Fig. 11 and Fig. 12.
- The model take in account the stochastic and time aspect of the behaviours of the biological system.

1) *Without the introduction of synchronization sort:* One can easily notice in Fig. 11 the occurrence of oscillations. This is not the expected behavior from the biological system but it is coherent with the choice of modelisation: cooperation sorts were used to model multiple regulators of a common target. It is important to notice that the intensity of the oscillation is linked with the size of the concurrence, i.e. the number of predecessors that a node in the network has. Despite the presence of the oscillations, the model reproduces expected dynamical behaviours namely the dynamic of components, the signal transduction and the taking into account the stochastic and time aspect of the model.

2) *With the introduction of synchronization sort:* From Fig. 12 we can see that the introduction of the synchronization sort significantly reduces the impact of concurrence by the introduction of the synchronization and the cooperation sorts. The result shows a clear elimination of the previously observed oscillation Fig. 11.

IV. 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 stochastic pi-calculus PH model from a biological system composed of biochemical reactions, extracted automatically from public databases, relevant to keratinocyte stimulation induced by Calcium. We then proposed a method to discretize time-series gene expression data, so they can be confronted to the PH simulations and logically explained by the PH static analyses. Finally we described a method to automatically estimate the temporal

¹ Available at <http://process.hitting.free.fr>

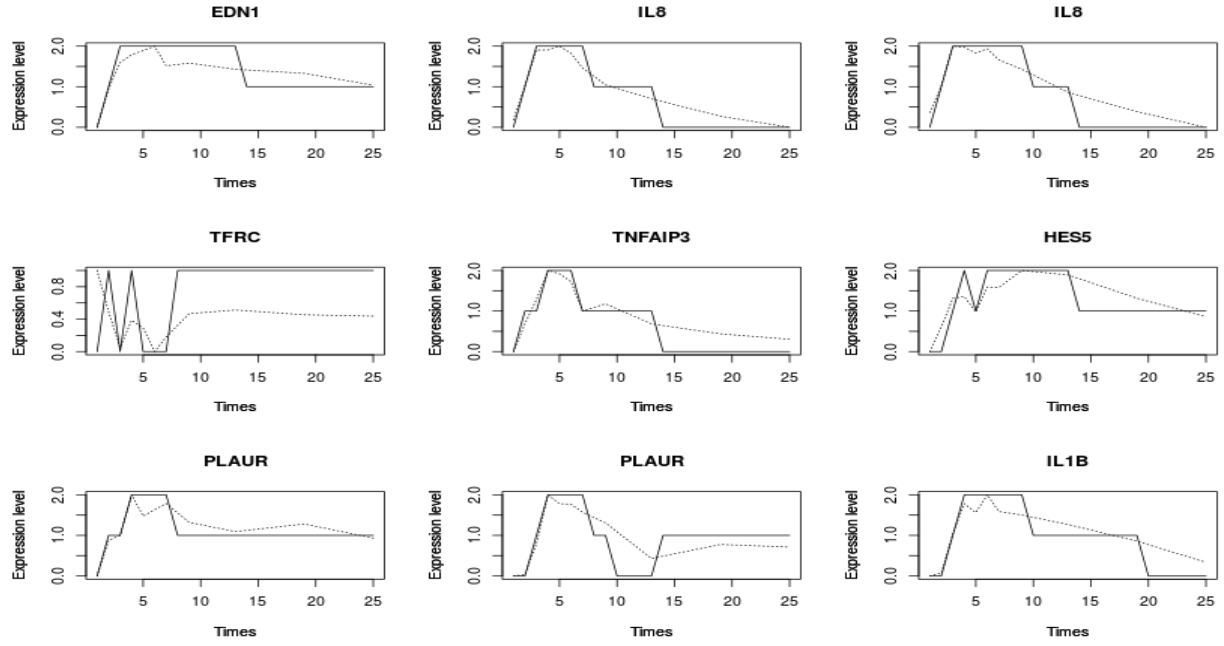


Fig. 9. Illustration of discretization of experimental data. Continuous lines are discrete data, dashed lines are continuous data

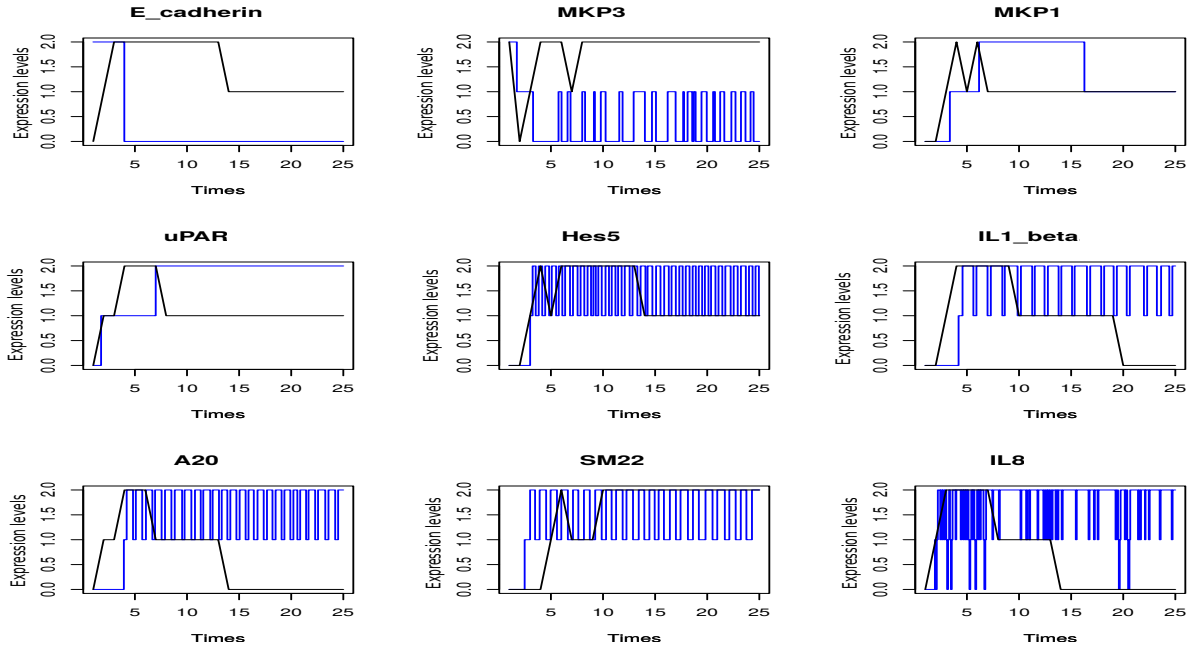


Fig. 11. Results of simulations without introducing the synchronization sort. In black line the expected behaviours come from the discretization of time-series data. In Blue line the simulation behavior.

and stochastic parameters for the PH simulation, so this estimation process will not be biased by over fitting. 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 analysis 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 our biological partners concrete hypotheses to test experimentally.

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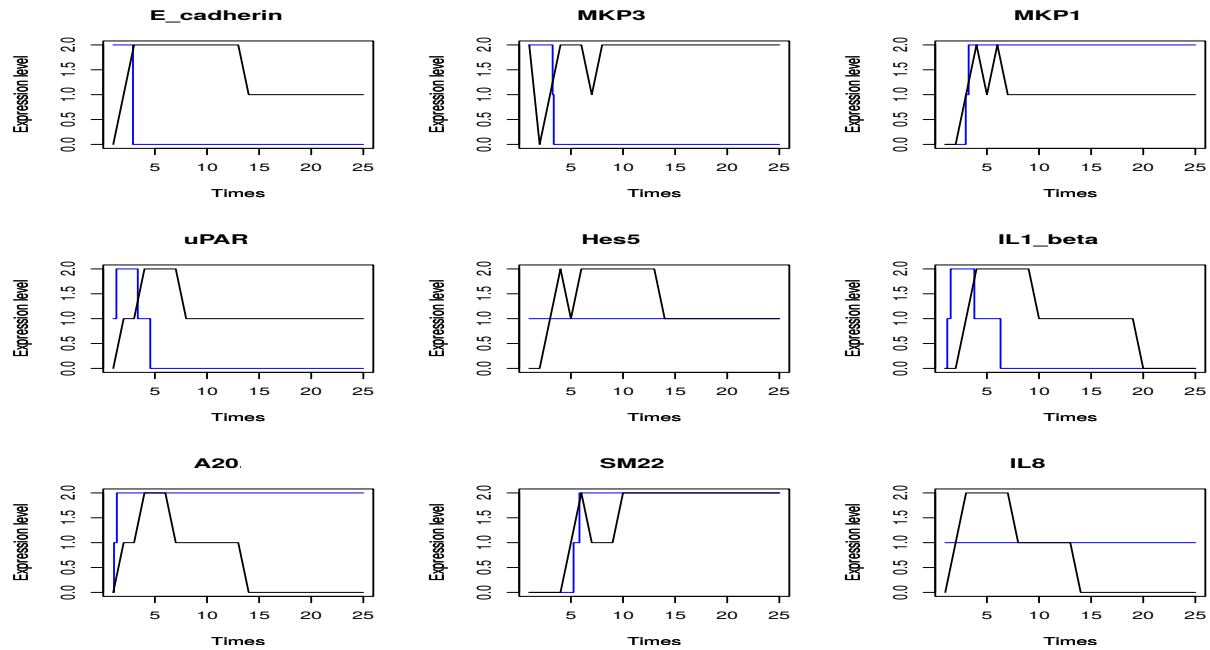


Fig. 12. Results of simulation by introducing the synchronization sort. In black line the expected behaviours come from the discretization of time-series data. In blue line the simulation behavior.

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