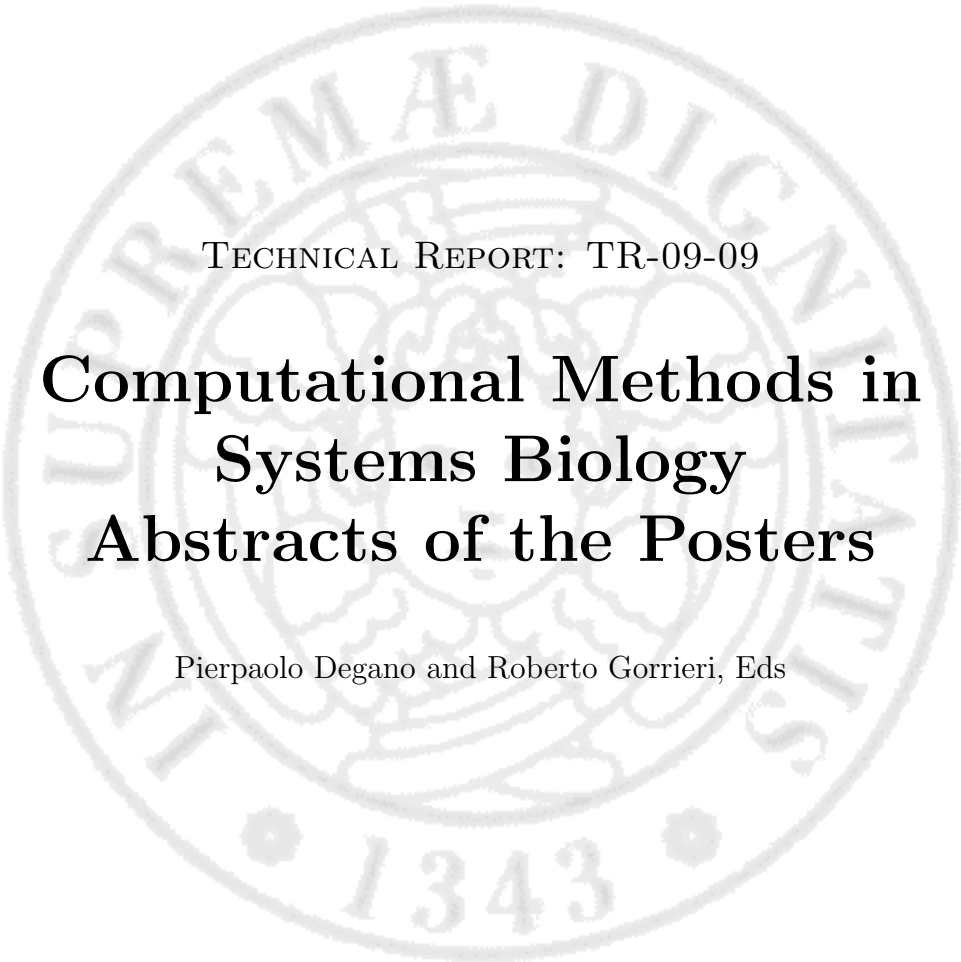
Università di Pisa
Dipartimento di Informatica



Technical Report: TR-09-09

Computational Methods in Systems Biology Abstracts of the Posters

Pierpaolo Degano and Roberto Gorrieri, Eds

Address: Largo B. Pontecorvo 3, 56127 Pisa, Italy. Tel: +39 050 2212700 Fax: +39 050 2212726

Computational Methods in Systems Biology
Abstracts of the Posters

Pierpaolo Degano and Roberto Gorrieri, Eds

Preface

This note contains the abstracts of the seven posters, selected out of eleven submitted, that have been presented at the 7th Conference on Computational Methods in Systems Biology (CMSB 2009), held in Bologna, August 31st - September 1st, 2009.

The first CMSB was held in Trento in 2003, to bring together life scientists, computer scientists, engineers and physicist. The goal was to promote the convergence of different disciplines aiming at a new understanding and description of biological systems, firmly ground in formal models, supported by computational languages and tools, and offering new methods of analysis. The conference then moved to Paris in 2004, Edinburgh in 2005, Trento in 2006, Edinburgh in 2007 and Rostock/Warnemünde in 2008.

Each poster submission was refereed by at least two members of the Programme Committee. We would like to thank their authors for the bright presentations of their work-in-progress.

We would like to thank all the people who contributed to the organization of CMSB 2009, and the generous support from the Alma Mater Studiorum – Università degli Studi di Bologna and from Microsoft Research Cambridge. We are also grateful to Andrei Voronkov, who allowed us to use the wonderful free conference software system EasyChair, which we used for the electronic submission of papers, the refereeing process and the Programme Committee work.

The Program Chairs of CMSB 2009

Pierpaolo Degano
Università di Pisa, Italy

Roberto Gorrieri
Università di Bologna, Italy

Steering Committee

Finn Drabløs, Norwegian Univ. of Science and Technology, Trondheim, Norway
François Fages, INRIA Rocquencourt, France
David Harel, Weizmann Institute of Science, Israel
Monika Heiner, TU Cottbus, Germany
Michael Hörnquist, Linköping University, Sweden
Satoru Miyano, University of Tokyo, Japan
Gordon Plotkin, University of Edinburgh, UK
Corrado Priami, MSR - U. Trento Centre for Comp. & Systems Biology, Italy
Adelinde M. Uhrmacher, University of Rostock, Germany

Programme Committee

Pierpaolo Degano, Università di Pisa, Italy (co-chair)
Finn Drabløs, Norwegian University of Science and Technology, Norway
François Fages, INRIA Rocquencourt, France
Stephen Gilmore, University of Edinburgh, UK
Roberto Gorrieri, Università di Bologna, Italy (co-chair)
Monika Heiner, TU Cottbus, Germany
Adaoha Elizabeth C. Ihekwaba, CoSBI, Italy
Marta Kwiatkowska, University of Oxford, UK
Pietro Liò, Computing Lab Cambridge, UK
Satoru Miyano, University of Tokyo, Japan
Mark van Rossum, University of Edinburgh, UK
Grzegorz Rozenberg, Leiden University, Leiden, NL
Carolyn Talcott, Stanford Research Institute, USA
Adelinde M. Uhrmacher, University of Rostock, Germany

Organizing Committee

Cinzia Di Giusto, Università di Bologna, Italy
Roberto Gorrieri, Università di Bologna, Italy
Cristian Versari, Università di Bologna, Italy (chair)
Antonio Vitale, Università di Bologna, Italy
Gianluigi Zavattaro, Università di Bologna, Italy

Table of Contents

BioDiVinE: A Tool for Parallel Analysis of Multi-Affine ODE Models*

J. Barnat, L. Brim, I. Černá, S. Dražan, J. Fabriková, J. Láník, and D. Šafránek

Department of Computer Science, Faculty of Informatics
Masaryk University Brno, Czech Republic
safranek@fi.muni.cz

1 Introduction

The most widely-used modelling frameworks for the analysis of the dynamics of biological systems are based on the deterministic continuous approach of ordinary differential equations (ODE). The reduction of continuous models to discrete automata by a sequence of reductions, approximations, and abstractions allows formal methods for the automated analysis of temporal properties to be applied [6, 7, 4]. When dealing with large models from systems biology, standard discrete state-space exploration techniques do not provide acceptable response times for answering user queries and high-performance parallel algorithms are required. Owing to dynamical dependencies among state variables, the *state-space explosion problem* arises during reduction to discrete automata.

In the poster we present a prototype tool BioDiVinE [9] for parallel analysis of biological models based on mass action kinetics. In particular, the tool adapts the rectangular abstraction approach of multi-affine ODEs mathematically introduced in [8] and algorithmically tackled in [14, 6]. We contribute to the domain by means of a scalable algorithm. In particular, the contribution of BioDiVinE is three-fold. First, the tool provides a parallel on-the-fly state space generator for the rectangular abstraction (RATS). Second, the state space generation algorithm employs several heuristics [2] for reducing the extent of approximation by guiding the state generator to avoid spurious simulations. Finally, the embedded enumerative on-the-fly LTL model checker allows direct application of efficient parallel model checking algorithms to analysis of biological models. Our experiments [2] show that ODE models involving up-to 20 variables resulting in reachable state spaces having around 10^7 states can be sufficiently analysed (with responses in the order of tens of seconds) on a common cluster. BioDiVinE also provides a graphical module that allows two-dimensional visualisation of reachable state spaces.

2 Related Work

In our previous work [3] we have dealt with parallel model checking analysis of piece-wise affine ODE models [12]. The method allows fully qualitative analysis, since in the piece-wise affine approximation generating of the state space does not require to numerically enumerate the equations. Therefore that approach, in contrast to this one, is primarily devoted for models with unknown kinetic parameters. The price for this feature is higher time complexity of the state space generation. In particular, time appears there more critical than space while causing the parallel algorithms not to scale well.

In the current version of BioDiVinE all the kinetic parameters are required to be numerically specified. In such a situation there is an alternative possibility to do LTL model checking directly on numerical simulations [15, 10]. However, in the case of unknown initial conditions there appears the need to provide large-scale parameter scans resulting in huge number of simulations. On the contrary, the analysis conducted with BioDiVinE can be naturally generalised to arbitrary intervals of initial conditions by means of rectangular abstraction.

* This work has been partially supported by the Academy of Sciences of CR grant No. 1ET408050503 and the FP6 project No. NEST-043235 (EC-MOAN).

3 Toolset Description

BioDiVinE employs aggregate power of network-interconnected workstations (nodes) to analyse large-scale state transition systems whose exploration is beyond capabilities of sequential tools. System properties can be specified either directly in Linear Temporal Logic (LTL) or alternatively as processes describing undesired behaviour of systems under consideration (negative claim automata). From the algorithmic point of view, the tool implements a variety of novel parallel algorithms [11, 1] for cycle detection (LTL model checking). By these algorithms, the entire state space is uniformly split into partitions and every partition is distributed to a particular computing node. Each node is responsible for generating the respective state-space partition on-the-fly while storing visited states into the local memory.

The state space generator constructs the rectangular abstraction transition system for a given multi-affine system. The scheme of the tool architecture is provided in Figure 1. Library-level components are responsible for constructing, managing and distributing the state space. They form the core of the tool. The tool provides two graphical user interface components *SpecAff* — allowing editing of biological models in terms of chemical reactions, and *SimAff* — allowing visualisation of the simulation results.

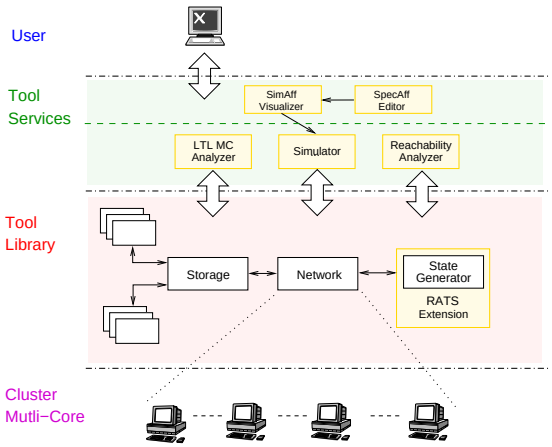


Fig. 1. BioDiVinE Toolset Architecture

The input (biochemical) model is specified by the following data:

- list of chemical species,
- list of partitioning thresholds given for each species,
- list of chemical reactions.

The biochemical model is then automatically translated into a multi-affine system of ODEs forming the mathematical model that can be analysed by BioDiVinE algorithms. The mathematical model consists of the following data:

- list of variables,
- list of (multi-affine) ODEs,
- list of partitioning thresholds given for each species,
- list of initial rectangular subspaces (the union of these subspaces forms the initial condition),
- Büchi automaton representing an LTL property (this data is not needed for simulation).

An example of a simple three-species model representing a single biochemical reaction $A + B \rightarrow C$ performed with rate 0.5 $M^{-1}s^{-1}$ is showed in Figure 2. The respective mathematical model is

showed in Figure 3 on the left in the textual `.bio` format. For each variable there is specified the equation as well as the list of real values representing individual threshold positions. The initial condition is defined in this particular case by a single rectangular subspace: $A \in \langle 6, 10\rangle, B \in \langle 4, 6\rangle$, and $C \in \langle 0.0001, 2\rangle$. The state space generated for this setting is depicted in Figure 3 on the right. Figure 4 demonstrates visualisation features of BioDiVinE.

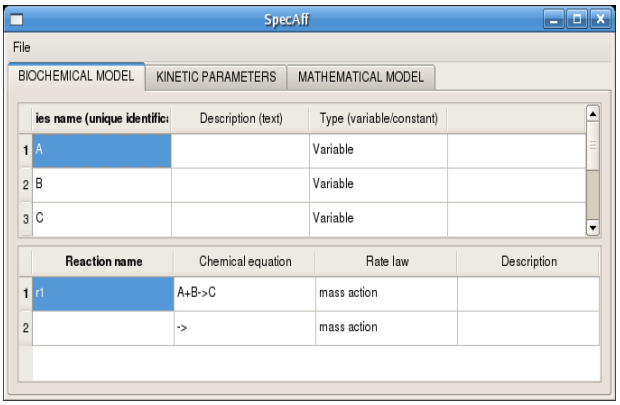


Fig. 2. A biochemical model specified in BioDiVinE GUI

```
VARS:A,B,C

EQ:dA = (-0.5)*A*B
EQ:dB = (-0.5)*A*B
EQ:dC = 0.5*A*B

TRES:A: 0.0001, 6, 10
TRES:B: 0.0001, 4, 6
TRES:C: 0.0001, 2, 4, 6

INIT: 6:10, 4:6, 0.0001:2
```

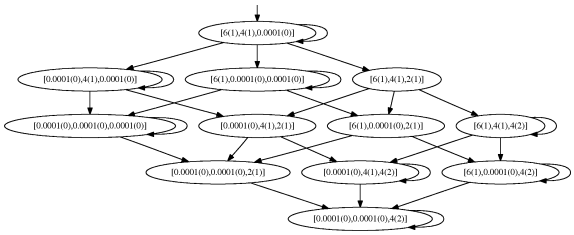


Fig. 3. A multi-affine ODE model and its state space generated by BioDiVinE

For model checking analysis, BioDiVinE relies on the parallel LTL model checking algorithms of the underlying DiVinE library [5]. A given LTL formula is translated into a Büchi automaton which represents its negation. That way the automaton represents the never claim property. The automaton is automatically generated for an LTL formula and merged with the mathematical model by `divine.combine` utility. An example of a model extended with a never claim property is showed in Figure 5. In particular, the automaton specified in terms of DiVinE language represents a never claim for the safety LTL formula $\mathbf{G}(A \leq 10)$ expressing that concentration of species A keeps under the given level.

For any multi-affine model extended with a never claim automaton as showed in Figure 5, the parallel model checking algorithms can be directly called. We have performed several experiments [2] in order to show scaling of the algorithms when distributed on several cluster nodes. Figure 6 shows scaling of model checking conducted on a simple model of a reaction network representing a catalytic reaction scaled for different numbers of intermediate products.

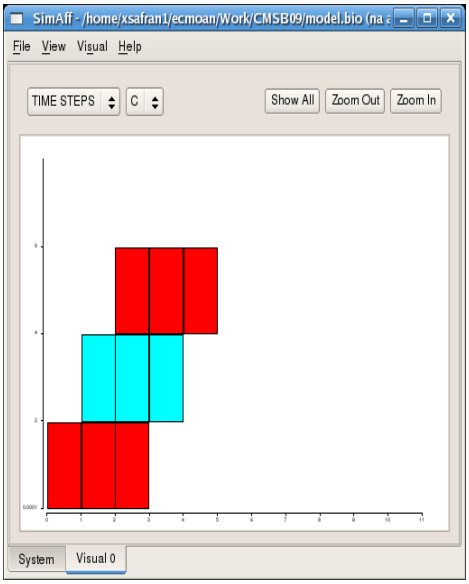


Fig. 4. A visualisation of the state space in BioDiVinE GUI

```
VARS:A,B,C

EQ:dA = (-0.5)*A*B
EQ:dB = (-0.5)*A*B
EQ:dC = 0.5*A*B

TRES:A: 0.0001, 6, 10
TRES:B: 0.0001, 4, 6
TRES:C: 0.0001, 2, 4, 6

INIT: 6:10, 4:6, 0.0001:2
```

```
process LTL_property {
state q1, q2;
init q1;
accept q2;
trans
q1 -> q2 { guard A>10; },
q1 -> q1 {},
q2 -> q2 {};
}

system sync property LTL_property;
```

Fig. 5. A multi-affine model extended with a never claim automaton

$$S + E \rightleftharpoons ES_1 \rightleftharpoons ES_2 \rightleftharpoons \cdots \rightleftharpoons ES_k \rightarrow P + E$$

$$\begin{aligned}
\dot{S} &= ES_1 - 0.01 \cdot E \cdot S \\
\dot{E} &= ES_1 - 0.01 \cdot S \cdot E + ES_1 \\
\dot{ES_1} &= 0.01 \cdot E \cdot S - ES_1 - ES_1 \\
\dot{ES_2} &= \cdot ES_1 - 2 \cdot ES_2 + ES_3 \\
&\vdots \\
\dot{ES_{k-1}} &= ES_{k-2} - 2 \cdot ES_{k-1} + ES_k \\
\dot{ES_k} &= ES_{k-1} - 2 \cdot ES_k
\end{aligned}$$

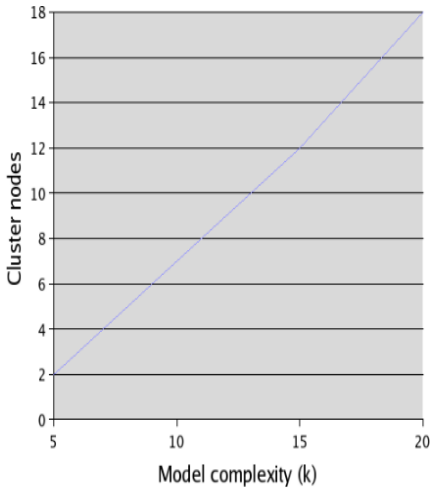


Fig. 6. Scaling of model checking algorithms on a homogeneous cluster

4 Conclusion

In this poster abstract we have presented the tool BioDiVinE for parallel model checking analysis of multi-affine ODE models. The tool currently supports rectangular abstraction of multi-affine systems providing discrete (over)approximation of the continuous state space. Properties of the model are specified in terms of LTL formulae. Parallel model checking algorithms can be used to either find an example of a particular behaviour or to decide that certain property is satisfied by all trajectories of the system starting at states given by particular initial conditions. The practicability of model checking is naturally limited by the level of overapproximation involved. Current applications of BioDiVinE show its usage for analysis of safety properties.

For future work we aim to employ BioDiVinE for analysis of biological models developed in the EC-MOAN project [13]. We also plan to improve the GUI in order to bring the tool closer to the community of biologists.

References

1. J. Barnat, L. Brim, and J. Chaloupka. From Distributed Memory Cycle Detection to Parallel LTL Model Checking. *Electronic Notes in Theoretical Computer Science*, 133(1):21–39, May 2005.
2. J. Barnat, L. Brim, I. Černá, S. Dražan, J. Fabriková, and D. Šafránek. Computational Analysis of Large-Scale Multi-Affine ODE Models. 2009. Accepted to High Performance Computational Systems Biology (HIBI) 2009.
3. J. Barnat, L. Brim, I. Černá, S. Dražan, J. Fabriková, and D. Šafránek. On Algorithmic Analysis of Transcriptional Regulation by LTL Model Checking. *Theoretical Computer Science*, 2009. in press.
4. J. Barnat, L. Brim, I. Černá, S. Dražan, and D. Šafránek. Parallel Model Checking Large-Scale Genetic Regulatory Networks with DiVinE. In *Proc. of From Biology to Concurrency and Back*, volume 194 of *ENTCS*, pages 35–50. Elsevier, 2007.
5. J. Barnat, L. Brim, I. Černá, P. Moravec, P. Ročkai, and P. Šimeček. DiVinE – A Tool for Distributed Verification (Tool Paper). In *Computer Aided Verification*, volume 4144/2006 of *LNCS*, pages 278–281. Springer, 2006.
6. G. Batt, C. Belta, and R. Weiss. Model checking liveness properties of genetic regulatory networks. In *Conference on Tools and Algorithms for the Construction and Analysis of Systems*, volume 4424 of *LNCS*, pages 323–338. Springer, 2007.
7. G. Batt, D. Ropers, H. de Jong, M. Page, and J. Geiselmann. Symbolic Reachability Analysis of Genetic Regulatory Networks using Qualitative Abstractions. Technical Report RR-6136, INRIA, 2007.
8. C. Belta and L.C.G.J.M. Habets. Controlling a class of nonlinear systems on rectangles. *IEEE Transactions on Automatic Control*, 51(11):1749–1759, 2006.
9. BioDiVinE Toolset. Masaryk University, 2009. http://divine.fi.muni.cz.
10. L. Calzone, F. Fages, and S. Soliman. BIOCHAM: an environment for modeling biological systems and formalizing experimental knowledge. *Bioinformatics*, 22(14):1805–1807, 2006.
11. I. Černá and R. Pelánek. Distributed explicit fair cycle detection (set based approach). In *Model Checking Software. 10th International SPIN Workshop*, volume 2648 of *LNCS*, pages 49 – 73. Springer, 2003.
12. H. de Jong, J. Geiselmann, C. Hernandez, and M. Page. Genetic network analyzer: qualitative simulation of genetic regulatory networks. *Bioinformatics*, 19(3):336–344, 2003.
13. *FP6 EU Project EC-MOAN (Escherichia Coli-Modelling and Analysis)*, 2007. `http://www.ec-moan.org`.
14. M. Kloetzer and C. Belta. Reachability analysis of multi-affine systems. *Transactions of the Institute of Measurement and Control*, 2008. in press.
15. X. Liu, J. Jiang, O. Ajayi, X. Gu, D. Gilbert, and R. Sinnott. BioNessie. *Global Healthgrid: e-Science Meets Biomedical Informatics - Proceedings of HealthGrid 2008*, (138):147–157, 2008.

Mechanistic Insights into Metabolic Disturbance during Type-2 Diabetes and Obesity using Qualitative Networks

Antje Beyer[1⋆], Peter Thomason[2], James Scott[2], and Jasmin Fisher[3]

[1] Department of Genetics, University of Cambridge, Cambridge, UK
[2] National Heart and Lung Institute, Imperial College, London, UK
[3] Microsoft Research, Cambridge, UK

Abstract. The high-fat diets in the modern life-style of developed countries lead to metabolic disturbance and inflammation which can ultimately result in obesity and type-2 diabetes. The transcription factor MLXIPL is probably a key player in the development and maintenance of such metabolic disturbances. In this work we are using the Qualitative Networks framework to model a metabolic network related to fat metabolism, which plays an important role in type-2 diabetes and obesity. The model is based on gene expression data obtained at 8 days and 15 weeks after a fat-feeding process. We show that the model is consistent with the experimental data and therefore allows in silico testing of new hypotheses. Using the model, we demonstrate that acetyl CoA and MLXIPL regulate the level of fatty acid production in a synergistic way, as well as highlight the necessity of further regulators of MLXIPL in addition to the known ones. Furthermore, the analysis predicts various new modes of interactions between components in the network. This modelling work suggests new avenues to explore experimentally and further facilitates our understanding of the complex interconnectivity between metabolic networks operating in obesity and type-2 diabetes.

1 Introduction to the Model and Results

High-fat diets in modern life style are one of the main reasons for the development of metabolic diseases like obesity and type-2 diabetes mellitus (T2D). Nowadays T2D affects over 110 million people worldwide and, as well as obesity, it highly increases the risk of cardiovascular disease, blindness, amputation and kidney failure. With cardiovascular disease being a major cause of mortality, T2D and obesity pose a significant threat to global health. In T2D and obesity, metabolic and inflammatory pathways play important roles as their dysregulation can lead to insulin resistance which is a characteristic symptom of these diseases.

The transcription factor MLXIPL is probably a key player in the development and maintenance of such metabolic disturbances. The computational

⋆ This work was done while this author was an intern at Microsoft Research Cambridge, UK.

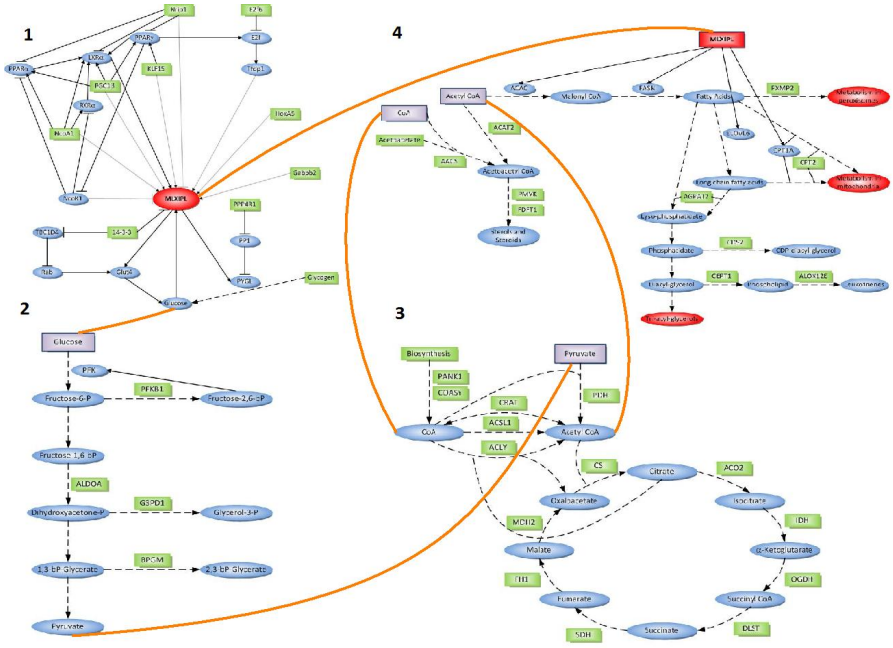


Fig. 1. The four main parts of the metabolic network with their interactions as used in the computational model. (1) network of MLXIPL regulatory connections, (2) glycolysis, (3) network surrounding acetyl CoA and Krebs cycle, (4) fatty acid metabolism.

model described in this work is an executable model comprising transcriptional and post-translational regulations of MLXIPL together with four interconnected metabolic pathways regulated by MLXIPL (Figure 1). These processes all take place in a single cell and are key players in T2D and obesity. The computational model was constructed from a metabolic and transcriptional interaction network using the Qualitative Networks framework [1], which is an extension of Boolean networks. The model is based on gene expression data obtained at 8 days and 15 weeks after a fat-feeding process as described in a study by Scott and colleagues [2, 3]. The model is used to analyze the steady state of the metabolic network at these time points. In order to account for the different time points, we use two different environmental conditions matching 8 days and 15 weeks, respectively. These are modeled by fixing inputs to the network according to their values in the experimental data for the different time points.

In order to be able to analyze the steady state of the model, we break it into four modules (Figure 1). The MLXIPL module consists of 31 components and the steady state analysis computes the attractors of the model from 3^{31} different initial states. Similarly, the fatty acid metabolism module contains 33 components and 3^{33} states; the glycolysis module has 18 components and 3^{18}

states; and the CoA module has 36 components and 3^{36} states. Analysis of the full model would require analysis of approximately 5×10^{47} states and is currently computationally infeasible.

Although there is a good amount of knowledge on MLXIPL function, its regulation is not fully understood. The present computational model aims to provide more insights into this regulation process as well as the importance of specific regulatory connections. The computational analysis revealed that the model is consistent with the experimental data and hence allows testing new hypotheses in silico. Using the model, we demonstrate that acetyl CoA and MLXIPL regulate the level of fatty acid production in a synergistic way, as well as highlight the necessity of further regulators of MLXIPL in addition to the known ones. Furthermore, the analysis predicts various new modes of interactions between components in the network.

2 Concluding Remarks

This modelling work suggests new avenues to explore experimentally and further facilitates our understanding of the complex interconnectivity between metabolic networks operating in obesity and type-2 diabetes.

We have chosen to use the Qualitative network framework for various reasons. As the available data is qualitative it is impossible to construct models describing exact quantities of the different components over time (e.g., differential equations or process calculus). The advantages of Qualitative networks over Boolean networks is in allowing a larger range of possible values. In particular, some of the enzymatic reactions would be impossible to model with just two possible values. Furthermore, the analysis framework using symbolic state exploration (using BDDs, cf., [1]) enables handling networks of this magnitude.

References

1. Schaub, M.A., Henzinger, T.A., Fisher, J. Qualitative networks: a symbolic approach to analyze biological signalling networks. *BMC Systems Biology*, 1:4, 2007.
2. Kooner, J.S., Chambers, J.C., Aquilar-Salinas, C.A., Hinds, D.A., Hyde, C.L.,Warnes, G.R., Gomez Perez, F.J., Frazer, K.A., Elliot, P., Scott, J., Milos, P.M., Cox, D.R., Thompson, J.F. Genome-wide scan identifies variation in MLXIPL associated with plasma triglycerides. *Nature Genetics* 40:149-151, 2008.
3. Scott, J. Oxidative stress in adipose tissue is a unifying trigger for inflammation and insulin resistance. (manuscript in preparation and personal communication)

Towards a minimal calculus for complexation

Cinzia Di Giusto, Cristian Versari, and Antonio Vitale

Dipartimento di Scienze dell'Informazione, University of Bologna

Abstract. We present here a termination preserving encoding of Minsky Machines in a particularly restricted fragment of the κ-calculus where only one species with no fields is admitted and only binary rules are allowed.

1 Motivation

The κ language was introduced in [1] to formally model proteins interactions and it has been studied since for its powerful way to characterize molecular biology dynamics: essentially κ can be regarded as a restricted kind of graph rewriting system. The nodes of the graph represent molecules, the edges the bonds between molecules and the rewriting rules the change of configuration due to an interaction between molecules.

Although the core of the calculus is very simple, still it is very powerful. The full calculus can be easily proved to be Turing-complete. Nevertheless, there are few works assessing the computational power of κ and its fragments: in [2] it has been proved that the binary version of the language (i.e. only rules with at most two reactants in the left hand side are allowed) is Turing equivalent by providing an encoding of Turing machines in the language. Unfortunately, in order to encode the idea of unlimited space on the tape, the encoding does not preserve termination: i.e. a rule generating new tape cells is always active even if the computation in the original machine has stopped. More recently in [3] several fragments of the calculi have been studied w.r.t. their expressiveness: i.e. there have been studied properties like decidability and undecidability of reachability and termination.

We aim here to find a *minimal fragment* of the κ calculus where it is possible to define a termination preserving encoding of Minsky Machines (MM) [4] (a two counter machine where only increments or decrements can take place) which are known to be a Turing equivalent formalism.

2 The language

First we briefly introduce the original κ language (as defined in [1]) and then define the sub calculus we consider.

The basic elements defining a κ system are molecules. Each molecule is given a species that defines its finite interaction points (sites) and its finite internal state values. A molecule may be connected to another via one of these sites. A configuration of a system is described by a *solution* which is a set of molecules possibly interconnected. More formally a solution is given by a sequence of molecules where information on their internal state and their bindings with other molecules are explicitely stated. This

way we obtain a distributed description of a graph: i.e. molecules are the nodes and the bindings the arcs between nodes.

A model in the κ language is completely described by defining the initial solution together with a set of rewriting rules. The semantics allows rewritings of finite graphs whose nodes are in specific states into finite graphs in such a way that changes to a solution are always *localized* to the rewriting part. A model evolves by applying one rule at a time starting from the initial solution.

More precisely we use a finite set of names (species) $\mathcal{A}$, a finite set of sites $\mathcal{S} = \{1, \cdots, l\}$, a finite set of fields $\mathcal{F} = \{1, \cdots, p\}$, a finite set of values $\mathcal{V}$ and a countable set of bonds $\mathcal{B}$. The syntax of molecules and solutions is given below.

$$\begin{aligned} a &::= N[u](\sigma) && \text{(molecule)} \\ N &::= A \in \mathcal{A} && \text{(species)} \\ S &::= \emptyset \mid a, S && \text{(solution)} \end{aligned}$$

Each molecule has a *name* defining its species and it is given an *interface* σ and an *internal state* u that correspond to maps $\mathcal{S} \mapsto \mathcal{B} \cup \{\varepsilon\}$ and $\mathcal{F} \mapsto \mathcal{V}$ respectively.

Rewriting rules also called reactions are either *creations* or *destructions*. Creations may change state, produce new bonds between two unbound sites, or synthesise new molecules. Their format is:

$$\begin{aligned} &A_1[u_1](\sigma_1), \ldots, A_n[u_n](\sigma_n) \\ &\qquad \rightarrowtail \; A_1[u'_1](\sigma'_1), \ldots, A_n[u'_n](\sigma'_n), B_1[v_1](\phi_1), \ldots, B_k[v_k](\phi_k) \end{aligned}$$

Destructions behave the other way around, they may change state but construction of neither bonds nor new molecules is allowed. Their format is:

$$A_1[u_1](\sigma_1), \ldots, A_n[u_n](\sigma_n) \rightarrowtail A_{i_1}[u'_{i_1}](\sigma'_{i_1}), \ldots, A_{i_m}[u'_{i_m}](\sigma'_{i_m})$$

Using the terminology introduced in [3] we will consider the fragment of the κ calculus denoted with κ^{-n} where no destruction of molecules is allowed. Moreover we restrict this calculus by allowing only one species with no fields (i.e. no internal state) and only binary rules[1]. We denote this calculus with κ_2^{-n}. Roughly speaking we are dealing with a calculus where all the molecules are of the same kind, they have no fields and the rewriting rules may have at most two molecules on the left hand side. The expressive power of κ_2^{-n} seems, thus, weakened by the significant restrictions on species and rules: the presence of a unique species and the lack of internal fields in this language makes difficult the representation of information. Notice that the encodings provided in [3] require several species, fields and moreover rules are not binary. In this restricted scenario it should be clear that the state of a molecule is completely defined by its bonds with other molecules, thus any modification in the molecule's bonds actually change its state. Since the state of a molecule lies in its bonds any rule aiming to modify its state have to consider some of its adjacent neighbours. We overcome the restrictions of a unique species and no fields by using a subset of the molecule's sites to encode both the molecule specific role (i.e. the species) and its internal status (i.e. the fields' values).

[1] For a precise definition see [1,3]

An encoding of MM into κ_2^{-n} is indeed possible since bonds allow to mimic the presence of fields and species. In detail, by connecting on special sites we can devote one molecule to a certain group of operations thus obtaining a sort of pseudo-species. Similarly fields can be mimicked by a group of sites and the presence or absence of bonds denotes the current value of the corresponding pseudo field. For instance, a field F with values in 1..10 may be represented by 10 sites $F_1, \ldots, F_{10}$. and the presence of a bond in the site F_i would be interpreted as i being the current value of the pseudo field F.

It is worth noticing that with this representation the state of a molecule can be checked only testing the presence of bonds between two molecules, hence recalling that we only admit binary rules, even if the storage is possible, the propagation of information between molecules is heavily hindered. Nevertheless the capability of testing the *absence* of bonds allows the partial overcoming of such limitation.

3 The encoding

We first recall the definition of a Minsky machine (MM) and then show the encoding. A MM [4] is a machine with *two registers* R_1 and R_2 holding arbitrary large natural numbers and a *program* P consisting of a finite sequence of numbered instructions of the following type:

- j : Succ(R_i): increments R_i and goes to the instruction $j + 1$;
- j : DecJump(R_i, l): if the content of R_i is not zero, then decreases it by 1 and goes to the instruction $j + 1$, otherwise jumps to the instruction l;
- j : Halt: stops the computation and returns the value in the register R_1.

A state of the machine is given by a tuple (j, v_1, v_2) where i indicates the next instruction to execute (the program counter) and v_1 and v_2 are the contents of the two registers. The user has to provide the initial state of the machine.

We now give some intuitions on a possible encoding from MM to κ_2^{-n}.

The registers of the encoded MM are represented as chains of linked molecules: the number of molecules represents the content of the register. Moreover two molecules are devoted to the role of program counter. This configuration is depicted in Figure 1. More precisely the molecules in the center (of pseudo species *PC*) store the state of the program counter, while the chains of molecules (of pseudo species U) on the left and right of *PC* represent the integer values of the two registers of the MM.

The increment and decrement of one register is then propagated from *PC* to the proper end of the chain by means of a sequence of value changes of specific pseudo fields. The increment is encoded straightforwardly by adding a new molecule at the proper end of the chain, while the decrement is encoded by setting the value of a particular pseudo field of the molecule which should be removed, since physical deletion of molecules is not allowed in the calculus. More precisely the propagation of a signal from *PC* to the proper molecule is performed in the following way: We choose a finite set of *properties* that identifies uniquely the state/configuration of the molecule, then we define an injective function that depending on the property we want to be valid returns a

number between 1 and n (where n is the combinatorial factor of all the properties combined together). This number represents the site for the bonds. If a molecule satisfies a property X then all sites apart from the site corresponding to X are bonded. This way, by checking the absence of bonds in a site and by setting properly the interface of two facing molecules in the chain we can propagate information.

The presence of a unique chain of molecules constantly connected after each step ensures the correctness of the encoding, which turns out to be deterministic and termination preserving. Hence, we have the following result:

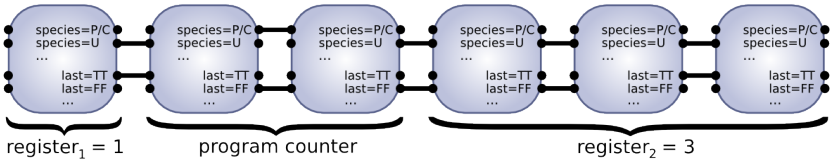


Fig. 1. Schema of the encoding: species and sites with roles

Theorem 1. *There exists a correct encoding of MM in κ_2^{-n} deterministic and termination preserving.*

This encoding is a first step towards the definition of the requirements needed in order to determine the minimal fragment – Turing equivalent – of the κ calculus.

References

1. Danos, V., Laneve, C.: Formal molecular biology. TCS. **325**(1) (2004) 69–110
2. Curien, P.L., Danos, V., Krivine, J., Zhang, M.: Computational self-assembly. Theor. Comput. Sci. **404**(1-2) (2008) 61–75
3. Delzanno, G., Di Giusto, C., Laneve, C., Gabbrielli, M., Zavattaro, G.: The κ-lattice: Decidability boundaries for qualitative analysis in biological languages. In: CSMB 09
4. Minsky, M.: Computation: finite and infinite machines. Prentice Hall (1967)

Analyzing the Glutathione Ascorbate Redox cycle using Petri Nets *

Hermenegilda Macià*, M. Isabel González-Sánchez**, Valentín Valero***, and Edelmira Valero**

* Department of Mathematics, Escuela Superior de Ingeniería Informática. Albacete. University of de Castilla-La Mancha. Spain
** Department of Chemistry-Physics, Escuela de Ingenieros Industriales. Albacete. University of Castilla-La Mancha. Spain
***Department of Computer Science, Escuela Superior de Ingeniería Informática. Albacete. University of Castilla-La Mancha. Spain
{Hermenegilda.Macia, MIsabel.Gonzalez, Valentin.Valero, Edelmira.Valero}@uclm.es

Keywords: Petri nets, metabolic pathways, biological systems.

Abstract. The Glutathione Ascorbate Redox cycle is modelled by Petri nets. For that purpose, we have defined the specific Petri net model that corresponds to the network of chemical and enzymatic steps involved in the cycle, and we have applied structural techniques of analysis of Petri nets in order to obtain some properties of the biological system. On the other hand, some computer simulations have been performed by using some existing tools on Petri nets to analyze the system behaviour.

1 Introduction

The glutathione-ascorbate redox pathway in chloroplasts is a complex network of spontaneous, photochemical, and enzymatic reactions for detoxifying hydrogen peroxide. Our model has been constructed to analyze the dynamic behaviour of the pathway under, for instance, oxidative stress conditions. For that, the model includes an electron source whose flux is distributed among three competitive routes. A detailed description of this metabolic pathway can be found in [6], where the study was based on ordinary differential equations (ODEs). We now apply the Petri net formalism [3] to model and analyze this cycle to have a better understanding of the pathway physiology. The results here obtained may contribute to the prediction of results in the design of experiments.

2 Methods

We have built a discrete Petri net model shown in Fig.1 from the description of the cycle following the steps described in [2]. Then, we can do a qualitative

* Supported by the Spanish government (cofinanced by FEDER founds) with the project TIN2006-15578-C02-02, and the JCCM regional projects PEII09-0232-7745, PAI-05-036 and PAI-08-0175-8618

analysis of the metabolic pathway using both the INA [4] and the Snoopy [5] Petri net tools that help us to understand its biological behaviour. We can also perform simulations of this metabolic pathway considering a continuous time model of Petri Nets [1].

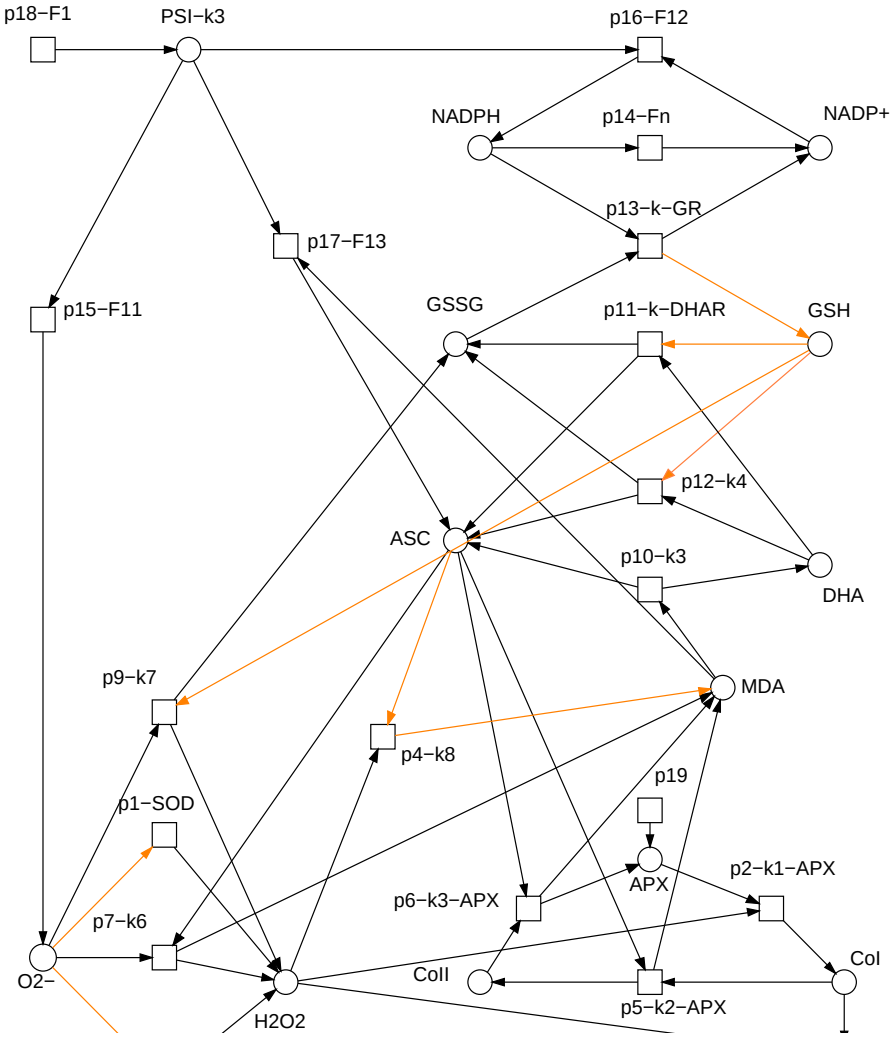


Fig. 1. The Petri net model for the Glutathione Ascorbate Redox cycle (orange arcs are of weight 2)

3 Results

We have applied structural techniques of analysis of Petri nets obtaining Petri nets invariants. The P-invariants are:

P-Inv1 = {NADPH, $NADP^+$}
P-Inv2 = { 2 GSSG, GSH}
P-Inv3 = {ASC, DHA, MDA}

that correspond to the following biological interpretation. This means that the pathway under study is constituted by three moiety-conserved cycles coupled in series to attain a very high amplification capacity [7] against an increase in hydrogen peroxide concentration. In its evolution since the appearance of oxygen in the atmosphere, the cell has developed a very efficient defense tool against oxygen toxicity, although it needs a continuous supply of NADPH.

The system has also three T-invariants:

T-Inv1 = {p18-F1, p16-F12, p14-Fn }
T-Inv2 = {p1-SOD, 2 p15-F11, 4 p18-F1, 2 p17-F13, p2-k1-APX,
p6-k3-APX, p5-k2-APX }
T-Inv3 = { p1-SOD, 2 p15-F11, 4 p18-F1, 2 p17-F13, p4-k8}

that correspond to the following biological interpretations:

- T-Inv1 (see Figure 2) represents the balance between the photochemical production of NADPH and its consumption by the Calvin cycle.

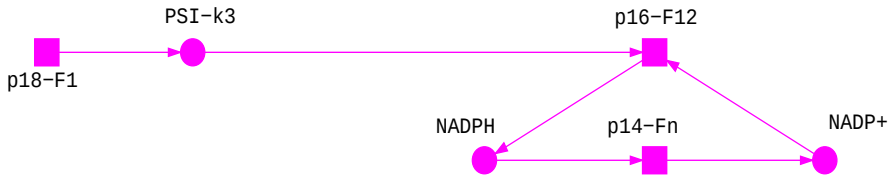


Fig. 2. T-Inv1

- T-Inv2 (see Figure 3) illustrates how the enzyme ascorbate peroxidase works to eliminate H_2O_2 at the expense of the reducing power of ascorbate.
- T-Inv3 (see Figure 4) illustrates the functioning of the spontaneous steps involved in the cycle when the enzymatic system is inoperative.

A better understanding of the system is obtained by means of simulations, by considering a continuous time model of Petri Nets [1]. Simulations have shown the same results as in [6].

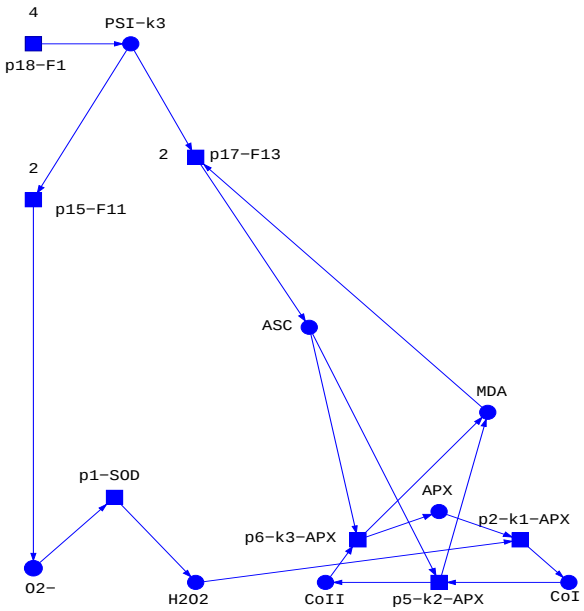


Fig. 3. T-Inv2

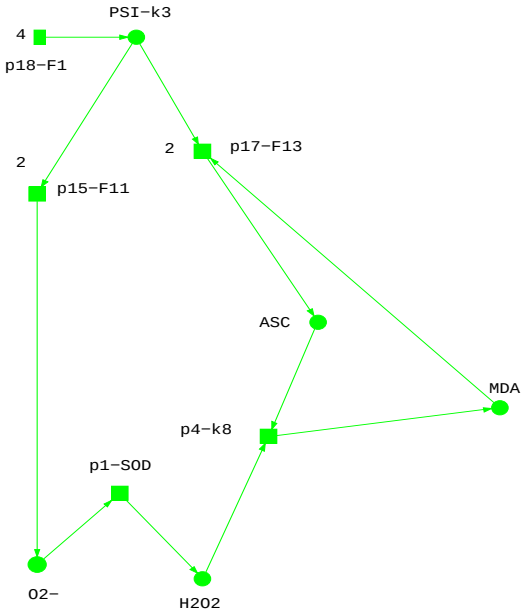


Fig. 4. T-Inv3

4 Conclusions and future Work

We have applied the Petri net formalism to model the Glutathione Ascorbate Redox cycle in chloroplasts. Then, we have obtained Petri net invariants and we have obtained their corresponding biological interpretations as the flux models and conservation relations that help us to understand this behaviour and to extend this model by considering some new features such as dark-light conditions.

References

1. R. David and H. Alla. *Discrete, Continuous and Hybrid Petri Nets*. Springer, 2005.
2. D. Gilbert and M. Heiner. *From Petri Nets to Differential Equations - an Integrative Approach for Biochemical Network Analysis*. Proc. ICATPN 2006, Turku, June, Springer LNCS 4024, pp. 181-200, 2006.
3. J.L. Peterson. *Petri Net Theory and the Modeling of Systems*. Prentice-Hall, 1981.
4. P.H. Starke. *INA: Integrated Net Analyzer*. 2001. http://www.informatik.hu-berlin.de/ starke/ina.html.
5. Data Structures University of Technology in Cottbus, Dep. of Computer Science and Software Dependability. *Snoopy*. 2009. http://www-dssz.informatik.tu-cottbus.de/.
6. E. Valero, M. I. González-Sánchez, H. Macià, and F. García-Carmona. *Computer Simulation of the Dynamic Behavior of the Glutathione-Ascorbate Redox Cycle in Chloroplasts*. Plant Physiology, Vol. 149, pp. 1958-1969, 2009.
7. E. Valero, R. Varón, and F. García-Carmona. *Kinetic analysis of a model for double substrate cycling: highly amplified ADP (and/or ATP) quantification*. Biophysical Journal, Vol. 86, pp. 3598-3606, 2004.

Spatial Multi-Level Modelling and Simulation for Systems Biology

Carsten Maus, Matthias Jeschke, and Adelinde M. Uhrmacher

University of Rostock
Institute of Computer Science, Modelling and Simulation Group
Albert-Einstein-Str. 21, 18059 Rostock, Germany

1 Introduction

Spatial constraints influence the dynamics of various biological processes, e.g. cytoplasmic molecular crowding [1] or membrane lipid rafts [10], just to mention a few of them. With advanced experimental laboratory techniques like high-resolution microscopy, spatial methods gain increasing importance in computational Systems Biology as well.

Simulation methods taking space into account have been developed for different levels of detail, i.e. spatial and temporal resolutions. They vary from microscopic to mesoscopic and finally macroscopic scales each representing different abstraction levels [11]. However, simulations at levels of high detail are rather costly and at higher abstraction levels some processes may be represented insufficiently detailed for the simulation study objective. Here, multi-level approaches come into play combining different abstraction levels and thus supporting details on demand while reducing computational costs. One example is the combination of an abstract population-based level, i.e. particles of the same species type are indistinguishable from each other, and a more detailed individual level where each particle has its own identity showing individual behaviour.

2 The Starting Points

Our work has two starting points, a multi-algorithm approach for spatial simulations combining the Next Subvolume Method with Brownian dynamics [8] and a multi-level formalism rooted in Discrete Event Systems Specification [12].

2.1 Spatial Multi-Resolution Simulation Approach

In [7] and [8] we presented a multi-resolution approach that combines two different spatial simulation algorithms. Populations of particles are simulated by applying the Next Subvolume Method (NSM) [4]. Spatial aspects are represented by a lattice-based discretization of space leading to multiple sub-volumes. In each subvolume the stochastic Gillespie algorithm [5] simulates volume-internal reaction events. In addition, diffusion of particles into adjacent subvolumes are taken into account. At individual level, Brownian dynamics is currently responsible for moving the entities, which occupy a certain space, and inducing reactions. Typically, macro-molecules of particular interest are simulated individually.

Both levels of abstraction influence each other in various ways. Individual particles constrain the space that is available in a subvolume and thus, have an impact on the reactions and diffusion taking place in it. Individual macro-molecules also form a border for population-based particles. By attempting to diffuse into another subvolume particles might collide with the macroscopic molecule. Depending on different parameters, e.g. the orientation of the individual and the diffusion speed of the particle, this collision might be *reactive*, i.e. both the particle and the individual undergo a reaction. First experimental results showed the applicability of the approach to biological phenomena like molecular crowding [8].

2.2 Multi-Level-DEVS

To facilitate multi-level modelling in the Discrete Event Systems Specification (DEVS) [13], we developed an extended formalism named ML-DEVS [12]. The formalism lies – like all DEVS variants – in the tradition of general systems theory and thus supports the modular and hierarchical nesting of model components that are only able to communicate with each other through specific interfaces, i.e. sending and receiving events via their input and output ports. Like State Charts it supports a reactive systems metaphor. For their environment, all models are black-boxes hiding their internal structure and dynamics. Internally, a component can be of type *atomic* or *coupled* model. Atomic models can be regarded as timed automata changing their states depending on time-triggered internal or situation-triggered external events. Coupled models wrap further model components, again either of type atomic or coupled model.

In contrast to traditional DEVS variants, coupled models in the ML-DEVS formalism are not just containers but also have a state and dynamics of their own. This extension allows to describe high-level information and dynamics at the level of coupled models. Typical examples for high-level information are global variables describing environmental conditions like temperature or emergent phenomena resulting from components' behaviour. Also concentrations might be stored and updated globally in the coupled model, emphasising a population-based approach. The need for a comfortable access to global information in cell biological modelling, has also been acknowledged by others, e.g. in developing the Imperative Pi-Calculus [9].

In ML-DEVS, interactions between the two different levels are supported by downward and upward causation. For example, a component might signalise crucial internal changes to the outside by dynamically adding and removing ports. Those might influence the global dynamics of the coupled model. Global variables can directly be accessed by the components. Therefore, ports can be connected to global variables via value couplings. Furthermore, events can be produced at the level of the coupled model and be sent to its components inducing transitions in its components. Due to invariants defined at the level of the coupled model, structure changes invoked by the components might trigger the global dynamics.

Population Level

- Species
- Reaction rules
- Diffusion coefficient of each species
- Lattice of volumes
- Initial particle location/distribution

Individual Level

- Particle shape (e.g. radius)
- Movement function (diffusion coefficient for Brownian dynamics)
- Internal dynamics (state transitions)

Fig. 1. Information needed to describe particles at population and individual level.

3 Towards a Formal Description of Spatial Multi-Level Models

Multi-level-DEVS supports the description of processes at different abstraction levels and explicit interactions between them (see previous section 2.2). However, the formalism was not intended to define spatial aspects as described in section 2.1. Therefore, we developed a concept for an extended ML-DEVS formalism that is enriched by means of defining spatial properties and can be simulated based on the spatial multi-resolution approach described above.

In order to provide all information needed for the reaction-diffusion master equation simulated by the underlying NSM simulator, the diffusion coefficients of each species as well as the lattice of subvolumes and the initial particle distributions have to be defined at population level. Furthermore, besides the ability to describe dynamics as timed state automata, the formalism shall include also means for more intuitive and simple definitions of (bio-)chemical reactions at the level of the coupled model. Therefore, it is planned to adopt ideas from rule-based approaches like κ [2]. For describing molecules at individual level, model components must provide information about their shape. In the most simple case this is just a radius depicting the size of balls. For applying a simple Brownian dynamics simulator, a diffusion coefficient must be associated to individuals as well. Figure 1 lists relevant properties to describe particles at both levels of detail.

Again, both levels of abstraction are tied by causal relationships. Possible interactions between different abstraction levels are illustrated in Figure 2. Components constrain the space that is available in the subvolumes they occupy and thus affect the rates of reactions and diffusion taking place in it. They still form a border for population-based particles. By attempting to diffuse into another subvolume, particles might collide with the macroscopic molecule and thus, might trigger a reaction. This reaction could then lead to a state change inside the macro-molecule, which, in turn, can result in the introduction of new particles. The interaction of the components with their environment takes typically place by receiving and sending events via ports; to notify individuals about events taking place within close proximity their ports can now be connected to subvolumes. As in regular DEVS, a port to port connection can be used for covalent structures and a direct exchange between molecules [3].

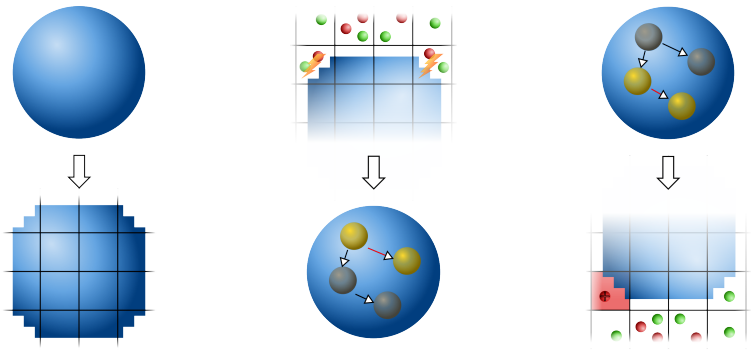


Fig. 2. (Left) Representation of an individual at the population level grid by approximating the space it occupies. **(Middle)** Collisions of particles with the macro-molecule can trigger state changes of the individual. **(Right)** Introduction of new particles at population level caused by state changes at individual level.

4 Conclusion and Outlook

The concepts of the formalism are schematically shown in Figure 3. Based on DEVS it inherits its modular, hierarchical construction of models. Thus, each of the components could be described again as a spatial multi-level model. At the lowest level and for the interaction of subsystems we still find the reactive systems metaphor, however at the global level of a coupled model the reaction-based perspective prevails.

Our next research steps will be dedicated to implementing the introduced formalism in JAMES II [6]. Conceptually, suitable strategies have still to be found that allow moving components to update their spheres of interest in terms of ports connected to subvolumes. Also currently, we assume Brownian Dynamics, but other, maybe more sophisticated methods for describing the movement of particles, could be used as well. All of this will have an effect on the performance of the simulation, here thorough evaluations are needed.

References

1. N. Chebotareva, B. Kurganov, and N. Livanova. Biochemical effects of molecular crowding. *Biochemistry (Moscow)*, 69:1239–1251(13), 2004.
2. V. Danos and C. Laneve. Formal molecular biology. *Theoretical Computer Science*, 325(1):69–110, 2004.
3. D Degenring, M Röhl, and A M Uhrmacher. Discrete Event, Multi-Level Simulation of Metabolite Channeling. *BioSystems*, 75(1–3):29–41, 2004.
4. J. Elf and M. Ehrenberg. Spontaneous separation of bi-stable biochemical systems into spatial domains of opposite phases. *IEE Proceedings Systems Biology*, 1(2):230–236, 2004.
5. D.T. Gillespie. Exact stochastic simulation of coupled chemical reactions. *The journal of physical chemistry*, 81(25):2340–2361, 1977.

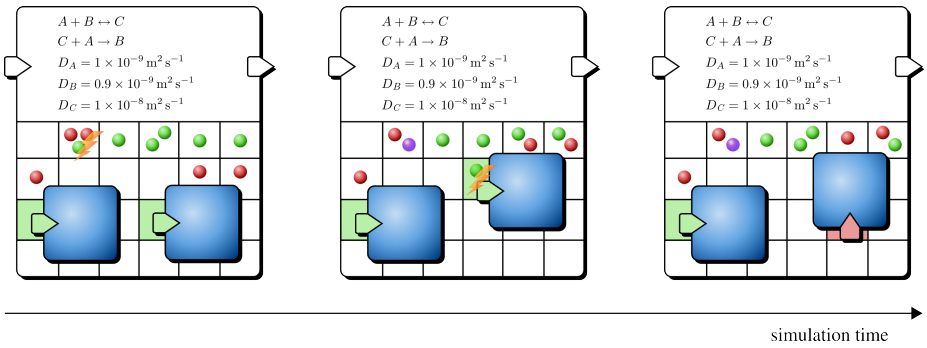


Fig. 3. Schematic overview of the extended ML-DEVS formalism for defining spatial multi-level models. Three different states of a simulated system are shown. Small particles at the population level can diffuse or undergo reactions. Macro-molecules at individual level can move as well. Their ports are connected to specific subvolumes of the grid (same colour) indicating at which location interactions between both levels can take place. The port configuration of an individual macro-molecule might change depending on events like a reaction with a population-based particle.

6. Jan Himmelspach and Adelinde M Uhrmacher. Plug'n simulate. In *Proceedings of the 40th Annual Simulation Symposium*, pages 137–143. IEEE Computer Society, 2007.
7. Matthias Jeschke and Adelinde M Uhrmacher. Multi-resolution spatial simulation. *Poster at the 6th Conference on Computational Methods in Systems Biology (CMSB)*, 2008.
8. Matthias Jeschke and Adelinde M Uhrmacher. Multi-resolution spatial simulation for molecular crowding. In L. Moench S.J. Mason, R.R. Hill and O. Rose, editors, *Proceedings of the 40th conference on Winter simulation*, pages 1384–1392. IEEE Computer Society, 2008.
9. Mathias John, Cédric Lhoussaine, and Joachim Niehren. Dynamic Compartments in the Imperative Pi-Calculus. In Pierpaolo Degano and Roberto Gorrieri, editors, *7th Conference on Computational Methods in Systems Biology (CMSB)*, LNCS. Springer Verlag, 2009.
10. D.V. Nicolau Jr, K. Burrage, R.G. Parton, and J.F. Hancock. Identifying Optimal Lipid Raft Characteristics Required To Promote Nanoscale Protein-Protein Interactions on the Plasma Membrane. *Molecular and cellular biology*, 26(1):313–323, 2006.
11. K. Takahashi, S.N. Arjunan, and M. Tomita. Space in systems biology of signaling pathways–towards intracellular molecular crowding in silico. *FEBS Lett.*, 579:1783–1788, 2005.
12. Adelinde M Uhrmacher, Roland Ewald, Mathias John, Carsten Maus, Matthias Jeschke, and Susanne Biermann. Combining micro and macro-modeling in devs for computational biology. In *Proceedings of the 39th conference on Winter simulation*, pages 871–880. IEEE Press, 2007.
13. B.P. Zeigler, H. Praehofer, and T.G. Kim. *Theory of modeling and simulation*. Academic Press, 2000.

Modeling the Cell Cycle Dependency of the Wnt/β-catenin Signaling Pathway in the Imperative π-Calculus

Orianne Mazemondet[1], Mathias John[2], Carsten Maus[2], Adelinde M. Uhrmacher[2], and Arndt Rolfs[1]

[1] University of Rostock, Albrecht-Kossel-Institute for Neuroregeneration
[2] University of Rostock, Institute of Computer Science, Modeling / Simulation Group

1 Introduction

We develop a model of the Wnt/β-catenin signaling pathway based on wet-lab experiments and existing models [12]. Our goal is to investigate the interplay between this pathway and another biological process, the cell cycle, by quantitatively analyzing the pathway's outcome, i.e. the protein β-catenin concentration. For our purpose some extensions of the model proposed by [12] were made, such as compartmentalization. We also abstracted certain reactions that appear of less interest to the objective of our study in order to keep the model as lean as possible. We chose the Imperative Pi-Calculus to implement our model since we aim to explore its modeling power regarding the inclusion of different sorts of reactions and also reaction kinetics. In this context, we provide, to the best of our knowledge, the first stochastic model that includes Michaelis-Menten kinetics in a Pi-Calculus based approach.

2 The Wnt/β-catenin Signaling Pathway

The Wnt/β-catenin signaling pathway is an intracellular network with decisive impact on neural progenitor cells (NPCs) i.e. on their development into neurons. On the biochemical level, in the absence of Wnt molecules, a degradation complex formed by diverse proteins e.g. Axin, GSK3-β, is binding to β-catenin which is consequently degraded. When Wnt molecules give signal to the receptors, the degradation complex gets deactivated, β-catenin stabilizes and, due to its constant production, increases in the cytosol. Hence β-catenin shuttles into the nucleus were it binds to the T cell factor (TCF) and activates gene transcription.
The cell cycle is a biological process that directs Cell growth. In vitro NPCs asynchronously traverse the different phases of the cell cycle, such that they are not sensitive to the Wnt signal simultaneously. We suspect that this delay influences the results of our studies on the pathway's activity. So far, in this context, it has neither been possible to prevent the cells from traversing the cell cycle, nor to experimentally determine the quantitative impact of the cell cycle on the

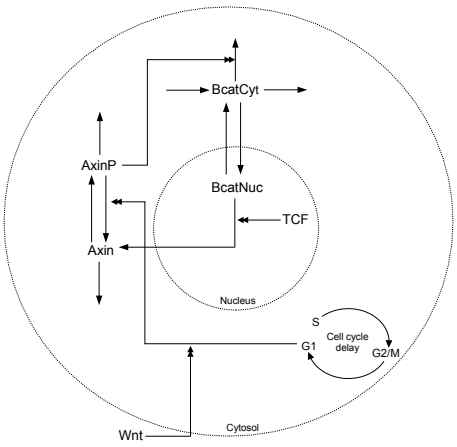


Fig. 1. Schematic overview of the model. Double-headed arrows indicate that reactants are not consumed. Dashed circles indicate the cytosol and nucleus.

measurement of the pathway's activity. The current model shall be a first step toward addressing this question.

3 Wnt Model

Our model describes the Wnt/β-catenin signaling pathway in a single cell with two compartments, the cytosol and the nucleus, represented by concentric spheres, see Figure 1. Following the work in [12], the main components of our model are β-catenin, Axin and TCF. In the cytosol de-/phosphorylated Axin ($Axin/AxinP$) and β-catenin ($BcatCyt$) are located. The nucleus contains species TCF and β-catenin ($BcatNuc$).
Two reactions are defined for Axin ($Axin$): it decays, i.e. the number of $Axin$ is simply reduced by one, and phosphorylates, i.e. it transforms to $AxinP$. Symmetrically, $AxinP$ dephoshporylates and decays. Notice that Axin's dephosphorylation only occurs in presence of the Wnt signal, i.e. when the pathway is active. The activation of the pathway is implemented by a sequence of reactions that mimics the delays of the cell cycle. β-catenin ($BcatCyt$) is produced, i.e. a reaction is defined that increases its amount by one, and decays. The key reaction in the cytosol is the β-catenin degradation, which is mediated by $AxinP$, i.e. the more $AxinP$ exists the faster the reaction. Similarly, in the nucleus located β-catenin ($BcatNuc$) mediates Axin production under consumption of TCF. β-catenin can move from the cytosol to the nucleus, i.e. transform from $BcatCyt$ to $BcatNuc$, and back.
Primarily, Mass action kinetics are assigned to the reactions in our model. Exceptions are given by the Axin mediated degradation of β-catenin and the β-catenin mediated production of Axin. These reactions follow Michaelis-Menten kinetics.

4 Stochastic Parametrization

Parameters needed were mainly taken from different sources in literature [9], [12], [16]. However, most of these were given for deterministic models and needed to be recalculated for our stochastic approach. As compartment volumes are a prerequisite for stochastic parameters we performed additional experiments.
Molecule numbers and stochastic rate constants were calculated according to the method described in [11]. We extracted the initial parameters, such as concentrations and kinetic rate constants from [12]. To calculate diffusion rate constants for the shuttling of β-catenin, we followed the work in [5] and applied Fick's first law to the motion of a single molecule. Therefore, we assumed, that the compartments have the shape of two concentric spheres and that in average a molecule travels between the compartment centers. For the cell cycle delays we took the duration of each phase of the cycle from [1] and combined it with experimental data to obtain the duration of a cycle and the amount of cells in each phase. In our model, the initial phase of a cell and the duration of that phase is randomly chosen with equal distribution. In order to apply Michaelis-Menten kinetics to Axin mediated β-catenin degradation, we had to make two assumptions:

1. the process of β-catenin binding to Axin before degradation is a typical enzymatic reaction. Phosphorylated Axin is the enzyme, β-catenin the substrate. No product exists as β-catenin is degradated.
2. the enzyme concentration is very low compared to the substrate.

Similar assumption apply to the β-catenin mediated production of Axin. These assumptions are not well justified and have to be seen as the basic weakness of our model. However, they allow us to take essential parameters from [12], where similar assumptions were made.

5 Implementing Michaelis-Menten Kinetics in the Imperative Pi-Calculus

In the basic calculus of stochastic biochemistry, [6], reactions have reactants of at most two different species and reaction kinetics follow the law of Mass action, i.e. their reaction rates yield the product of the numbers of reactants and the rate constant. However, modeling complex reaction networks based on these rules requires detailed knowledge about the system under study like binding orders or rate constants, which is usually not given. Thus, abstractions like reactions with Michaelis-Menten kinetics or Hill kinetics are widely used. E.g. in our model Michaelis-Menten kinetics were assigned to β-catenin mediated Axin production and Axin mediated β-catenin degradation. Therefore, these abstractions are supported by basic tools for stochastic modeling, e.g. [3, 7, 4, 2].
In [14, 13, 10] tools are presented that make use of the Pi-Calculus as a formalism for the stochastic modeling of biochemistry. In the Pi-Calculus reaction networks are modeled by mapping molecules to concurrent processes and reactions to process communication, see [15]. Each reaction has exactly two reacting species,

represented by sending and receiving processes. Reaction rates follow the law of Mass action per definition, thereby entirely excluding other kinetics. In this sense, modeling and simulation tools based on the Pi-Calculus lack basic functionality. Here, we show that in an extended Pi-Calculus, called the Imperative Pi-Calculus [8], reactions with Michaelis-Menten kinetics can be implemented. Potentially, this modeling technique defines an approach for implementing general kinetics in the Imperative Pi-Calculus. However, to what extend this is true is subject to current research.
The Imperative Pi-Calculus extends on the Pi-Calculus in two ways: On one hand it introduces a global store, that maps names to values. The store can be accessed by processes that read and assign values. On the other hand, it allows to define functional rate constants, possibly depending on globally stored values. Most importantly, assignments to global names imply an immediate recalculation of all rate constants that depend on the changed value. By this means not only dynamic changes of compartment structures can be modeled, like compartments entering or leaving other compartments, but it is also possible to adapt reaction rates to variable compartment volumes or temperature.
In order to implement Michaelis-Menten kinetics in the Imperative Pi-Calculus, the hard-wired law of Mass action needs to be bypassed. This can be done by modeling all those reactions whose kinetics depend on the substrate, enzyme, or product as communications with only one sender and one receiver. The rates of these communications entirely depend on their rate constants, since they represent reactions, where the amount of each reactant is one. Consequently, functional rate constants can be used to freely define the underlying kinetics, e.g. Mass action or Michaelis-Menten. To trace the amounts of the enzyme, product, and substrate, global names are defined. These are updated, whenever a reaction is performed that involves these species. Provided by the semantics of the Imperative Pi-Calculus, the corresponding rate constants are immediately recalculated. This ensures that all reaction rates are correctly adapted to the current amounts.

6 Conclusion and outlook

Currently, we are in the process of fitting the behavior of our model to the results in [12]. Our later goal is to extend our single cell model to an entire cell population communicating through Wnt molecules propagation. On the technical side, we wish to further explore the generality of our approach for the modeling of Michaelis-Menten kinetics in the Pi-Calculus.

References

1. S. Alam, A. Sen, L. A. Behie, and M.S. Kallos. Cell Cycle Kinetics of Expanding Populations of Neural Stem and Progenitor Cells In Vitro. *Biotechnology and bioengineering*, 88:332–347, 2004.

2. N. Chabrier-Rivier, F. Fages, and S. Soliman. The Biochemical Abstract Machine BIOCHAM. In *Computational Methods in Systems Biology*, pages 172–191, 2004.
3. F. Ciocchetta and J. Hillston. Bio-PEPA: An Extension of the Process Algebra PEPA for Biochemical Networks. *ENTCS*, 194(3):103–117, 2008.
4. Vincent Danos and Cosimo Laneve. Formal Molecular Biology. *TCS*, 325(1):69–110, 2004.
5. J. Elf and M. Ehrenberg. Spontaneous Separation of Bi-Stable Biochemical Systems into Spatial Domains of Opposite Phases. *Systems Biology*, 1(2):230–236, Dec 2004.
6. D.T. Gillespie. A General Method for Numerically Simulating the Stochastic Time Evolution of Coupled Chemical Reactions. *Journal of Computational Physics*, 22(4):403–434, December 1976.
7. M. Heiner, R. Richter, M. Schwarick, and C. Rohr. Snoopy - A Tool to Design and Execute Graph-Based Formalisms. *Petri Net Newsletter*, 74:8–22, April 2008.
8. M. John, C. Lhoussaine, and J. Niehren. Dynamic Compartments in the Imperative π Calculus. In *Computational Methods in Systems Biology, 7th International Conference*, LNCS, 2009 (to appear).
9. E.I. Krieghoff. *Analyzing the Regulation of the Subcellular Localization of beta-catenin by Fluorescence Recovery After Photobleaching*. PhD thesis, Friedrich-Alexander-Universitßt Erlangen-Nßrnberg, 2006.
10. C. Kuttler, C. Lhoussaine, and J. Niehren. A Stochastic Pi Calculus for Concurrent Objects. In *Second International Conference on Algebraic Biology*, number 4545 in LNCS, pages 232–246, July 2007.
11. C. Kuttler and J. Niehren. *Gene Regulation in the Pi Calculus: Simulating Cooperativity at the Lambda Switch*, volume 4230/2006 of *LNCS*, pages 24–55. Springer, 2006.
12. E. Lee, A. Salic, R. Krßger, R. Heinrich, and M. Kirschner. The Roles of APC and Axin Derived from Experimental and Theoretical Analysis of the Wnt Pathway. *PLoS Biol*, 1(1):e10, 10 2003.
13. A. Phillips and L. Cardelli. Efficient, Correct Simulation of Biological Processes in the Stochastic Pi Calculus. In *Computational Methods in Systems Biology*, pages 184–199, 2007.
14. C. Priami, A. Regev, E. Shapiro, and W. Silverman. Application of a Stochastic Name-Passing Calculus to Representation and Simulation of Molecular Processes. *Information Processing Letters*, 80:25–31, 2001.
15. A. Regev and E. Shapiro. The Pi Calculus as an Abstraction for Biomolecular Systems. In G Ciobanu and G Rozenberg, editors, *Modelling in Molecular Biology*, pages 219–266. Springer, 2004.
16. O. Schaeffer. *On the Use of Process Algebra Techniques in Computational Modelling of Cancer Initiation and Development*. PhD thesis, School of Computer Science University of Birmingham, 2008.

Structural Kinetic Modeling of Polyamine Metabolism in Mammals

David Urdiales-Nieto*, Francisco R. Villatoro *, Miguel A. Medina**,

Francisca Sánchez-Jiménez **, and José F. Aldana-Montes*

* Khaos Group, Department of Computer Languages and Computing Science,
University of Malaga, Malaga, Spain
** Procel Group, Department of Molecular Biology and Biochemistry,
University of Malaga, Malaga, Spain
durdiales@lcc.uma.es

Abstract. Structural kinetic modeling is a technique for the study of the stability and robustness of metabolic networks based on constructing a local linear model around a given steady state parametrized by means of the so-called saturation parameters. Such technique is applied to the study of an accurate model of mammalian polyamine metabolism. Our preliminary analysis shows the large robustness of this model requiring at least unrealistic values for three saturation parameters in order to yield instability.

1 Problem Formulation

Structural kinetic modeling [1] is an approach for analysis metabolic networks without recurring to an explicit kinetic model where only stoichiometric information is available. It is based on the analysis of the eigenvalues of the Jacobian matrix of the system of ordinary differential equations given by the model, evaluated at a given steady state. The analysis of bifurcations, including bifurcations of higher codimension, can be exactly determined from the spectrum of eigenvalues of the Jacobian, giving rise to specific dynamical behaviors with biological interest [2,3].

In structural kinetics the model is nondimensionalized using a change of variables which represents the Jacobian matrix in a convenient set of parameters, referred to as saturations, measuring the normalized degree of saturation of each reaction with respect to every substrate at the steady state (this concept is similar to the concept of "effective kinetic order" used in the power-law formalism). Changes in the values of the saturation parameters correspond to changes in the biochemical rate law of each reaction, allowing the analysis, for example, of the effects introduced in the dynamics by changes between competitive and noncompetitive inhibition in the Michaelis-Menten kinetics.

Structural kinetic analysis of metabolic networks can also be applied to metabolic networks with known detailed kinetics in order to quantify their stability and robustness, allowing the determination of their stabilizing sites [4,5]. For example, the

stabilizing effects of allosterically regulated enzymes can be studied by comparing the metabolic network with and without such allosteric regulation by properly changing the values of the corresponding saturation parameters. Moreover, the strength of each regulatory interaction can also be chosen arbitrarily allowing the analysis of its main effects on the whole dynamics.

In this work, the structural kinetic modeling is applied to the analysis of an explicit and detailed kinetic model, that of polyamine metabolism in mammals [6], in order to recover further information on the robustness and stability properties of such a model. We expect to recover some insight into the role of regulation and control in polyamine metabolism.

The contents of this paper are of follows. Section 2 briefly reviews the technique of structural kinetic modeling and presents its application to the polyamine metabolic model. Section 3 is devoted to the main results of this paper and its corresponding discussion. Finally, the last section summarizes the main conclusions and highlights further work.

2 Structural Kinetic Modeling

The dynamical behavior of a metabolic network can be described by a set of differential equations given by

$$\frac{\mathrm{d}\,S(t)}{\mathrm{d}t} = N v(S, k), \tag{1}$$

where S is the m-dimensional vector of biochemical reactants, N the $m \times r$ stoichiometric matrix, and $v(S, k)$ is the r-dimensional vector of reaction rates, nonlinear functions depending on the substrate concentrations S and on a set of kinetical parameters k. Let us assume that this system has at least a positive steady-state S^0, such that $N v(S^0, k) = 0$. In structural kinetics [1], Eq. (1) is rewritten as

$$\frac{\mathrm{d}\,x}{\mathrm{d}t} = \Lambda \mu(x) \tag{2}$$

in terms of new variables $x(t)$ given by the following change of variables

$$x_i(t) := \frac{S_i(t)}{S_i^0}, \qquad \Lambda_{ij} := N_{ij} \frac{v_i(S^0)}{S_i^0}, \qquad \mu_j(x) := \frac{v_j(S)}{v_j(S^0)}, \tag{3}$$

with $i=1,\ldots,m$, and $j=1,\ldots, r$. The corresponding Jacobian of the normalized system at the steady state $x^0 = 1$ is given by

$$J_x = \Lambda \left.\frac{\partial \mu(x)}{\partial x}\right|_{x^0=1} =: \Lambda \theta_x^{\mu}, \tag{4}$$

where the elements $\mathbf{\Lambda}_{ij}$ have the units of an inverse time and consist of the elements of the stoichiometric matrix properly normalized, and each element $\theta_{x_i}^{\mu_j}$ of the matrix θ_x^{μ} measures the normalized degree of saturation of the reaction v_j with respect to a substrate S_i at the steady state S^0 [1]. The stability of the dynamics of Eq. (1) can be

studied by calculating the eigenvalues of the Jacobian as function of the values of the saturations $\theta_{x_i}^{\mu_j}$, which define the physiologically admissible ''parameter space'' of the system.

In this paper, Eqs. (2)-(4) are applied to the mathematical model of mammal polyamine metabolism model presented in Ref. [6]. This model consists of m = 13 time-dependent reactans and r = 21 kinetical reactions. The resulting parameter space for the Jacobian matrix is given by 51 saturations $\theta_{x_i}^{\mu_j}$. The exact expresions of each of the components of the matrix θ_x^{μ} are omitted here for the sake of brevity.

3 Results and Discussion

Polyamine homeostasis is widely known to be very robust to perturbations, as quantified by the sensibility analysis presented in Ref. [6]. Structural kinetics analysis can also be used to quantify such robustness by means of Monte Carlo simulations. The values of the parameters $\theta_{x_i}^{\mu_j}$ can be varied in intervals around the values $\theta_{x_i}^{\mu_j,0}$ given by the parameters of model chosen for simulations in silico shown in Table S1 in Ref. [6]. The intervals have been determined by changing the values of the parameters inside the experimental ranges from the available literature also shown in Table S1 in Ref. [6]. The whole ensemble of 10^5 simulations results in a stable steady state, i.e., the whole set of eigenvalues of the Jacobian have negative real part, except one eigenvalue which is practically null (smaller in absolute value than the machine epsilon).

Structural kinetics allows further analysis of the robustness of the kinetical laws used in the model to changes in the strength of its regulation, since the evaluation of the parameters $\theta_{x_i}^{\mu_j}$ in the intervals given by the largest integer not greater than and the smallest integer not less than $\theta_{x_i}^{\mu_j,0}$, i.e., in $\Im_{x_i}^{\mu_j} = \left(\left\lfloor \theta_{x_i}^{\mu_j,0} \right\rfloor, \left\lceil \theta_{x_i}^{\mu_j,0} \right\rceil \right)$ corresponds to spamming the full interval $[0,\infty)$ for each kinetical parameter. Although such values may be biologically unrealistic, the quantification of the stability of the steady state under such changes is a good indication of the robustness of the metabolic network [4,5]. Our Monte Carlo simulations in which a random value in $\Im_{x_i}^{\mu_j}$ if assigned to every $\theta_{x_i}^{\mu_j}$ shows that the 52.79% of all cases are locally unstable, show at least one eigenvalue with positive real part as shown in Table 1. In fact, only the 0.020%, 2.28%, and 5.24% of all cases show 4, 3, and 2, respectively, unstable eigenvalues, hence only the 7.72% can present Hopf bifurcations and large co-dimension bifurcations. The identification of the concrete combination of $\theta_{x_i}^{\mu_j}$ responsible for such instatibilities is difficult due to combinatorial explosion of the possible cases to be considered.

The possibility that instability due to extreme values of only one, two, or three $\theta_{x_i}^{\mu_j}$ has been studied by Monte Carlo simulations, where all the $\theta_{x_i}^{\mu_j}$ has its steady state value $\theta_{x_i}^{\mu_j,0}$ except one, two, or three receiving random values in the corresponding intervals $\Im_{x_i}^{\mu_j}$. This study has not evidenced any kind of instability when only one or two saturations are changed, assessing the robustness of the model.

Our study has detected a possible source of instability requiring a triple combination of (unrealistic) values of the saturations associated with the rate equations μ_5 and μ_6 of the polyamine oxidase (PAO) for N-acetyl-spermidine (aD) and N-acetyl-spermine (aS), respectively (equations referred to as PAO for aD and PAO for aS in Table 1 of Ref. [6]). Such reactions are modelled by Michaelis equations with competitive inhibition, hence $\theta_{aD}^{\mu 5}$ and $\theta_{aS}^{\mu 6}$ belongs to the interval $[0,1]$ and $\theta_{aD}^{\mu 5}$ and $\theta_{aS}^{\mu 6}$ belongs to $[-1,0]$. Figure 1 shows the separatrices between the regions of instability and instability for $\theta_{aD}^{\mu 5}$ (left plot) and $\theta_{aS}^{\mu 6}$ (right one) as function of $\theta_{aS}^{\mu 5}$ (horizontal axis) and $\theta_{aD}^{\mu 6}$ (vertical ones). Note that each curve is labelled by the value of either $\theta_{aD}^{\mu 5}$ (left plot) or $\theta_{aS}^{\mu 6}$ (right one).

The interpretation of the instability illustrated in Fig. 1 requires a careful analysis of the mathematical expression for the saturations. The saturation of PAO for *aS* with respect to *aS* is given by

$$\theta_{aS}^{\mu 6} = \frac{[aS]^0 / K_{MaS}^{PAO}}{1 + \frac{[aS]^0}{K_{MaS}^{PAO}} + \frac{[aD]^0}{K_{MaD}^{PAO}} + \frac{[D]^0}{K_{MD}^{PAO}} + \frac{[S]^0}{K_{MS}^{PAO}}} \in [0,1],$$

where the 0 superindex indicate concentrations at steady state. The instability onset requires that the reaction of PAO for aS has saturation and order of magnitude smaller than its physiological value, which requires low concentrations of spermidine and spermine in the steady state, which can only be attained in polyamine defficient genetically modified cells. Experimentally, this instability is difficult to observe since the regulation of polyamine metabolism results in the conversion of putrescine into spermidine and spermidine into a spermine. Otherwise, unphysical values for the Michaelis constants K_{MD}^{PAO} and K_{MS}^{PAO}, about two orders of magnitude larger than the experimentally observed ones, are required. Both conditions indicate that the instability observed in our simulations has a limited biological value.

$[0,10^{-5}]$	$[10^{-5},10^{-4}]$	$[10^{-4},10^{-3}]$	$[10^{-3},10^{-2}]$	$[10^{-2},10^{-1}]$	$[10^{-1},1]$	$[1,10]$	Total
0.07%	0.48%	2.26%	1.72%	8.83%	38.11%	1.32%	52.79%

Table 1: Percentage of eigenvalues with positive real part (larger than 10^{-12}).

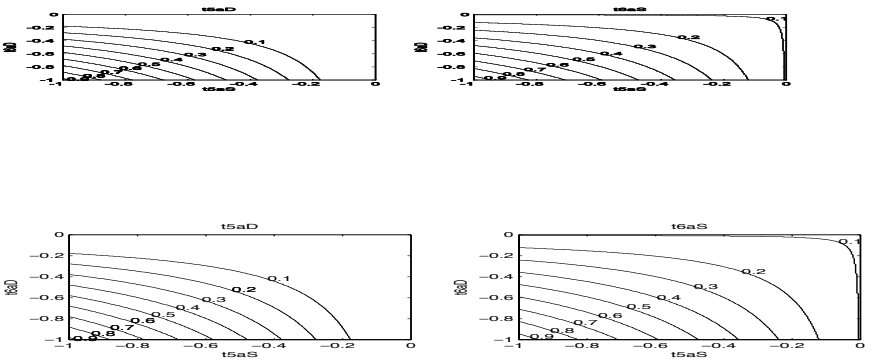


Figure 1: Boundary of the region of instability in the parametric space as function of $\theta_{aD}^{\mu 5}$ and $\theta_{aS}^{\mu 6}$ as function of $\theta_{aS}^{\mu 5}$ and $\theta_{aD}^{\mu 6}$, where the unstable region is behind the each curve in direction to the left-bottom corner of the plot.

4 Conclusions and Future Work

The technique of structural kinetic modeling has been applied to a detailed model of polyamine metabolism in mammals. The robustness of the model on the changes in the values of its parameters has been quantified by the application of a Monte Carlo method to a large ensemble of realizations. The technique has also identified some instabilities in the model but which cannot be reached by biologically relevant values of the parameters.

Further research is required. First, further Monte Carlo simulations are required in order to identify the possibility of Hopf bifurcations of codimension one and two. Second, the analysis of the importance of allosteric regulation must be elucidated. And third, current approach is semiautomatic but apparently easily automatizable resulting in the development of new bioinformatic tools for the analysis of large metabolic networks lacking a detailed kinetic model.

References

1. Steuer, R., Gross, T., Selbig, J., & Blasius B. (2006). *Proc. Natl. Acad. Sci. U. S. A.* 103, 11868-11873.
2. Schuster, S., Fell, D. A. & Dandekar, T. (2000) *Nat. Biotechnol.* **18,** 326–332.
3. Famili, I., Förster, J., Nielsen, J. & Palsson, B. O. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 13134–13139.
4. Steuer, R. Nesi, A. N., Fernie, A. R., Gross, T., Blasius, B., & Selbig, J. (2007) *Bioinformatics* **23**, 1378-1385.
5. Grimbs, S., Selbig, J., Bulik, S., Holzhütter, H.-G., & Steuer, R. (2007) *Mol. Syst. Biol.* **3**, 146.
6. Rodríguez-Caso, C., Montañez, R., Cascante, M., Sánchez-Jiménez, F., & Medina, M.A. (2006) *J. Biol. Chem.* **281**, 21799-21812.

Author Index