A SystemC-based Platform for Assertion-based Verification and Mutation Analysis in Systems Biology

Daniele Coati, Rosario Distefano, Nicola Bombieri, Franco Fummi
Dept. Computer Science
University of Verona - Italy
Email: name.surname@univr.it

Michela Mirenda, Carlo Laudanna
Dept. Pathology and Diagnostics
University of Verona - Italy
Email: name.surname@univr.it

Rosalba Giugno
Dept. Clinical and Molecular Biomedicine
University of Catania - Italy
Email: giugno@dmi.unict.it

***Abstract*—Boolean models are gaining an increasing interest for reproducing dynamic behaviours, understanding processes, and predicting emerging properties of cellular signalling networks through in-silico experiments. They are emerging as a valid alternative to the quantitative approaches (i.e., based on ordinary differential equations) for exploratory modelling when little is known about reaction kinetics or equilibrium constants in the context of gene expression or signalling. Even though several approaches and software have been recently proposed for logic modelling of biological systems, they are limited to specific modelling contexts and they lack of automation in analysing biological properties such as complex attractors, molecule vulnerability, dose response. This paper presents a design and verification platform based on SystemC that applies methodologies and tools well established in the electronic-design automation (EDA) field such as assertion-based verification (ABV) and mutation analysis, which allow complex attractors (i.e., protein oscillations) and robustness/sensitivity of the signalling networks to be simulated and analysed. The paper reports the results obtained by applying such verification techniques for the analysis of the intracellular signalling network controlling integrin activation mediating leukocyte recruitment from the blood into the tissues.**

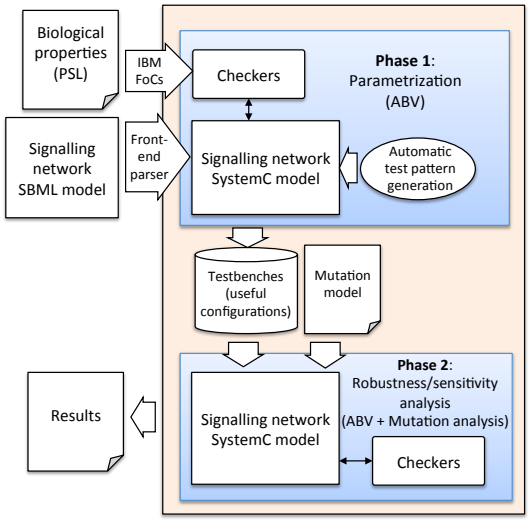

Fig. 1. Overview of the proposed platform

I. Introduction

A central goal of Systems Biology is the construction of models for reproducing dynamic behaviours and predicting emerging properties of bio-molecular networks. In this context, modelling approaches can be classified into two categories: *quantitative* and *qualitative* models. Quantitative modelling allows for a natural representation of molecular and gene networks and provides the most precise prediction. Nevertheless, the lack of kinetic data (and of quantitative data in general) hampers its use in many situations [1].

In contrast, qualitative models simplifies the biological reality and are often able to reproduce the system behaviour. They cannot describe actual concentration levels nor realistic time scales. As a consequence, they cannot be used to explain and predict the outcome of biological experiments that yield quantitative data. However, given a biological network consisting of input (e.g., receptors), intermediate, and output (e.g., transcription factors) signals, they allow studying the input-output relationships through discrete simulations [2].

Even though different qualitative approaches have been successfully used to extrapolate insights of medium-large signalling networks, they have shown having two main limitations: (i) they are limited to specific modelling context, and (ii) they lack of automation in analysing biological properties such as complex attractors, molecule vulnerability, and dose response [1].

This paper presents a platform for modelling and simulating signalling networks (see Figure 1). The platform is based on SystemC, which is the de-facto reference standard language for system-level modelling and simulation of Hardware/Software/Network electronic systems. The platform provides a front-end parser to import signalling network models from Systems Biology Markup Language (SBML) [3], which is the de-facto reference representation format for communicating and storing computational models of biological processes. The platform relies on assertion-based verification (ABV), by which the biological properties to observe can be formally defined through the property specification language (PSL) and, then, automatically synthesized and integrated as checkers into the simulation system (phase 1 in Figure 1). An automatic test pattern generator allows exploring the solution

space of the network parameters to identify which network configurations lead the model to satisfy the properties (i.e., parametrization). Such *useful* configurations are then used in the second phase, in which mutation analysis is applied to verify the robustness/sensitivity properties of the network.

The platform has been applied for modelling and analysing the signalling network controlling LFA-1 beta2 integrin activation mediating leukocyte recruitment from the blood into the tissues [4]. In particular, simulation has been conducted to understand how the concerted action of the signalling proteins generate a concurrent modular mechanism of regulation of integrin activation, which is characterized by dynamic properties such as oscillations and hysteresis.

The paper is organized as follows. Section II presents the related work. Section III introduces the case study, which will be used as a model system in the subsequent sections. Section IV presents the platform. Section V reports the experimental results, while Section VI is devoted to the concluding remarks.

II. Related work

Boolean networks have been successfully applied for modeling gene regulatory and signaling networks in several different biological systems [5], [6], [7]. They have also been used to analyse human signalling networks associated with deseases to predict pathogenesis mechanisms and potential therapeutic targets [8], [9].

Many Software tools are available for Boolean dynamic modeling of biological systems, such as *BooleanNet* [10], *Boolnet* [11], *SimBoolNet* [12], and *ChemChains* [13]. Several software packages also support multi-valued logical dynamic modeling, such as *GINSim* [14], and *ADAM* [15]. In addition to logic operation-based Boolean networks, threshold Boolean networks have been used in modeling biological networks at both cellular and population levels [16], [17]. Piecewise linear models are a hybrid of Boolean models and differential equation-based continuous models, and have been successfully applied due to their attractive combination of continuous time, quantitative information and few kinetic parameters [18], [19]. The methodologies and modeling approaches described above apply to threshold Boolean models and piecewise linear models as well. In particular, one can apply the software packages *BooleanNet* for qualitative modelling of biological networks on piecewise linear models.

Recently, some approaches have been proposed to derive logic-based ordinary differential equations by multivariate polynomial interpolation. Some implemented examples are *SQUAD* [20] and *Odefy* [21]. They transform Boolean models into systems of continuous differential equations. The dynamic descriptions are derived automatically from the Boolean ones without adding any further knowledge. As a consequence, the resulting models, although being able to be fitted against experimental data, must be considered as phenomenological models in contrast to mechanistic (kinetic) models that require more detailed information on the kinetics and parameters of the involved processes.

Differently from all these approaches, the proposed platform relies on modeling, verification, and fault injection techniques extensively applied and optimized in the EDA context. In particular, it relies on SystemC, which provides an event-driven simulation and allows handling temporal characteristics of the network proteins like delay times and lifetimes [22]. The platform also aims at automating most of the modeling and simulation steps, thanks to EDA methodologies and tools for ABV and mutation analysis. Finally, differently from all qualitative or semi-quantitative modelling approaches based on boolean models in literature, the proposed platform allows reproducing and observing oscillating behaviours even when neither negative nor positive feedbacks appear in the network [23].

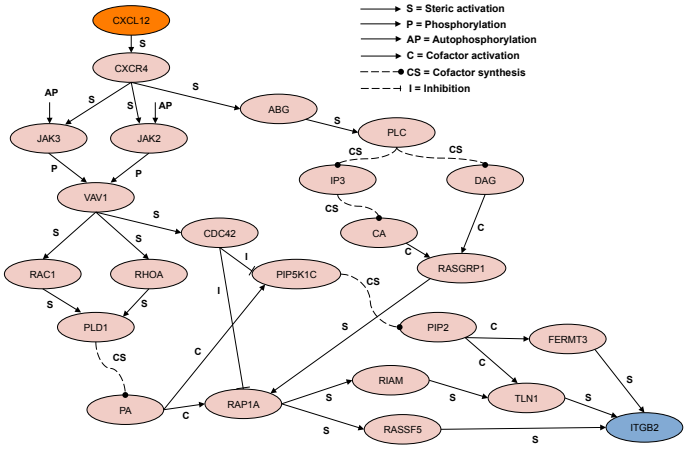

Fig. 2. The signalling network of the leukocite integrin activation

III. The case study

In order to better explain how ABV and mutation analysis are applied for the property analysis of signaling networks, we first present the case study, which will be used as a model system in the subsequent sections.

The case study is the signalling network controlling LFA-1 beta2 integrin activation mediating leukocyte recruitment from the blood into the tissues. The mechanism of leukocyte recruitment is a fundamental homeostatic process of the immune response. It is modeled as a concurrent ensemble of cellular events consisting of a stereotyped sequence of leukocyte behaviors on the vascular endothelium and including tethering, rolling, integrin activation, arrest and diapedesis [24]. In this context, a critical event is integrin activation since it mediates cell arrest underflow and diapedesis. Integrin (ITGB2) activation is controlled by a complex signal transduction network (see Fig. 2), mainly generated by chemotactic factors, and involving different intracellular molecules, with a particularly important role for JAK protein tyrosine kinases, RHO and RAP small GTPases, lipid kinases and a number of cytoskeletal proteins [25].

Notably, cell motility requires an on-off kinetic of integrin activation, leading to iterative adhesion-de-adhesion events and, thus, ensuring cell migration. Controlling dynamics of the cell adhesion is crucial to control cell migration. Such a on-off, oscillatory, kinetics of integrin triggering likely depends

on on-off kinetics of the signalling transduction machinery triggered by chemokines and controlling integrin-mediated cell adhesion.

Although a qualitative characterization of such a complex mechanisms is, at least partially, available, a quantitative description is lacking, thus limiting the possibility of applying any quantitative kinetic modelling approach in literature. In addition, since neither negative nor positive feedbacks appear in the network, any qualitative or semi-quantitative modelling approach based on boolean models in literature do not allow reproducing and observing oscillating behaviors [23].

IV. The SystemC-based platform

In Systems Biology, a signalling network consists of a set of biological elements, such as, *proteins* or *co-factors*. The elements behave as concurrent objects and interact each other through activation or inhibition actions to form signal transduction chains. An element can be activated (or inhibited) by an *upstream element*, and it can activate (or inhibit) a *downstream element*.

A. Modelling and implementation of biological elements

The proposed modelling approach relies on two concepts: Boolean modelling and finite state machines (FSMs). FSMs allow us to formally represent the boolean model of each protein behaviour in terms of states (e.g., inactive, active), transitions between states, and guard conditions (i.e., boolean conditions).

Figure 3(a) depicts the proposed FSM template, while Figure 3(b) shows, as an example, the VAV1 protein model of the case study in Figure 2. Each protein changes state (i.e., a transition occurs) when the guard condition is evaluated to be true. The condition may be set on a particular reaction *event* (e.g., activation via phosphorylation, steric, auto-phosphorylation, cofactor or inhibition via phosphatase) generated by any upstream protein or on any environment status. As an example, VAV1 moves from *Inactive* to *Active* (which represents the activation through phosphorilation) as soon as either JAK3 or JAK2 activates VAV1 (see Figure 2). Such a transition occurs after a *delay time*, which represents the time spent by a JAK protein to encounter the molecules of VAV1 and to carry out the activation through a phosphorilation process. Once activated, VAV1 seeks for the steric activation of its own protein targets (RAC1, RHOA, CDC42). t represents the time elapsed, which is constantly updated during simulation, while *lifetime* represents the maximum time from the activation instant in which the protein carries out its biological function. VAV1 continues to seek for steric activation of target proteins as long as it is bounded with the upstream protein compound (i.e., CXCR4+JAK) and the lifetime has not expired.

The template includes two sets of input data that can affect the protein behaviour and one set of generated output:

- *Upstream inputs (U_S)*: They are inputs whose values are generated during simulation and depend on the topological interaction of the modelled protein with upstream proteins. Some examples are the activation via phosphorylation, steric, cofactor, or inhibition.

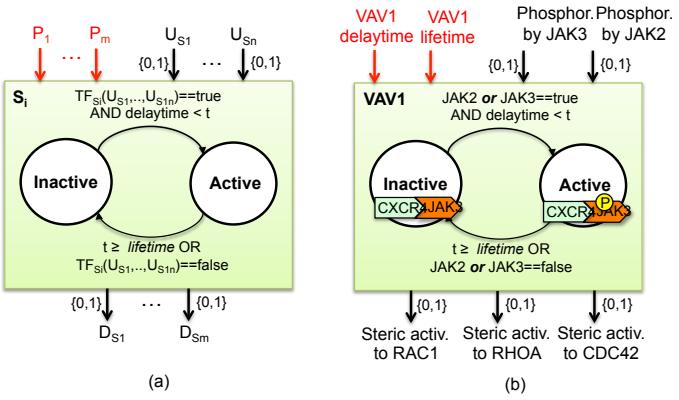

Fig. 3. The network protein modelling through Finite State Machines. The protein template (a), the VAV1 protein example (c), and the proposed mutation model applied for mutatoin analysis (c)

- *Parameters (P)*: They are inputs whose values depends on the environment characteristics and status, which are unknown at modelling time. Some examples are the *delay time* (i.e., time spent by the protein to encounter a protein target), the protein *lifetime*, the molecular concentrations of the downstream proteins (which affect the delay time), the initial state of the protein, etc. For each parameter, the simulation platform generates different values with the aim of observing how such values affect the system dynamics (i.e., *parametrization*).
- *Downstream outputs (D_S)*: They are outputs whose values are calculated at simulation time and depend on the role of the protein towards downstream proteins (e.g., the ouputs of the VAV1 module are set to true when VAV1 start seeking for RAC1, RHOA, or CDC42 proteins) .

Each protein is implemented through a SystemC *module*, with both the topological and parameters inputs and outputs as SystemC *ports*. The protein behaviour represented by FSM in Figure 3 is implemented through a SystemC *process*, which is sensitive to any *event* on the input signals. An activation/inhibition from an upstream protein is represented by an input (boolean) signal set to *true*. Being event-driven, the process *wakes up* and updates both the internal state and the output signals whenever a new event on inputs occurs. Each network node is implemented as a SystemC module through processes. The element modules are finally connected and simulated at system level.

The proposed FSM model, which is shared by each network element, allows the corresponding SystemC implementation to be automatically generated from a SBML description (see Figure 1). SBML is a representation format, based on XML, for communicating and storing computational models of biological processes. It is a free and open standard with widespread software support and a community of users and developers. SBML can represent many different classes of biological phenomena, including metabolic networks, cell signaling pathways, regulatory networks, infectious diseases, and many others. Since SBML is the de-facto a reference standard for representing computational models in systems biology

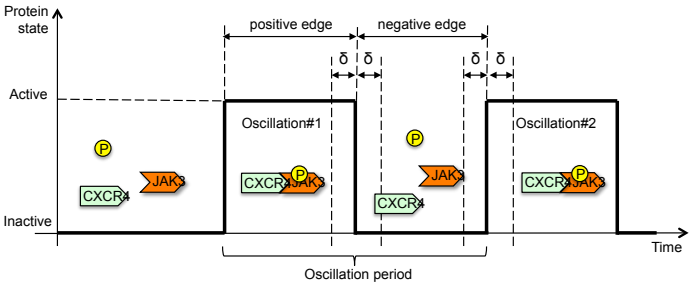

Fig. 4. The state periodic oscillation of VAV1 as example of complex attractor.

today, the proposed platform includes a front-end parser for the automatic SBML-SystemC translation.

B. Phase 1: Network Parametrization through Assertion-based Verification

In EDA, functional verification based on assertions represents one of the main applied and investigated techniques that combines dynamic and static verification [26]. Assertions are a formal descriptions of what behaviour is expected during the model simulation and allow detecting bugs as well as driving the test pattern generation [27].

The proposed platform applies simulation-based ABV, by which assertions are defined in PSL, automatically synthesized into *checkers* through the IBM FoCs synthesizer [28], and plugged to the SystemC model as in [26]. The checkers monitor observable signals of the model under verification during simulation and raise a failure signal when a failure is found. In the context of signalling networks, they aim at monitoring the protein states, whose temporal activity is a key to understand crucial biological properties such as steady states (simple attractors) and oscillations (complex attractors) [2].

Figure 4 shows an example of state dynamics of a protein to be observed and for which an assertion should be defined. The assertion that describes a periodic oscillations activity (*positive edge* and *negative edge* are constant in every Active and Inactive state, respectively, at each oscillation) and that considers a percentage of natural tolerance (δ) in such a periodicity is defined as follows:

```
define pos_edge:=(ta-delta) <= t &
       t <= (ta+delta);

define neg_edge:=(ti-delta)<= t &
       t <= (ti+delta);


assert (
G((state=ACT)->X((state=INACT)&(pos_edge=true)))
&
G((state=INACT)->X((state=ACT)&(neg_edge=true)))
);
```

where *ta* and *ti* are temporal counters initialized at the first oscillation, and that hold the time elapsed from the first state transition ($Inactive \rightarrow Active$ and $Active \rightarrow Inctive$, respectively). They are used to measure the positive edge and negative edge values, respectively. t is the counter set, from the second oscillation on, at each state transition, and it is used to measure the period of subsequent oscillations to be compared with the first ones.

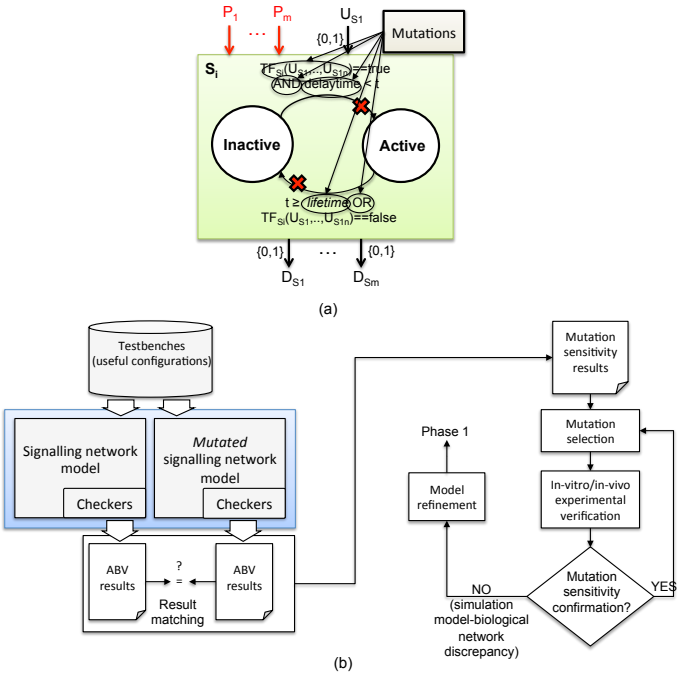

Fig. 5. The robustness/sensitivity analysis flow (a) and some example of FSM mutations for reproducing real protein mutations.

In the proposed platform, ABV is applied for the *parametrization phase* (see Figure 1), which aims at identifying the parameter settings (see Section IV-A) that lead the network to satisfy the property described in the assertions. The protein network is connected to an *automatic test pattern generator (ATPG)*, which generates the parameter values of each protein. The set of all parameter values of every proteins represents a *configuration*. The ATPG generates a configuration and runs (i.e., executes) a dynamic simulation of the network behaviour for such a set of input values for a given simulation time. Then, the ATPG generates a new different configuration for a new simulation. The run ends when all the possible configurations have been simulated and, as a result, it generates a set of *useful configurations*, i.e., all and only configurations that lead the network to satisfy the given properties.

The useful configurations are then applied in the analysis of the network robustness and sensitivity, as described in the following section.

C. Robustness/Sensitivity Analysis through ABV and Mutation Analysis

In Systems Biology, *robustness* and *sensitivity* analysis is a systematic evaluation of the network response if the model is confronted with *failures*. Such an analysis goal is twofold: First, it aims at understanding the network behaviours, complexity, and its reaction to internal/external failures; Second, it aims at validating the simulation model against in-vitro/in-vivo experimental results.

In general, robustness represents the persistence of a system characteristic behaviour under perturbations or conditions of uncertainty. Biological systems robustness (such as stability) encompasses a relative not an absolute property since no system can maintain stability for all its functions when encountering any kind of perturbation. In relatively simple systems, like for instance that studied in this work, robustness is often equivalent to a dynamical regime. Investigations of genetic oscillators thus focus on the persistence of a regular periodic solution, which does not preclude quantitative changes (in period or amplitude of the oscillations) to occur [29].

In this the proposed platform we specify (i) which characteristic behaviour or property should remain unchanged under perturbation

Mutated protein	Aperiodic config.	Useful conf. (periodic)	Oscill. (#)	Period ms
Golden model	-	2,008,188	2	50(±20%)
CXCR4	0	0	0	0
JAK3	2,008,188	0	2-4	-
JAK2	2,008,188	0	2-4	-
ABG	0	2,008,188	2	50(±20%)
VAV1	0	0	0	0
PLC	0	2,008,188	2	50(±20%)
RAC1	860,652	1,147,536	1-3	50(±20%)
RHOA	860,652	1,147,536	1-3	50(±20%)
CDC42	2,008,188	0	2-4	-
IP3	0	2,008,188	2	50(±20%)
DAG	17,640	1,990,548	1-2	50(±20%)
PLD1	0	0	0	0
PIP5K1C	0	0	0	0
CA	0	2,008,188	2	50(±20%)
RASGRP1	0	2,008,188	2	50(±20%)
PA	0	0	0	0
RAP1A	0	2,008,188	2	50(±20%)
PIP2	0	0	0	0
RIAM	0	2,008,188	2	50(±20%)
RASSF5	0	2,008,188	2	50(±20%)
TLN1	792,036	1,216,152	2-3	50(±20%)
FERMT3	952,140	1,056,048	2-4	50(±20%)

TABLE I
EXPERIMENTAL RESULTS

through ABV, and ii) for which type of perturbations this invariance property holds through a mutation model.

Figure 5(a) shows an overview of the proposed mutation model, which relies on fault injection in the FSM model [30] of the network proteins. Each fault represents the real effects (mutations) of the protein behaviour due to natural diseases, such as gene transcription alterations, cellular environmental disturbs, etc. For the sake of clarity, the figure shows how the mutation model implements only some well known protein alterations (i.e., total inactivity, alternation in the transcription function, variations in delay time and lifetime). The extension of such a model and a complete matching of natural diseases, protein mutations, and FSM mutations is part of our current and future work.

Figure 5(b) shows an overview of the robustness/sensitivity analysis flow implemented in the proposed platform. Given the useful configurations generated in phase 1 and the properties to observe, the platform simulates such stimuli on the network golden model (mutation free) and on the mutated model, in which one mutation is activated at a time. The ABV results are then matched to classify which mutation has generated the highest result divergence with respect to the golden model. The divergence is measured by comparing the number of configurations that still lead to oscillations and how much the number of such oscillations is preserved. Such a ranking (mutation sensitivity results in Figure 5(b)) is analysed to select, among the most sensitive mutation, which one to reproduce experimentally (in-vitro/in-vivo). If such a sensitivity is experimentally confirmed, the flow iterates to confirm any other mutation, while a non confirmation means that the starting model does not fully represent the signalling network and has to be refined.

The first part of the flow (left-most side of Figure 5(b)) is automatic (an extended version of [31] allows for automatic mutation injection in the SystemC code, while an adapted version of [32] allows for automatic ABV and generation of mutation sensitivity ranking). The second part of the flow (right-most side of Figure 5(b)) consist of in-vitro/in-vivo experiments.

V. EXPERIMENTAL RESULTS

We applied the proposed platform to analyse the case study presented in Section V, and, in particular, to analyse the on-off kinetics of integrin triggering, which is represented by the oscillatory state of ITGB2 between inactive and activate affinity state. ABV has been applied through the parametrization phase (see Section IV-B) by considering, as variables, the delay time of each protein in the range 2-8 ms and the lifetime set to 50 ms. This range of values has been chosen as a reasonable approximation of the available experimental data in literature. The automatic test pattern generator (ATPG) has been set to exhaustively explore the protein delay time solution space through 3 ms steps (i.e., 2, 5, 8 ms). Overall, the ATPG generated $\simeq 2.14 \cdot 10^9$ different configurations.

An assertion has been defined to identify all and only the configurations that lead to at least one *periodic* oscillation of ITGB2 within a total simulated time of 200 ms, where the periodicity has been set with a tolerance of $\pm 20\%$ ($\delta/2$ in Figure 4). Such a period represents the average stopping time of a cell when it interacts with the blood vessel epithelium. Notably, although accurate experimental measurement of on-off dynamics of integrin triggering is, at the present, unavailable, the extremely rapid kinetics of leukocyte arrest under flow conditions, occurring in the experimentally-determined range of few milliseconds clearly suggest that it is reasonable to consider this rapid time-frame as a correct reference time to simulate on-off dynamics of integrin triggering. Furthermore, since directional leukocyte motility (chemotaixs) appears to maintain constant speed, at least in the context of a chemotactic gradient, it is reasonable to observe regular oscillatory dynamics of signaling mechanisms controlling integrin triggering.

Table I (first row - *Golden model*[1]) reports the parametrization results, which required 150 runtime hours. The table reports the number of configurations, among all the generated ones, that led to periodic oscillations (column *Useful conf. (periodic)*), the number of oscillations, and the approximate oscillation period. In this phase, we didn't consider the configurations that led to aperiodic behaviours since, from a biological point of view, they are not of interest.

The same assertion has been then applied in the second phase, in which, we analysed the network robustness/sensitivity through mutation analysis. For the sake of clarity and for the lack of space (without loss of meaningfulness), we report and analyse only the mutation effects over the Inactive-Active state transition, for each network protein (see Table I and Figure 6). Each injected mutation represents either a disease as well as a drug effect that selectively prevents a specific protein to carry out its biological function. Each row of the table reports the effects of a mutation on the specific protein, which allows us to classify the proteins into three categories. *Robust* (as for proteins *ABG, PLC*, etc.), for which the number of useful configurations does not change after mutation and that underlines that the network is robust for any failure on those proteins. *Sensitive* (as for proteins *CXCR4, VAV1, PLD1*, etc) in which the mutation leads ITGB2 to an inactive steady state (no oscillations) for the whole simulation time. The third category, *Modifying*, includes the proteins for which the ITGB2 oscillation still exists even though the periodiciy is totally or partially lost (e.g., proteins *JAK*, *RAC1*, *RHOA*, etc.)

In general, the mutation analysis underlines that the network, considering the periodic oscillation property, is extremely sensitive to the pathway involving *PIP5K1C*, while it is robust to the pathway involving *RASGRP1*. The results also show that proteins *JAK2/3* and *CDC42* are essential to guarantee the oscillation periodicity. All the proteins classified as *Sensitive* and *Modifying* are more interesting to verify and further investigate experimentally in-vitro such a signalling network.

VI. CONCLUSIONS

This paper presented a design and verification platform based on SystemC that applies methodologies and tools widely used and consolidated in the EDA field such as ABV and mutation analysis for modelling and simulation of biological signalling networks. The paper showed how the platform has been successfully applied for parametrizing and studying important properties (i.e., robustness and sensitivity) of the signalling network controlling LFA-1 beta2 integrin

[1]The golden model represents the network with no mutation injected.

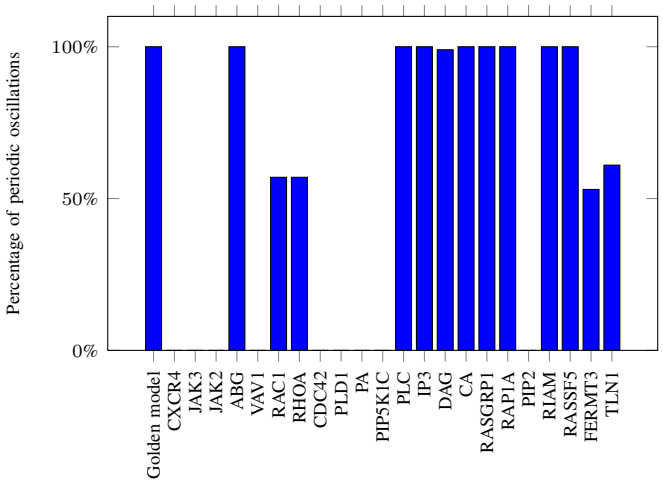

Fig. 6. Experimental results

activation mediating leukocyte recruitment from the blood into the tissues. In particular, the paper showed how, through ABV and mutation analysis, the platform allowed us to classify each network protein depending on their influence in such an on-off kinetics of integrin triggering.

ACKNOWLEDGMENT

This work has been supported by the Electronic System Design research group University of Verona (http://www.di.univr.it/?ent=bibliocr&id=57&tipobc=4&lang=en), the Laboratory of Cell Trafficking and Signal Transduction University of Verona (http://dp.univr.it/ laudanna/LCTST/index.html), and the Center for BioMedical Computing (http://www.cbmc.it/)

REFERENCES

[1] N. Le Novere, "Quantitative and logic modelling of molecular and gene networks," *Nat Rev Genet*, vol. 16, no. 3, pp. 146–158, Mar. 2015. [Online]. Available: http://dx.doi.org/10.1038/nrg3885

[2] R. Samaga and S. Klamt, "Modeling approaches for qualitative and semi-quantitative analysis of cellular signaling networks," *Cell Communication and Signaling*, vol. 11, no. 1, 2013. [Online]. Available: http://dx.doi.org/10.1186/1478-811X-11-43

[3] "The Systems Biology Markup Language," http://www.sbml.org.

[4] N. Bombieri, R. Distefano, G. Scardoni, F. Fummi, C. Laudanna, and R. Giugno, "Dynamic modeling and simulation of leukocyte integrin activation through an electronic design automation framework," *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)*, vol. 8859, pp. 143–154, 2014.

[5] S. Li, S. Assmann, and R. Albert, "Predicting essential components of signal transduction networks: A dynamic model of guard cell abscisic acid signaling," *PLoS Biology*, vol. 4, no. 10, pp. 1732–1748, 2006.

[6] C. Giacomantonio and G. Goodhill, "A boolean model of the gene regulatory network underlying mammalian cortical area development," *PLoS Computational Biology*, vol. 6, no. 9, 2010.

[7] R. Schlatter, K. Schmich, I. Vizcarra, P. Scheurich, T. Sauter, C. Borner, M. Ederer, I. Merfort, and O. Sawodny, "On/off and beyond - a boolean model of apoptosis," *PLoS Computational Biology*, vol. 5, no. 12, 2009.

[8] R. Zhang, M. V. Shah, J. Yang, S. B. Nyland, X. Liu, J. K. Yun, R. Albert, and T. P. Loughran, "Network model of survival signaling in large granular lymphocyte leukemia," *Proceedings of the National Academy of Sciences*, vol. 105, no. 42, pp. 16 308–16 313, 2008.

[9] A. Saadatpour, R.-S. Wang, A. Liao, X. Liu, T. Loughran, I. Albert, and R. Albert, "Dynamical and structural analysis of a t cell survival network identifies novel candidate therapeutic targets for large granular lymphocyte leukemia," *PLoS Computational Biology*, vol. 7, no. 11, 2011.

[10] I. Albert, J. Thakar, S. Li, R. Zhang, and R. Albert, "Boolean network simulations for life scientists," *Source Code for Biology and Medicine*, vol. 3, 2008.

[11] C. Mssel, M. Hopfensitz, and H. Kestler, "Boolnet-an r package for generation, reconstruction and analysis of boolean networks," *Bioinformatics*, vol. 26, no. 10, pp. 1378–1380, 2010.

[12] J. Zheng, D. Zhang, P. Przytycki, R. Zielinski, J. Capala, and T. Przytycka, "Simboolnet–a cytoscape plugin for dynamic simulation of signaling networks." *Bioinformatics (Oxford, England)*, vol. 26, no. 1, pp. 141–142, 2010.

[13] T. Helikar and J. Rogers, "Chemchains: A platform for simulation and analysis of biochemical networks aimed to laboratory scientists," *BMC Systems Biology*, vol. 3, 2009.

[14] A. Gonzalez, A. Naldi, L. Snchez, D. Thieffry, and C. Chaouiya, "Ginsim: A software suite for the qualitative modelling, simulation and analysis of regulatory networks," *BioSystems*, vol. 84, no. 2, pp. 91–100, 2006.

[15] F. Hinkelmann, M. Brandon, B. Guang, R. McNeill, G. Blekherman, A. Veliz-Cuba, and R. Laubenbacher, "Adam: Analysis of discrete models of biological systems using computer algebra," *BMC Bioinformatics*, vol. 12, 2011.

[16] F. Li, T. Long, Y. Lu, Q. Ouyang, and C. Tang, "The yeast cell-cycle network is robustly designed," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 14, pp. 4781–4786, 2004.

[17] C. Campbell, S. Yang, R. Albert, and K. Shea, "A network model for plant-pollinator community assembly," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 1, pp. 197–202, 2011.

[18] M. Chaves, E. Sontag, and R. Albert, "Methods of robustness analysis for boolean models of gene control networks," *IEE Proceedings: Systems Biology*, vol. 153, no. 4, pp. 154–167, 2006.

[19] H. De Jong, J.-L. Gouz, C. Hernandez, M. Page, T. Sari, and J. Geiselmann, "Qualitative simulation of genetic regulatory networks using piecewise-linear models," *Bulletin of Mathematical Biology*, vol. 66, no. 2, pp. 301–340, 2004.

[20] A. Di Cara, A. Garg, G. De Micheli, I. Xenarios, and L. Mendoza, "Dynamic simulation of regulatory networks using squad," *BMC Bioinformatics*, vol. 8, 2007.

[21] J. Krumsiek, S. Plsterl, D. Wittmann, and F. Theis, "Odefy - from discrete to continuous models," *BMC Bioinformatics*, vol. 11, 2010.

[22] R. Distefano, F. Fummi, C. Laudanna, N. Bombieri, and R. Giugno, "A systemc platform for signal transduction modelling and simulation in systems biology," in *Proceedings of the 25th Edition on Great Lakes Symposium on VLSI*, ser. GLSVLSI '15, 2015, pp. 233–236.

[23] M. Wynn, N. Consul, S. Merajver, and S. Schnell, "Logic-based models in systems biology: A predictive and parameter-free network analysis method," *Integrative Biology (United Kingdom)*, vol. 4, no. 11, pp. 1332–1337, 2012.

[24] K. Ley, C. Laudanna, M. Cybulsky, and S. Nourshargh, "Getting to the site of inflammation: the leukocyte adhesion cascade updated," *Nat. Rev. Immunol.*, vol. 7, pp. 678–689, 2007.

[25] A. Montresor, M. Bolomini-Vittori, L. Toffali, B. Rossi, G. Constantin, and C. Laudanna, "Jak tyrosine kinases promote hierarchical activation of rho and rap modules of integrin activation," *J Cell Biol.*, vol. 203(6), pp. 1003–1019, 2013.

[26] N. Bombieri, F. Fummi, V. Guarnieri, G. Pravadelli, F. Stefanni, T. Ghasempouri, M. Lora, G. Auditore, and M. Marcigaglia, "Reusing rtl assertion checkers for verification of systemc tlm models," *Journal of Electronic Testing: Theory and Applications (JETTA)*, vol. 31, no. 2, pp. 167–180, 2015.

[27] M. Boulé and Z. Zilic, *Generating hardware assertion checkers: for hardware verification, emulation, post-fabrication debugging and on-line monitoring*. Springer, 2008.

[28] Y. Abarbanel, I. Beer, L. Glushovsky, S. Keidar, and Y. Wolfsthal, "FoCs: Automatic generation of simulation checkers from formal specifications," in *Proc. of CAV*, 2000, pp. 538–542.

[29] N. Barkai and S. Leibler, "Robustness in simple biochemical networks," *Nature*, vol. 387, no. 6636, pp. 913–917, 1997.

[30] K.-T. Cheng and J.-Y. Jou, "A single-state-transition fault model for sequential machines," in *Proc. of IEEE ICCAD*, 1990, pp. 226–229.

[31] P. Lisherness and K.-T. (Tim) Cheng, "SCEMIT: A SystemC error and mutation injection tool," in *Proc. of ACM/IEEE DAC*, 2010, pp. 228 –233.

[32] N. Bombieri, F. Fummi, V. Guarnieri, and G. Pravadelli, "Testbench qualification of systemc tlm protocols through mutation analysis," *IEEE Transactions on Computers*, vol. 63, no. 5, pp. 1248–1261, 2014.