Model checking for studying temporal behavior in cell differentiation

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***Short Abstract* — We use computational modeling and formal analysis techniques to study the temporal behavior of a logical model of the naïve T cell differentiation. The model is analyzed formally and automatically by performing temporal logic queries via statistical model checking.**

I. Introduction

In this work, we apply temporal logic model checking to automatically analyze the behavior of the model of a signaling network that controls T cell differentiation. The model used in this work (described in [1]) couples exogenous signaling inputs to T cell phenotype decisions. This model was developed using a discrete, logical modeling approach, and simulated using a random asynchronous approach in the *BooleanNet* tool [2]. Since the underlying semantic model of the simulation tool is essentially a discrete-time Markov chain, we need to verify probabilistic (stochastic) models. The verification problem for stochastic systems amounts to computing the probability that a given temporal logic formula is satisfied by the system. One approach uses precise numerical methods to compute exactly the probability that the formula is true. However, these methods suffer from the state explosion problem, and do not scale well to large-scale systems. Statistical model checking can be effectively used for verifying temporal logic specifications for systems affected by the state explosion problem [3][4]. 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. In return, statistical model checking is more scalable and hence more useful for large models.

II. Results

We encode relevant properties of the model as temporal logic formulae, which are then verified via statistical model checking. We use Bounded Linear Temporal Logic (BLTL) as our specification language. BLTL restricts the well-known Linear Temporal Logic (LTL) with time bounds on the temporal operators.

Experimental observations from [5] show that the induction/expansion of Foxp3+ regulatory T (Treg) cells by low dose antigen is inversely correlated with the levels of signaling via the mTOR pathway, suggesting a complex interaction between cell surface receptors, signaling molecules and important transcription factors. The model in [1] captures critical signaling events, from stimulatory signals at receptors, through activation of transcription factors, to production of proteins representing different phenotypes, but analysis of the model is complicated by the wide range of model behaviors and timing of key events.

In [1] analysis was performed on the behavior of critical elements in the model averaged across 1000 simulation trajectories for multiple stimulation scenarios. When naïve T cells are stimulated with low antigen dose, they can differentiate into Treg cells expressing Foxp3. Similarly, model simulations that mimic the low antigen dose case result in steady state with Foxp3=1. Model simulation results show that the behavior of IL-2 gene expression early after stimulation is similar for both low and high antigen dose. This is not so straightforward to measure in experiments as IL-2 is measured outside of cells, where it is consumed quickly after being expressed and secreted. What is not clear from averaged simulation trajectories is whether IL-2 reaches value 1 on all trajectories, but at different update rounds, or whether it reaches value 1 on only 80% of trajectories. To test this, we consider the property F^{20} (IL2 = 1) (IL-2 always becomes one by round 20). Statistical model checking shows that the probability that this property holds is close to 1. We have also computed the probability that IL-2 remains at level 0 until its inhibitor, Foxp3, becomes 1. This property, (IL2 = 0) U^{15} (FOXP3 = 1), is returned as a low-probability event. Thus, by defining appropriate queries, we were able to show that our model predicts an initial increase in IL-2, irrespective of antigen dose scenario.

Another observation from experiments is that removal of antigen 18 hours after stimulation results in a mixed population of Treg and Th (helper) cells. Studies of the model have indicated that early events and relative timing of the Foxp3 activating and inhibiting pathways play a crucial role in this differentiation. With model checking, we were able to show that early events involving CD25, mTORC1, and mTORC2 are good predictors of the differentiation outcome. We tested several properties that include relative timing (*e.g.*, F^{10} (MTORC1=1 & MTORC2=1 & CD25 = 0 & (F^{18} (CD25 = 1)))), and most of them return probability estimate close to 0.5, reflecting the mixed population that we observe as a result of differentiation. By varying the time indicator in properties (*e.g.*, 10 and 18 above), we find the relationship between early events and phenotype decisions: early increase in CD25 vs. mTOR is critical for Treg cells, while delayed, or very brief increase in CD25 results in Th population.

III. Conclusion

Model checking is an efficient approach for studying cell signaling network models, as it allows for answering a variety of questions about the system. Instead of manually analyzing simulation trajectories and large output files, one creates properties that can be automatically verified. We uncovered several relationships between early behaviors of elements in our T cell model and differentiation outcomes. The framework we have developed holds considerable promise for further analysis of this and other models of cell signaling and differentiation.

References

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