

# Studies of biological networks with statistical model checking: application to immune system cells

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## ABSTRACT

We use computational modeling and formal analysis techniques to study temporal behavior of a discrete logical model of the naive T cell differentiation. The model is analyzed formally and automatically by performing temporal logic queries via statistical model checking. The results obtained using model checking provide details about relative timing of events in the system, which would otherwise be very cumbersome and time consuming to obtain through simulations only.

## Categories and Subject Descriptors

B.6.1 [Logic Design]: Design Styles Combinational logic. F.4.1 [Mathematical Logic And Formal Languages]: Mathematical Logic - temporal logic.

## General Terms

Design, Logic, Verification.

## Keywords

Formal methods, Immune system, Boolean networks, T cell, Statistical model checking.

## 1. INTRODUCTION

In this work, we apply temporal logic model checking to automate analysis of the model of a T cell differentiation control network. The model used in this work (described in [3]) couples exogenous signaling inputs to T cell phenotype decisions. The goal of our study is to provide details about relative timing of events in the system that contribute to the differentiation of T cell into a regulatory (Treg) vs. helper (Th) phenotype.

Different T cell phenotype ratios play an important role in T-cell mediated immunity, in both autoimmune diseases and in cancer. Previous studies have indicated that the timing of T cell stimulation, antigen dose and the duration of antigen stimulation, strongly influence the T cell phenotype choice [3][5]. The model we are studying was developed using a discrete, logical modeling approach, and simulated using a random asynchronous approach with the BooleanNet tool [1][3]. Model simulations allow for recapitulating a number of experimental observations and provide new insights into the system. However, to test new properties of the model, it is usually necessary to write new parts of the simulator code, or to manually analyze a significant amount of simulation data. This approach quickly becomes tedious and error-prone. Thus,

to study this system, we apply computational modeling approaches together with formal methods.

Since the underlying semantic model of the simulation tool is essentially a discrete-time Markov chain, it can be analyzed as a probabilistic (stochastic) system verification problem. Such problems amount to computing the probability that a given temporal logic formula is satisfied by the system. Statistical model checking can be effectively used for verifying temporal logic specifications for systems affected by the state explosion problem [6][7]. The technique relies on simulation, thereby avoiding a full state space search. This implies that the answer to the verification problem (*i.e.*, the probability that the property holds) is only approximate, but its accuracy can be arbitrarily constrained by the user. Our formal approach allows for querying the system very efficiently, and for quickly obtaining information about transitions that are allowed (or not allowed) by the model configuration, as well as understanding ordering of events and causal relationships.

## 2. NETWORK MODEL

The model in [3] represents a generalization of the Boolean network model, and allows for modeling elements of the network as discrete (not only Boolean) variables. The model consists of 38 elements, and includes ligands outside of the cell, receptors, signaling molecules inside the cell, transcription factors, and several genes of interest. Several scenarios with different initial conditions are of special interest for immunologists:

1. High stimulation of T cell receptor (TCR) – experiments show that such stimulations result in differentiated Th cells (referred to as High antigen (Ag) dose scenario) [5];
2. Low stimulation of TCR – experiments show that such stimulations result in a mixed population of both Th and Treg cells with a significant number of Treg cells (Low Ag dose scenario) [5];
3. High stimulation of TCR, and removal of stimulus after 18 hours – experiments show that, similar to low stimulation, such stimulation results in a mixed population with a significant number of Treg cells (High Ag dose + Ag removal scenario) [4].

We implemented these scenarios by setting different initial states and analyzing trajectories from initial to steady state. Steady state in which element Foxp3 is at level 1 is used as a marker of differentiated Treg cells, and steady state in which element IL-2 is at level 1 is used as a marker of differentiated Th cells.

## 3. RESULTS

We obtain model simulation traces using the BooleanNet [1] simulation tool. We have combined BooleanNet with a parallel statistical model checker, by encoding relevant properties of the model as temporal logic formulae. The properties are then verified via statistical model checking by using Bounded Linear Temporal Logic (BLTL) [2] as our specification language. Verification of BLTL properties of logical models can be performed efficiently and automatically on a multi-core system. In particular, each core runs a

BooleanNet simulation and checks its trace with respect to the BLTL property. A designated core carries out the required statistical computations. We have used the OpenMP API for shared-memory parallel programming in C++.

We tested a large number of system properties using model checking, but due to space constraints, we describe here just a few examples.

### 3.1 High stimulation level

When naïve T cells are stimulated with high Ag dose, they differentiate into Th cells and have high levels of IL-2. Although differentiated Th cells do not express Foxp3, there is a brief transient period shortly after stimulation during which some cells express Foxp3 [3]. Averaged simulation results show that the peak magnitude of Foxp3 transient is approximately 10%. However, it is not clear from these averaged time courses, whether this means that 10% of all trajectories exhibit such Foxp3 behavior, or this occurs in most of the trajectories, but at different simulation rounds, thus leading to a lower average peak. In order to further investigate whether there are additional transient or steady states that can be reached in this scenario, we use model checking. We also use model checking to get more specific information about the transient behavior of Foxp3.

We define several properties to test the probability of Foxp3 occurrence in case of high stimulation scenario. For example, testing the property  $F^{29} (FOXP3 == 1)$  returns the probability that Foxp3 becomes 1 within 29 simulation rounds. Another property,  $F^{10} (FOXP3 == 1 \ \& \ F^{19} (FOXP3 == 0))$ , tests the transient behavior of Foxp3, that is, the probability that Foxp3 becomes 1 by round 10 and returns back to 0 by round 19. Our results show that the probability that Foxp3 transiently increases to level 1 is about 24%, confirming our hypothesis that more than 10% of trajectories exhibit this transient Foxp3 behavior. We also conclude from additional tests that the transient is more probable to last only very briefly (one simulation round) after high Ag dose stimulation.

### 3.2 Low stimulation level

When naïve T cells are stimulated with low Ag dose, they can differentiate into Treg cells expressing Foxp3. Similarly, model simulations that mimic the low-stimulation scenario result in steady state with Foxp3 at level 1 [3]. Model simulation results show that IL-2 gene expression increases early after stimulation in both low- and high-stimulation scenarios. While in high Ag dose scenario IL-2 increases and remains at steady level 1 in all trajectories, in low Ag dose scenario averaged simulation results show that the peak magnitude of IL-2 is approximately 80%, after which all trajectories decrease back to level 0.

The transient behavior of IL-2 is not straightforward to measure in experiments, since IL-2 is measured outside of cells where it is consumed quickly after being expressed and secreted. It is not clear from averaged simulation trajectories whether IL-2 reaches value 1 in all trajectories, but at different update rounds, or it reaches value 1 in 80% trajectories only. With statistical model checking, we test properties similar to the ones for Foxp3,  $F^{29} (IL2 == 1)$  and  $F^{10} (IL2 == 1 \ \& \ F^{19} (IL2 == 0))$ , and we find that the probability that IL-2 reaches value 1 in all trajectories is close to 1.

### 3.3 Analysis of varying stimulation outcome

Another observation from experiments is that removal of antigen at 18 hours after stimulation results in a mixed population of Treg and Th cells [4]. Studies of the model have indicated that early events and relative timing of the Foxp3-activating and Foxp3-inhibiting paths play crucial role in differentiation [3]. With model

checking, we were able to carry further and more efficient studies of early behavior in these elements.

The Ag removal scenario results in a mixed population of cells with different phenotypes (i.e., steady states). As shown in [3], depending on the time (simulation round) of stimulation removal, the presence and frequency of phenotypes vary. Eleven different phenotypes were observed in Ag removal simulations. Antigen removal at simulation round 6, leads to a mixed population that carries all eleven phenotypes, while other scenarios, with earlier or later removal, typically lead to a less diverse population.

Using model checking, we were able to compute a probability for different attractors to be observed by a specific time point. In addition, we compute the probability of Foxp3 transient behavior, which is characteristic for one of the 11 attractors, using the following property:  $(G^5 (FOXP3 == 0)) \ \& \ (F^{12} (FOXP3 == 1 \ \& \ F^3 (FOXP3 == 0))) \ \& \ G^{29} ((step < 16 \ | \ FOXP3 == 1))$ . The results suggest that the variability present across trajectories ending in Treg phenotype causes longer times to reach steady state. This property is observed in all attractors that express Foxp3 and have similar probabilities of occurrence.

## 4. CONCLUSION

Model checking is an efficient approach for answering a variety of questions about the cell signaling network dynamics. Instead of manually analyzing simulation trajectories and large output files, one creates properties that can be automatically verified. Our goal in this work was to uncover events following varying stimulation conditions and leading to different cell phenotypes. To this end, by using model checking in analyzing the model of T cell differentiation we were able to find the most probable set of events, as well as temporal behavior of model elements resulting in mixed population of cells or in specific phenotypes. We tested a large number of properties, confirmed or rejected existing hypotheses, and specified a number of directions for future experiments and systems studies.

## 5. ACKNOWLEDGMENTS

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