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Computational modelling of mycobacterium infection and innate immune response in zebrafish

Viana de Carvalho, R.

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6 Discussion and conclusion

O wee

The blossoms fall but why the hurry?
Coming of age I want the spring slowing down.
Unfortunately all places of pleasure
are different from the days of my youth.

What relaxes me is just the wine,
What distracts my mind is the poetry.
This idea, Tao Yuanming would understand -
My life falls later than yours!

(Du Fu, China, 712-770)

可惜

花飞有底急 老区愿春迟
可惜欢愉地都非少壮时

宽心应是酒 与这莫过时
此意陶潜解 吾生后汝期

杜甫, 中国 (712 - 770)

Abstract

In this thesis we provided a comprehensive overview on the steps that are involved in the modeling process and simulation of biological phenomena; from the choice of the method to the validation of the results. We gradually implemented a model with which we would be able to study the complex interplay of the components involved in the *Mycobacterium marinum* infection process and innate immune response in zebrafish embryos. In itself this process is a model for deeper understanding of tuberculosis infection in humans using zebrafish as model organism. Each chapter is a building block in the modeling process, which gradually forms a model that can represent cause-and-effect among these components involved in the biological behavior. In this chapter we present our discussion and conclusion about the work presented in this dissertation.

6.1 Modeling mycobacterial infection process

In Chapter 1 we introduced the modeling process and the methods that can be used to model a biological system. It is important to the modeling process to identify the characteristics of the problem, the data available and to be able to generate the hypothesis to define the properties of the model. The next step is to choose the formal method to use for the implementation of the model. In this Chapter 1 we emphasize the Petri net formalism, its characteristics of scalability, extensibility and effectiveness to model a biological system. The Petri net formalism is also combined with an intuitive graphical representation with a strong mathematical foundation, integrating qualitative and quantitative aspects in range of tools that support implementation, simulation and analysis. The characteristics presented in Chapter 1 suggest that Petri net is an important method to model, simulate and analyze biological behavior.

We started the modeling process by defining the central biological problem of this dissertation: the *Mycobacterium marinum* infection process and innate immune response in zebrafish. In Chapter 2 we presented the characteristics of the biological behavior and the modeling decision used to create the first concept of our biological case study. The resulting model provides a qualitative representation of the dynamics of the infection process and the primary defense mechanism. The model in this described chapter shows to be able to reproduce the steps of the infection process and granuloma formation as implemented in a qualitative colored Petri net method. Throughout this first model, questions about how the bacteria proliferate and why the immune cells do not respond effectively to the infection, guided us to analyze other aspects of the infection process.

Motivated by the conclusions of Chapter 2, we started to analyze the aspects of the interaction between bacteria and immune cells that influence the infection process. The challenge addressed in Chapter 3 was to assemble the information about the different components involved in activation, inhibition and control of the infection. We analyzed the range of concurrent events that occur simultaneously, and how their molecular and cellular components are interconnected. Through an extensive literature research, in this chapter we have modeled the important regulatory pathways explored by the bacteria to evade the host immune cell and to be able to proliferate. We opted to implement the pathways in sub-models to represent the biological scales in which they occur: intracellular, intercellular and molecular level. Therefore, we explored the scalability of the Petri net formalism using the QPN method. We connect each sub-model in a hierarchical structure representing the biological scales. The model provides an overview of the important signaling pathways that occur between the host-pathogen interactions.

In possession of two independent but interrelated models, other questions about the modeling process emerged: How to combine a colored Petri net model that represent the dynamics of the infection, with the qualitative Petri net model that represent signaling process; and can these be

connected in a natural hierarchy? Moreover, how to visualize the infection process from a different perspective while maintaining the Petri net formalism? In Chapter 3 we therefore, started this discussion by suggesting a hybrid Petri nets or Nets-within-Nets approach. The HPN would be a good solution in case continuous and stochastic information is used. The Nets-within-Nets is more an abstract approach for large nets that are independent and complex. So far, the complexity of our models and the information available does not yet demand such methods.

The hierarchical combination in natural scales was explored in Chapter 4. In this chapter, we coupled the previous two models in one hierarchical structure extending the QPN model to its colored extension. We use the QPN^C adding a color-set “**Dot**” to represent the signaling process on the pathways implemented at the molecular and cellular level. In addition we implemented a 3D visualization environment that reads the Petri net model simulating the infection process model as if it would happen *in vivo*. Using a different visualization method from the Petri net formalism, but keeping its strong mathematical foundation, we compared the simulation result with an image from *in vivo* experiment to confirm the strength of the qualitative aspects of the model.

The model presented so far, refers to the qualitative aspects of the infection process and its characteristics. For testing and prediction of different scenarios, however, a quantitative model is required that, through support of empirical data would enable to do quantitative analysis. Therefore, an further extension of the model taking stochastic aspects of the infection into account is presented in Chapter 5. As a basis for this model, we collected and analyze data from zebrafish infection studies. In the analysis we found the average granuloma distribution per region in the zebrafish. We use this information as basis for the quantitative model experiments. To create the quantitative model, we used another characteristic of the Petri net formalism related to the integration between qualitative and quantitative methods. We exported the QPN^C model from Chapter 4 to a stochastic Petri net model, which assign rates (probabilities) to the transitions to occur. We redesigned the model in order to use the data collected as basis for the model. We use the data to specify probabilities for an infected macrophage to form a granuloma in a specific region. Therefore, a new structure related to the movement process of an infected macrophage was added to support this quantitative approach. Moreover, we considered the other activities in the infection process as instantaneous (time independent), replacing the stochastic transitions that are not related to the movement for immediate transitions.

For this model, we performed a number of simulations considering the average of granuloma concentration as a probability rate for the stochastic simulations. The results of a simulation scenario indicate that our model behaves quantitatively similar to the empirical data, validating our hypothesis about the model structure and behavior. For a second simulation scenario, we add a stochastic parameter to disturb the concentration and infection distribution. The result shown the same behavior for the infection though the concentration of granuloma and the percentage of

distribution per region changed. Therefore, we demonstrated the robustness of our model by comparing the simulation results with the behavior observed from the *in vivo* experiments. Further refinement in the model based on availability of more data can lead to simulations that can approximate the model to the *in vivo* scenarios even better.

6.2 Model implementation

There are many tools available on internet as well as web platforms that can be used for implementation of Petri net models [9, 23, 29, 43, 45, 47, 64, 95, 121, 125, 131]. Most of the tools are confined to specific classes and/or not support extensions, portability or analysis. However, few tools can provide an extensible experience with Petri net methods [22, 63, 87]. We assessed that Snoopy software [50] is the most complete tool available to design and evaluate models in Petri nets. It supports a set of related important Petri net classes, i.e. QPN, SPN, CPN and HBPN and their colored extensions. Snoopy provides analysis techniques, e.g. animation, simulation, and also exports properties between classes and to external analysis tools i.e. Charlie [56] and Marcie [55]. Recently, the Snoopy Steering and Simulation Server tool (S4) [58, 59] was released as an extension of the Snoopy simulation to perform stochastic simulations in multi-core servers. This extension provides a better performance for simulations of big models as well as to change stochastic properties of the model while it is running. The Snoopy is platform independent and freely available for all relevant platforms, i.e. Linux, Windows and MacOS platforms.

6.3 Conclusion and future work

Here we present our final conclusions about our study on modeling of the *Mycobacterium marinum* infection process and the innate immune response in zebrafish. We have applied the modeling process from the concept of the problem up to simulation and validation of the results. We gradually increment the model complexity through a refinement process, where different and complementary models have been created in sub-models and these have been gradually connected. This was an important process on the modeling decision and turned to be a valuable approach to model a complex biological system. The literature research, gathering the biological processes and separate the processes in sub-models, was a challenge that was feasible just because of the powerful of the Petri net formalism. The result was a readable and extensible model that can be animated, simulated and analyzed independently or in as a whole.

Another contribution of this thesis is related to the hierarchical structure of the model. Coupling different biological scales still an open challenge in modeling biological systems. Using the Petri net formalism, we proposed a simple and effective solution to connect events that occur simultaneously

at molecular, intercellular and intracellular scale. The color Petri net extension bridges each scale using a simple color “dot” which realizes the flow between the scales, representing the presence or absence of an entity, signals and/or activity in the sub-nets.

The migration from qualitative model to quantitative also demanded an effort to collect and analyze data related to the biological problem. We use stochastic model, extending the QPN^C previously modeled to SPN^C. The stochastic process consist of define probability rates for a specific event to occur. Since our data was related to the concentration of granuloma per region in the zebrafish, we use the relative proportion distribution as a probability rate of one granuloma been formed in a specific region. We innovated the stochastic model by using the probabilities only for the infection migration (time delays), considering the other events as instantaneous (no time dependent). This approach not only simplifies the simulation, but also provides a controlled extension of the model by gradually replacing a specific instantaneous event for a stochastic (probability) one once data is available. Therefore, different “what-if” scenarios can be performed as part of the simulation process leading for new insights about the infection process.

In this thesis we studied the importance to identify the characteristics of the biological system to be modeled, the collection of information/data and how they influence the modeling decision. A modular implementation is essential since you can gradually increase the complexity of the model by adding new sub-models structure. Obviously to achieve the modular implementation it is necessary to separate the process in related groups identifying the key elements that bridge each sub-model. Moreover, a visual representation that can depict the modeled process is also important to depict the biological behavior. Therefore, it is important to choose a model formalism that can combine a method with a strong mathematical foundation and graphical representation that better suits to your problem. The validation and analysis of the model is an important step since it can leads to new refinements and conclusions that can drive future experiments.

As result of our studies, we presented computational models that are able to represent qualitative and quantitative the *Mycobacterium* infection process and innate immune response in zebrafish. It is a starting point for *in silico* experiments, new refinements can be performed along the availability of biological data. As future work, new simulations considering bacteria mutants can leads to new discovering on the localized behavior of the infection. Moreover, quantitative information about protein concentrations (i.e. calcium or anti-inflammatory cytokines), and pathways inhibitions (i.e. apoptosis or necrosis) can be used as part of simulation experiments that can bring new insights in the research and treatment of mycobacterial infection in humans.

Biological systems are inherently complex in nature. They are composed of multiple functional processes, which involve complex interconnected networks in a cross talk interaction. Principally, creating a multi-scale model to represent a biological system, it is important to identify and separate the processes that belong to each level of interaction. It is difficult to visualize these levels in the

biological literature, which brings another challenger to the modeling process. We have learned from modeling the *Mycobacterium* infection process using Petri nets the importance of simplification, modularization and extensibility in the modeling process. Through the simplification and modularization, we defined each scale in sub-models by simplifying their boundaries condition into black boxes, in which the connection between models is given by simple signals. The extensibility of the modeling process allowed gradually increasing the complexity of the model, while we were collecting and analyzing more information about the infection process and the innate immune response.

A key observation from this thesis is that the methodology used to model the behavior of the mycobacterium infection process using Petri nets can be used to represent other diseases e.g. diabetes. Most of the components are related to the immune system response and the modularization aspect facilitates the replacing process of modules, i.e. pathways that are specific related to the disease without changing the whole structure of the system. It is our hope that the techniques and practices presented here will guide future efforts in modeling biological process using high-quality multi-scale model implementation using Petri nets.

