

# Logical modelling of cellular networks

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*Organisers:*

Anna Niarakis (anna.niaraki@univ-evry.fr)

Denis Thieffry (thieffry@ens.fr)

*Venue:*

Stavros Niarchos Foundation Cultural Center - Leof. Andrea Siggrou 364, Kallithea 176 74





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Satellite Workshop 6 of 17th European Conference of Computational Biology  
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## Motivation, goals and scope

Logical models have long been used to explore the dynamical behaviors of regulatory networks. This workshop will provide an overview of recent methodological developments and applications on the use of the logical formalism for the modelling of regulatory networks.

Fostered by the Consortium for Logical Models and Tools (CoLoMoTo - <http://colomoto.org>), a consortium dedicated at promoting the exchange of models and the improvement of existing methods, this workshop will cover the following topics:

- Computational methods: attractor identification, model-checking, reduction techniques, network inference, ...
- Modelling tools: GINsim, CellNOpt, BoolSim, MaBoSS, CellCollective, EpiLog ...
- Biological applications: signalling networks, cell differentiation, cell reprogramming...

The program includes two keynote talks by Laurent Tournier (Jouy-en-Josas, France) and by Ina Koch (Frankfurt, Germany), along with 13 contributed talks.

## Keynote 1

### **Analyzing interconnections of asynchronous Boolean networks with biological applications.**

Laurent Tournier

UR1404, MaIAGE (Mathématiques et Informatique Appliquées de Génome à l'Environnement), INRA, Institut National de la Recherche Agronomique, Domaine de Vilvert, Bâtiment 210, F-78350 Jouy-en-Josas, France.

We propose to review some mathematical and computational tools for analyzing interconnections of asynchronous Boolean models of biological regulatory networks. The general idea, rather classic in control theory, is to recover as much information as possible on the dynamics of a large interconnected network from the dynamical behaviors of its smaller modules. From an analytical point of view, this can be viewed as a model reduction technique, where one wants to compute the attractors of a large network by decomposing it into several subnetworks. From a modeling point of view, the concept of interconnection is also very useful to address for instance the interplay between known modules and to test different hypotheses on the nature of their mutual regulatory links. It is particularly well adapted in biology as it exploits the general modularity of biological systems.

## Keynote 2

### Concepts for functional analysis of signaling pathways in complex networks based on Manatee invariants

Ina Koch, Leonie Amstein, Jennifer Scheidel, Jörg Ackermann

Molecular Bioinformatics Group, LOEWE program Ub-Net of the State of Hesse, Cluster of Excellence Frankfurt Macromolecular Complexes, Institute of Computer Science, Johann Wolfgang Goethe-University, Frankfurt am Main, Germany.

Complex regulatory processes often characterize signaling pathways. Paths from the receptor to the cell's response typically contain feedback loops, cross-talks, and / or signal amplification. Often, the underlying biological data are incomplete. In many cases, the kinetic parameters are unknown and hardly accessible, such that kinetic modeling becomes difficult or even impossible. Nevertheless, qualitative or semi-quantitative network models provide useful knowledge, for example, on networks pathways, functional modules, dynamic systems behavior, consistency, and completeness of the network. Especially for signaling pathways in medical applications, a huge amount of qualitative data exist such that the construction of big models becomes possible even if kinetic data are missing.

Semi-quantitative modeling formalisms, such as Petri nets [1], provide besides an intuitive graphical interface many sound analysis techniques. For biochemical networks, methods based on the evaluation of the steady state are important, such as invariant analysis techniques. Their application to metabolic networks as well as to signal transduction networks or gene regulatory systems, demonstrate the usefulness of transition invariants, also known as elementary modes. Invariant analysis allows for decomposition into smaller, biologically meaningful modules and supports to unravel system-wide functional modules [2].

The computation of transition invariants can lead to an interruption of the signaling pathways. Thus, transition invariants do not reflect the complete paths from receptor activation to the cellular response. To combine transition invariants in a proper way, we define Manatee invariants that ensure complete pathways in signaling networks. These Manatee invariants enable to perform in silico knockout analyses of signaling networks.

The first part of the talk focuses on these two concepts of Manatee invariants and the in silico knockout analysis. After a brief introduction of Petri nets, we will define invariants and motivate the concept of Manatee invariants, using a small case study. First, we consider feasible transition invariants [3] as combinations of interrelated transition invariants in a specific way to describe the signal flow from signal reception to cellular response. Within this framework, we introduce Manatee invariants as special case of feasible transition invariants. The second part of the talk introduces the in silico knockout analysis, which is based on Manatee invariants, to, on the one hand, verify the network and, on the other hand, to generate new hypotheses of network behavior [4].

To illustrate the conceptual ideas, we consider a small Petri net model of a part of the TNFR1-mediated NF- $\kappa$ B signaling pathway, modeled using MonaLisa [5, 7]. In the network, especially processes of transcription factor, NF- $\kappa$ B, regulation exhibit feedback loops and amplifications. Such characteristic intertwined structure elements of signaling systems lead to transition invariants that do not cover all entire signal flows from the receptor to the response. We demonstrate that Manatee invariants cover the entire signaling pathway. Applying Manatee invariants, e. g., to the knockout of NF- $\kappa$ B, we are able to detect correct relations to upstream and downstream signaling processes [7].

## References

1. Reisig W (1985) Springer, *Petri Nets: An Introduction*. EATCS Monographs on Theoretical Computer Science, vol. 4.
2. Koch I, Reisig W, Schreiber F (Eds.) (2011). *Modeling in Systems Biology: The Petri Net Approach*. Springer.
3. Sackmann A, Heiner M, Koch I (2006) *BMC Bioinformatics*, 7:482 (2006).
4. Scheidel J, Amstein L, Ackermann J, Dikic I, Koch I (2016) *PLoS Comp Biol* 12(12): e1005200.
5. Einloft J, Ackermann J, Nöthen J, Koch, I (2013) *Bioinformatics* 29: 1469-1470
6. Balazki P, Lindauer K, Einloft J, Ackermann J, Koch I (2015) *BMC Bioinformatics* 16: 215.
7. Amstein L, Ackermann J, Scheidel L, Fulda S, Dikic I, Koch I (2017) *BMC Systems Biol* 11:72.

## **Advances in computational methods for the modelling of signalling networks**

Enio Gierga<sup>1</sup>, Panuwat Trairatphisan<sup>2</sup>, Attila Gabor<sup>2</sup>, Julio Saez-Rodriguez<sup>1,2,3,\*</sup>

<sup>1</sup>RWTH Aachen University, Faculty of Medicine, Joint Research Centre for Computational Biomedicine (JRC-COMBINE), Aachen, Germany.

<sup>2</sup>European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, UK.

<sup>3</sup>Institute for Computational Biomedicine, Heidelberg University, Faculty of Medicine, Bioquant Zentrum, Heidelberg, Germany.

\* Corresponding author: [julio.saez@bioquant.uni-heidelberg.de](mailto:julio.saez@bioquant.uni-heidelberg.de)

The modelling of cell signalling mechanisms offers a huge potential to understand the regulatory processes behind cell behaviour in a normal, treated and disease condition. Many methods to model signalling pathways tend to refine a network from prior knowledge network (PKN) in a way to better reflects the data obtained after perturbing some components of this network. Following this line, we have developed CellNOptR (Terfve et al. BMC Sys Bio 2012; [www.cellnopt.org](http://www.cellnopt.org)) to build models of signalling networks offering a fully detailed mechanistic description of the processes involved. Besides the multiple logic based approaches originally implemented in CellNOpt, newly added features further extend the method in terms of quality of generated models and insights we can obtain from them. A new Integer Linear Programming (ILP) implementation of CellNOpt outperforms the previous stochastic based optimization approach in terms of speed, while at the same time giving the complete family of best scoring models within a user-specified tolerance from the optimal value. We also developed Omnipath, a framework to build curated PKN with prior knowledge from multiple sources (Türei et al. Nat Methods 2016; [www.omnipathdb.org](http://www.omnipathdb.org)) in order to infer additional possible signalling mechanisms despite the canonical ones. Furthermore, a newly implemented probabilistic framework allows a quantitative description of interactions in the network as probabilities, while keeping computational time low. Finally, additional functionalities allow us to perform systematic post-hoc analysis to determine the reliability degree of model parameters. We will show the value of these new features through specific cases where we address previous limitations (i.e. unguaranteed convergence to the optimal solutions, incomplete PKN, reliability of parameters, etc.) to provide improved insight into biomedical questions.

## Deciphering yeast physiology by a multi-scale framework integrating cell cycle and metabolism

Lucas van der Zee<sup>1</sup>, Edoardo Saccenti<sup>2</sup>, Hans V. Westerhoff<sup>1</sup>, Jens Nielsen<sup>3</sup> and Matteo Barberis<sup>1,\*</sup>

<sup>1</sup> Synthetic Systems Biology and Nuclear Organization, University of Amsterdam, Amsterdam, The Netherlands.

<sup>2</sup> Laboratory of Systems and Synthetic Biology, Wageningen University & Research, Wageningen, The Netherlands.

<sup>3</sup> Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden.

\* Corresponding author: [matteo@barberislab.com](mailto:matteo@barberislab.com)

Cell cycle and metabolism are coupled networks. Cell growth and division require synthesis of macromolecules which is dependent on metabolic cues. Conversely, metabolites involved in storage carbon-, lipid- and nucleotide metabolism have been observed to fluctuate periodically during the cell cycle progression. Connections among cell cycle and metabolism, spanning high-throughput and manually curated interactions, have been recently elucidated. Furthermore, computational models of cell cycle and metabolism are being developed for some time. However, to date no effort has been made to integrate, and to investigate the mutual regulation of, these two systems in any organism.

A multi-scale framework is presented that integrates a Boolean cell cycle model with a constraint-based model of metabolism in budding yeast, incorporating mechanistic and high-throughput interactions. Features (directionality and effect) are incorporated for the mechanistic interactions. Conversely, an evolutionary optimization algorithm has been developed to generate models that incorporate high-throughput interactions iteratively, to explore directionality and effect. Model results are verified against metabolic pathway activity and enzyme concentrations. Through Boolean logic, activity of cell cycle nodes activates or inhibits metabolic reactions. Conversely, presence / absence of a metabolic flux promotes / prevents activity of cell cycle nodes, respectively.

Seven known interactions in which cell cycle components regulate metabolic enzymes were used to integrate the two models. Initially, 15% of the flux changes showed similar dynamics to the corresponding proteomics, and only five out of 22 KEGG pathway activity changes were correctly predicted. Implementing the *in silico* framework such that (i) it utilizes trehalose through Nth1 trehalase under the control of the Clb5,6/Cdk1 enzymatic activity (which promotes DNA replication dynamics) and (ii) it allows lipolysis of triacyl glycerol (TAG) under the control of the Cln1,2/Cdk1 enzymatic activity (which promotes budding), increased the alignment of flux changes with enzyme concentration changes to 50%. Omitting combinations of interactions from the framework with the exception of Nth1 activation by Clb5,6/Cdk1 results in the correct prediction of ~50% of the data. This result highlights the importance of storage metabolites for metabolic changes during the growth phase of the cell cycle. The multi-scale model further predicted the production of dTMP to be constrained to the G1/S transition, and that glucans were not produced during early M phase.

After optimizing the high-throughput set of interactions, as well as 18 random sets of interactions by the evolutionary algorithm, the real set of interactions showed higher scores with respect to the proteomic data, as well as a more consistent interaction pattern.



Through machine learning, relevant interactions between cell cycle and metabolism were identified. By engineering the interaction features to represent cell cycle phase and pathway characteristics, design criteria of cell cycle- mediated metabolic regulation maybe predicted. A similar machine learning approach highlights, among others, that sulfur metabolism is more active in S phase, oxidative phosphorylation is more active in G1 and S phases, while the central carbon metabolism is downregulated in G2/M phase.

The first computer model that integrates cell cycle and metabolism in budding yeast reveals marked changes in flux distributions through different cell cycle phases. Predictions of relevant interaction connecting these networks may be tested experimentally. Future integration of interactions in the direction from metabolism to cell cycle may be employed to capture the mechanistic basis of robustness of cell cycle networks, by highlighting metabolic causes of cell cycle arrest.

## Boolean Networks: Beyond Generalized Asynchronicity\*

Thomas Chatain<sup>1</sup>, Stefan Haar<sup>1</sup>, and Loïc Paulevé<sup>2</sup>

<sup>1</sup> LSV, ENS Paris-Saclay, INRIA, CNRS, France.

<sup>2</sup> CNRS & LRI UMR 8623, Univ. Paris-Sud – CNRS, Université Paris-Saclay, 91405 Orsay, France.

Boolean networks (BNs) are widely used to model the qualitative dynamics of biological networks, notably of signalling and gene regulation networks. BNs model dynamics of systems where several components (nodes) interact. They specify for each node an update function to determine its next value according to the configuration (global state) of the network. In addition, an update mode for scheduling the application of functions has to be specified to determine the set of reachable configurations.

A major concern is the impact of this chosen updating mode on model validation [2]. Indeed, it is usual to assess the accordance of a BN with measurement data, including time series: it is expected that the observed behaviours can be reproduced in the abstract model. With this perspective, the computation of reachable configurations in BNs is key. For example, let us assume we observe (in the concrete system) that a given component (e.g., gene) gets eventually activated: if the reachability analysis of the BN concludes that no reachable state has this component active, the model would likely be rejected by the modeller.

In biological applications, the analysis of BNs merely splits into two scientific sub-communities: the one preferring the synchronous updating mode, and the one preferring the (fully) asynchronous updating mode. The generalized asynchronous updating, which subsumes synchronous and asynchronous, seems a good compromise but it received very little attention in practice. It should be noted that most of computational tools rely only on either synchronous or asynchronous modes, which can provide a partial explanation.

Is the generalized asynchronous mode the ultimate updating mode when analysing reachable configurations in BNs for biological systems? If little is known on time and speed features of the system and the reachability analysis with generalized asynchronicity concludes on the absence of the observed state, can we safely invalidate the model?

We exhibit a simple example where the generalized asynchronous updating misses reachable configurations, which correspond to particular, but plausible, behaviours. Thus, the resulting analysis can be misleading on the absence of some behaviours, notably regarding the reachability of attractors, and may lead to rejection of valid models. It is worth noting that the network considered in the example is embedded in many actual models of biological networks.

Then, inspired by semantics of concurrent systems, we define a new updating semantics for BNs which enable new behaviours (new reachable states), that can be caused by specific ordering and duration of updates, for which none of the usual updating modes for Boolean networks can predict.

Our encoding results in BNs for which the asynchronous updating subsumes generalized asynchronicity (hence synchronous and asynchronous updating modes), and which correctly recovers the missing reachable configurations, while preserving key properties of the original BN. Importantly, we demonstrate that our new semantics ensures a correct

Boolean abstraction of any multivalued refinement, as one may expect to achieve when modelling biological systems with no assumption on its time features.

\* Paper published as part of AUTOMATA 2018 conference [1], preprint available at <https://hal.archives-ouvertes.fr/hal-01768359/file/main.pdf>; extended journal paper under review.

## References

1. T. Chatain, S. Haar, and L. Paulevé (2018). Boolean Networks: Beyond Generalized Asynchronicity. *Lecture Notes in Computer Science* 10875: 29-42.
2. M. Noual and S. Sené (2017). Synchronism versus asynchronism in monotonic boolean automata networks. *Natural Computing*.

## **Logical modelling: Inferring structure from dynamics**

Ling Sun, Therese Lorenz, and Alexander Bockmayr

Freie Universität Berlin, FB Mathematik und Informatik, Arnimallee 6, 14195 Berlin, Germany.

We present a theoretical framework for relating the structure and dynamics of gene regulatory networks in the context of the logical modelling formalism of René Thomas. The starting point is a mathematical characterization by necessary and sufficient conditions of asynchronous state transitions graphs among all directed graphs on a discrete state space. Based on this characterization, we develop network inference algorithms that, given an asynchronous state transition graph  $G$ , compute minimal logical models that generate  $G$ . Here, minimal logical model means an interaction graph with a minimal number of edges satisfying certain model conditions together with its logical parameters. In low dimension, we can use these algorithms to explore the structure of all logical models that are able to realize a given dynamic behavior. We illustrate this approach by two small case studies. The first is a simplified MAPK cascade network with homeostatic behavior in the dynamics. The second case study concerns multi-stationarity in cell differentiation.

### **References**

1. Lorenz, Therese. Vergleich von zwei- und mehrwertigen Modellen bioregulatorischer Netzwerke. Diploma Thesis, Freie Universität Berlin, 2011.
2. Lorenz, Therese and Siebert, Heike and Bockmayr, Alexander: Analysis and characterization of asynchronous state transition graphs using extremal states. *Bulletin of Mathematical Biology*, 75/6, 920-938, 2013
3. Streck A, Lorenz T, Siebert H: Minimization and equivalence in multi-valued logical models of regulatory networks. *Natural Computing*, 14/4, 555-566, 2015.
4. Sun, Ling. Relating the structures and dynamics of gene regulatory networks. Doctoral Thesis. Freie Universität Berlin, 2017.

## Automated pipeline for the inference of Boolean models from molecular interaction maps.

Saran Pankaew<sup>1</sup>, Sylvain Soliman<sup>2</sup>, Tomáš Helikar<sup>3</sup> and [Anna Niarakis](#)<sup>1</sup>

<sup>1</sup>GenHotel EA3886, Univ Evry, Université Paris-Saclay, 91025, Evry, France

<sup>2</sup>Inria Saclay-Île-de-France - Équipe Lifeware, France.

<sup>3</sup>Department of Biochemistry, University of Nebraska-Lincoln Lincoln, NE, USA.

The construction of a molecular interaction map and a mathematical model consists mainly of separate tasks due to their different objectives. A molecular interaction map focuses on mechanistic details represented in process description, aiming to serve as disease knowledge base in the form of a map. On the other hand, a Boolean model, which is an abstract representation of the system, emphasizes on the flow of information that is displayed as an activity flow. However, both constructions share information which is interchangeable, such as the mode of influence (activation or inhibition), logic operations (complex formation or basic activation) and most importantly, network topology.

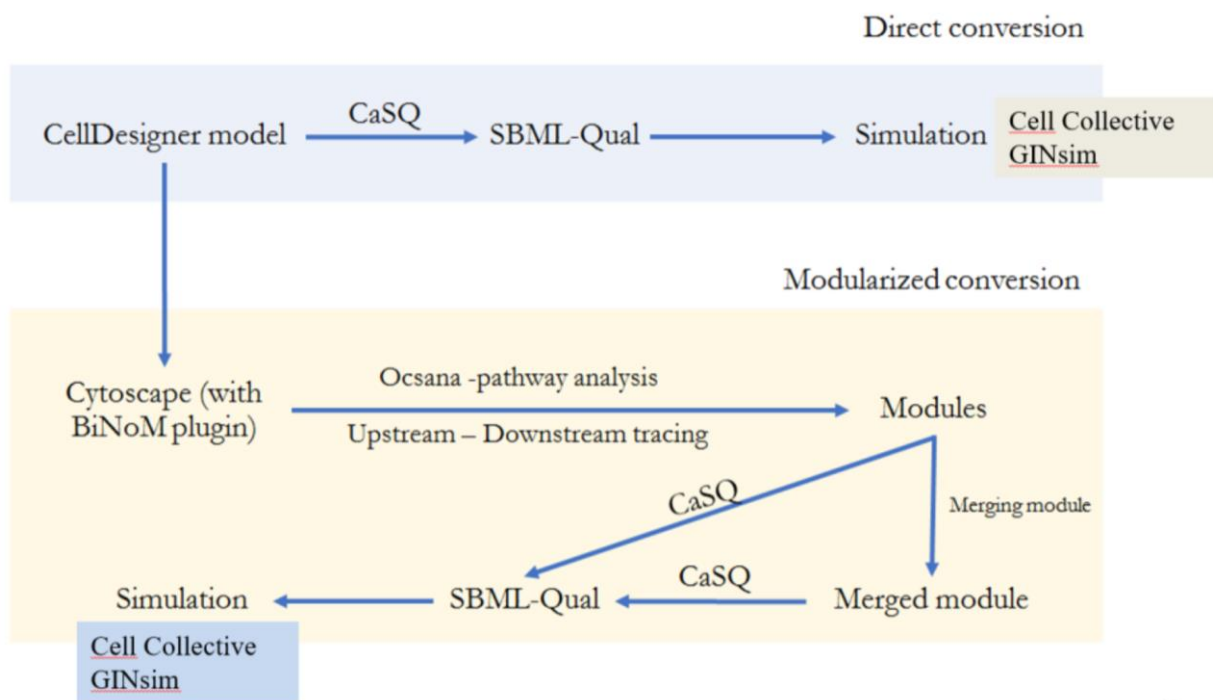
In this project, we are developing an automated pipeline to construct large-scale Boolean models of signal transduction using disease interaction maps as a basis, in a fully automated fashion (Figure 1). The proposed pipeline allows full range of network analysis, from topological features calculation, subnetwork extraction, modularized view, all the way to in depth dynamical analysis and *in silico* simulations. We make use of available tools [1-5] and we also develop a new one, CaSQ that can serve as a bridge between static representations and dynamical models. CaSQ infers preliminary logical (Boolean) rules based on topology and semantics of the disease network. The pipeline is used to infer a signal transduction model for RA synovial fibroblasts (RASFs) starting from a high quality human curated disease map for RA (Singh et al., 2018, unpublished). The goal is to understand in more details how the major functional outcomes of RASFs (apoptosis, pro-inflammatory response, chemokine secretion, bone erosion and matrix degradation) are articulated at the level of the underlying molecular network, and to what extent it might be possible to uncouple these functions and delineate means to control them separately or collectively.

Direct conversion of the disease map to an executable model was successful; however the resulting model was heterogeneous as a consequence of the translation of an integrated global map. In order to substract a cell specific network, we identified four major functional outcomes that concerned RASFs and we extracted the corresponding sub-networks from the original RA disease map. These subnetworks worked as functional modules, comprising each a various number of pathways. Subsequently, the modules were translated into executable Boolean models and simulations were performed in an effort to tune each module separately. The purpose of this procedure is to tune all sub-modules separately and then merge and fine-tune the whole model.

The inferred RASF specific model consists of 252 nodes and 390 interactions. Preliminary results focusing on apoptosis, show that the inferred model is able to reproduce a big number of biological phenomena. More specifically, simulations with FAS/FASL on (engagement of the cell death surface receptor Fas by Fas ligand (FasL) results in apoptotic cell death) resulted in activation of apoptosis while AKT2 (an anti-apoptotic factor) remained inactivated. Systematic testing of the AKT2 pathway revealed that when AKT2 was on the model was able to capture the activation of TNF, IL6, IL8, MMP3, RANKL, CXCL1-3 while FOXO1, BCL2 and BAX remained inactive, results that correlate

fully with the known role of AKT2. The model failed to reproduce MDM2 activation, reflecting a missing reaction in the original map.

More systematic testing is needed in order to obtain a fine-tuned model. In addition, simulations under specific initial conditions coupled with model reductions will be performed in order to calculate attractors of the system. However, the results obtained so far suggest that inference of preliminary Boolean rules based on topology and semantics can reduce significantly the time needed for the construction of a large scale Boolean model.



**Figure 1:** Schematic workflow of the proposed pipeline

## References

1. Funahashi A, Matsuoka Y, Jouraku A, Kitano H, Kikuchi N (2006). Celldesigner: A Modeling Tool for Biochemical Networks. In: Proceedings of the 2006 Winter Simulation Conference, pp. 1707–12.
2. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D et al. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13(11): 2498–504.
3. Bonnet E, Calzone L, Rovera D, Stoll G, Barillot E, Zinovyev A (2013). BiNoM 2.0, a Cytoscape plugin for accessing and analyzing pathways using standard systems biology formats. *BMC Syst Biol* 1: 7:18.
4. Naldi A, Berenguier D, Fauré A, Lopez F, Thieffry D, Chaouiya C (2009). Logical modelling of regulatory networks with GINsim 2.3. *Biosystems* 97 (2):134–9.
5. Helikar T, Kowal B, McClenathan S, Bruckner M, Rowley T, Madrahimov A et al. (2012). The Cell Collective: Toward an open and collaborative approach to systems biology. *BMC Systems Biology* 7;6:96.

## Automated Inference of Gene Regulatory Networks Using Explicit Regulatory Modules.

Clémence Réda<sup>1</sup> and Bartek Wilczynski<sup>2, \*</sup>

<sup>1</sup>Ecole Normale Supérieure Paris-Saclay, Cachan, France.

<sup>2</sup>Faculty of Mathematics, Informatics and Mechanics, Warsaw, Poland.

\*Corresponding author: [creda@ens-paris-saclay.fr](mailto:creda@ens-paris-saclay.fr)

Gene regulatory networks are a popular tool for modelling important biological phenomena, such as cell differentiation or oncogenesis. Efficient identification of the causal connections between genes, their products and regulating transcription factors, is key to understanding how defects in their function may trigger diseases. In recent years, we have seen great improvements in mapping of specific binding sites of many transcription factors to distinct regulatory regions. Recent gene regulatory network models use binding measurements; but usually only to define gene-to-gene interactions, ignoring regulatory module structure. Moreover, current huge amount of transcriptomic data, and exploration of all possible cis-regulatory arrangements which can lead to the same transcriptomic response, makes manual model building both tedious and time-consuming.

In our paper, we propose a method to specify possible cis-regulatory interactions in a given Boolean network, based on transcription factor binding evidence. This is implemented by an algorithm which turns ("expands") a regular Boolean network model into a "Cis Regulatory" Boolean network model, where these interactions are explicitly implemented. This type of model defines regulatory regions as additional nodes in the network. Then, we prove that new, valuable biological insights to the system dynamics can be inferred, while meaningful biological properties of regulatory functions are preserved by this transformation.

We use this modelling framework in order to design a pipeline which automatically enumerates all biological scenarii (as Boolean models) that can explain the experimental data provided. We show that the possibly multiple results obtained for a given dataset can be interpreted as different transcription factor binding site arrangements, and that the modular structure of these solution models allows to better understand the regulatory phenomena at play. This work is a proof-of-concept that current qualitative modelling frameworks can benefit from topological biological knowledge.

## **Dynamical modelling of T cell co-inhibitory pathways to predict anti-tumour responses to checkpoint inhibitors.**

Céline Hernandez<sup>1</sup>, Aurélien Naldi<sup>1</sup>, Wassim Abou-Jaoudé<sup>1</sup>, Guillaume Voisinne<sup>2</sup>, Romain Roncagalli<sup>2</sup>, Bernard Malissen<sup>2</sup>, Morgane Thomas-Chollier<sup>1</sup>, Denis Thieffry<sup>1</sup>

<sup>1</sup>Computational Systems Biology team, Institut de Biologie de l'Ecole Normale Supérieure (IBENS), CNRS UMR8197, INSERM U1024, Ecole Normale Supérieure, PSL Université, 75005 Paris, France.

<sup>2</sup>Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, INSERM U1104, CNRS UMR7280, 13288 Marseille, France.

In recent years, T cells were recognised to often display a reduced ability to eliminate cancer cells, caused by the expression of co-inhibitors at their surface. Antibodies blocking these co-inhibitors (checkpoint inhibitors) have become standard treatment for metastatic melanoma (Simpson et al. 2013), thus leading to a revival in the study of T cell co-inhibitors. However, our understanding of their immunobiology and their harmful role during anti-tumour responses remains fragmentary. Particularly, a mechanistic understanding at the systems-level of T cell function modulation by co-inhibitors has remained elusive.

To overcome these limitations, we aim to delineate the mechanisms through which co-inhibitory molecules, such as PD-1 and CTLA-4, impede T cell functions at the systems-level. To reach this goal, we use computational methods to map and model TCR co-signalling pathways, and ultimately predict cell responses to perturbations. First, we developed comprehensive annotated molecular maps (using the software CellDesigner, <http://www.celldesigner.org>) by curating scientific literature, automated queries to public databases and protein-protein graph reconstruction. Next, using the software GINsim (<http://www.ginsim.org>), these maps and protein networks were translated into a regulatory graph integrating current knowledge. The major challenge was then to properly model concurrent intracellular processes, along with feedback control mechanisms. To cope with this complexity, we explored network modules using a Rule-based formalism (Feret et al. 2009), in order to evaluate concurrent biological hypotheses and to specify logical rules that recapitulate observed component behaviour into the logical model. The resulting integrative model will be used to predict cell response to single or multiple perturbations, thus paving the way to delineate novel experiments, which in turn will be used to refine the maps and model.

This integrated systems-level view of the action mechanisms of key T cell co-inhibitors will provide a further rationale for designing and evaluating drugs targeting T cell co-inhibitory pathways in anti-cancer immunotherapy.



## **Integrative logical and experimental modeling: application to macrophage phenotype transition.**

Julien Dorier<sup>1</sup>, Anne Niknejad<sup>1</sup>, Isaac Crespo<sup>1</sup>, Andreas Roller<sup>2</sup>, Alessia Tarditi<sup>2</sup>, Leon P. Pradel<sup>3</sup>, Daniela Maisel<sup>2</sup>, Nikolaos Berntenis<sup>4</sup>, Robin Liechti<sup>1</sup>, Martin Ebeling<sup>4</sup> and Ioannis Xenarios<sup>1</sup>

<sup>1</sup>Vital-IT, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland.

<sup>2</sup>Pharmaceutical Sciences / Translational Technologies and Bioinformatics, Roche Innovation Center Munich, Germany.

<sup>3</sup>Discovery Oncology, Roche Innovation Center Munich, Germany

<sup>4</sup>Pharmaceutical Sciences / Translational Technologies and Bioinformatics, Roche Innovation Center Basel, Basel, Switzerland.

Contact: julien.dorier@sib.swiss

Macrophages are phagocytic immune cells derived from monocytes involved in both innate and adaptive immunity. During immune response, macrophages can be activated into at least two phenotypes, called M1 and M2, with different activities. M1-like macrophages are phagocytic, present antigens well, produce Th1-type cytokines and are cytotoxic. M2-like macrophages have pro-tumor, angiogenic and immuno-inhibitory effects. Despite their importance in the context of cancer progression, very little is known about macrophage activation mechanisms and how to interfere with them.

To help understand macrophage activation, we have created an in silico model based on a Boolean network framework. This model consists in a collection of networks and was obtained with optimusqual (Dorier et al. BMC Bioinformatics, 2016) using a training set formed by experimental datasets on macrophage activation, combined with a prior knowledge network taken from the literature. This model was used to predict macrophage response to various treatments and suggested possible treatments to drive transitions between macrophage subtypes.

## Logical modelling and analysis of cell adhesion properties along Epithelial to Mesenchymal Transition.

Gianluca Selvaggio<sup>1</sup>, Archana Pawar<sup>1</sup>, Florence Janody<sup>1,2,3</sup> & Claudine Chaouiya<sup>1</sup>

<sup>1</sup>Instituto Gulbenkian de Ciência (IGC), Rua da Quinta Grande 6, Oeiras, Portugal.

<sup>2</sup>Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Rua Alfredo Allen 208, Porto, Portugal.

<sup>3</sup>Instituto de Patologia e Imunologia Molecular da Universidade do Porto (Ipatimup), Rua Dr. Roberto Frias s/n, Porto, Portugal.

Contact: gselvaggio@igc.gulbenkian.pt

Carcinoma cells can reactivate a developmental program called Epithelial to Mesenchymal Transition (EMT). EMT involves a gradual loss of epithelial properties combined with a cumulative acquisition of mesenchymal features (e.g. higher invasiveness, motility, resistance to apoptosis and loss of cell-cell adhesion), which correlate with metastatic capacity of the tumour cells. Since first described in the '80s, a large number of proteins and pathways governing EMT have been identified. However, many questions still remain, e.g. concerning the molecular connections between these EMT regulators or the changes in the microenvironment that are critical.

This work aims to elucidate the series of molecular events leading to this phenotypic transition.

To this end, we defined a logical model of the molecular network involved in the regulation of cell adhesion properties. This network includes EMT key signalling cascades and their components at different levels (e.g. ligands, receptors, intracellular effectors and cellular responses).

Stable states of the model (attractors) qualitatively recapitulate degrees of cell-cell and cell- extracellular matrix (ECM) adhesions, mediated by E-cadherin and Integrins, respectively. Model attractors were thus classified and associated to the corresponding phenotypes (epithelial, mesenchymal, hybrid and transients). Variations of model inputs, representing changes in extracellular conditions, allowed to assess microenvironment influences on cell phenotypic commitments. The capacity of the system to adapt to input variations (plasticity) was assessed using model-checking techniques, producing a predictive reprogramming map. Furthermore, combinatorial model perturbations (knock-out, ectopic expression of network components) were performed to explore their impacts on the reprogramming map and on basins of attraction.

Model analyses suggested that to ensure a full EMT, the SRC oncogene should inhibit the activity of the type IIb family of Receptor Protein Tyrosine Phosphates (RPTP), which mediate homophilic cell-cell interactions. As a consequence, the perturbed model with over-expressing RPTP showed that the mesenchymal phenotype was not anymore acquired upon SRC activation. To test model predictions, we relied on the human mammary epithelial cell line MCF10A with conditional activation of SRC. We observed, as predicted by the model, that SRC downregulated PTPRK, the gene that encodes for RPTP-k, prior to the acquisition of a mesenchymal phenotype. Moreover, preventing the downregulation of RPTP-k by SRC activation restored cell-cell adhesions.

Taken together, our modelling approach permitted to reveal that RPTP-k might be a critical EMT inhibitor downstream of SRC. Furthermore, our model can serve as a tool to probe and explore cellular responses to internal mutations and environmental cues, hence to allow filling in knowledge gaps and supporting hypothesis driven drug development.

## **Identification of Diagnostic and Therapeutic Markers in Tumor Invasion using Logic-based Modeling.**

Faiz M. Khan

Department of Systems Biology and Bioinformatics, University of Rostock, Ulmenstrasse 69 (Building 3, 3rd Floor), Room 402, 18057 Rostock, Germany.

In living cells, a molecule interacts with other molecules in a form of networks which realizes cellular functionality. Mutations in molecular factors perturb the regulation of networks leading to dysregulation of cellular functionality which associates with complex diseases such as cancer. Unraveling mechanisms underlying diseases for the prediction of disease markers and therapeutic candidates has motivated the development of various systems biology approaches. Key challenges in systems biology approaches for mechanistic understanding of diseases are: (i) the large number of interacting components in molecular networks, and (ii) the nonlinear nature of spatio-temporal interactions constituting complex network structures including feedback/feedforward loops.

To address these challenges, I developed an integrative workflow (Khan et al. Nature Comm. 2017) by combining techniques from bioinformatics and systems biology. The workflow combines network structure, omics and biomedical data, and dynamic modeling (logic-based models). Using the proposed workflow, I analyzed large-scale molecular interaction map of E2F1, a transcription factor involved in tumor invasion. It identified core-regulatory networks for epithelial-mesenchymal transition (EMT) in bladder and breast cancers which are amenable for dynamical modeling. Using logic-based modeling formalism, the *in silico* stimulus-response analysis of the core networks detect molecular signatures for each cancer type. Further, I performed *in silico* perturbation experiments to identify therapeutic targets. The predicted molecular signatures and therapeutic targets were validated experimentally and through patient data. The computational analysis of biochemical networks can improve our understanding of disease processes in a mechanistic way. Ultimately, this shall provide the ability to manipulate and optimize processes towards treatment.

## Patient-specific prostate logical models allow clinical stratification of patients and personalized drug treatment.

Arnaud Montagud<sup>1,\*</sup>, Jonas Béal<sup>1</sup>, Pauline Traynard<sup>1</sup>, Luis Tobalina<sup>2</sup>, Julio Sáez-Rodríguez<sup>1,2,3</sup>, Emmanuel Barillot<sup>1</sup> and Laurence Calzone<sup>1</sup>

<sup>1</sup>Institut Curie, Inserm U900, Mines Paris Tech, PSL Research University, F-75005, Paris, France.

<sup>2</sup>RWTH Aachen University, Faculty of Medicine, Joint Research Center for Computational Biomedicine (JRC-COMBINE), MTZ Pauwelsstrasse 19, D-52074 Aachen, Germany.

<sup>3</sup>Institute for Computational Biomedicine, Heidelberg University Hospital, BIOQUANT-Zentrum, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany.

\*corresponding author: arnaud.montagud@curie.fr

Prostate cancer is one of the major diseases in developed countries whose treatment is undermined by its relapse rates. We have developed a logical model of prostate cancer that accounts for 147 nodes and 489 edges and considers major pathways responsible for prostate cancer development, such as antigen receptor, apoptosis, cell cycle, etc. This model has been extensively tested *in silico* by studying all single and double mutants and its robustness using our pipeline of tools [1].

A methodology to instantiate a generic logical model to a set of cancer patients was used to capture their diversity of functioning and response to perturbations by comparing the simulations to clinical data [2]. 333 TCGA prostate samples' data were used to build patient-specific models using our prostate model [3]. These simulations were successfully compared to available clinical data in the form of patients' Gleason score subgrouping. Two highly relevant cancer phenotypes, *Proliferation* and *Apoptosis*, exhibited Gleason-scores-specific probabilities.

Additionally, GDSC cell lines data were collected and prostate-cell-line-specific models were built using our prostate model [4]. We simulated the effects of two drugs (MEK and PI3K inhibitor) in the prostate-specific cell lines models under different growth conditions in order to find proper combinations of drug concentrations in these cell lines.

Present results facilitate the use of logical models in personalized medicine, by allowing the instantiation of patient-specific models, and facilitates the study of patient-specific drug treatments that depend on the specific patient's response. This work received funding from the EU Horizon 2020 program for PrECISE project under grant agreement 668858.

### References

1. A. Montagud *et al.* (2017). Conceptual and computational framework for logical modelling of biological networks deregulated in diseases. *Brief Bioinform* bbx163.
2. J. Béal *et al.* (2018). Instantiation of Patient-Specific Logical Models With Multi-Omics Data Allows Clinical Stratification of Patients. *Front Physiol*, in revision.
3. A. Abeshouse *et al.* (2015). The Molecular Taxonomy of Primary Prostate Cancer. *Cell* 163;:1011-25.
4. M.J. Garnett *et al.* (2012). Systematic identification of genomic markers of drug sensitivity in cancer cells. *Nature* 483: 570-5.

## Dysregulated signaling networks implicated in vascular aging of HIV+ subjects investigated by logical modeling

Lauren Benoodt<sup>1</sup>, Rohith Palli<sup>1</sup>, Adam Cornwell<sup>2</sup>, Meera V. Singh<sup>3</sup>, Alicia Tyrell<sup>5</sup>, Jun-ichi Abe<sup>6</sup>, Giovanni Schifitto<sup>4</sup>, Sanjay B. Maggirwar<sup>3</sup> and Juilee Thakar<sup>1,3,5</sup>

<sup>1</sup>Biophysics and Computational Biology graduate program, <sup>2</sup>Department of Biomedical, Genetics, <sup>3</sup>Department of Microbiology and Immunology, <sup>4</sup>Department of Neurology, <sup>5</sup>Department of Biostatistics and Computational Biology, <sup>6</sup>Department of Cardiology, Division of Internal Medicine, MD Anderson Center.

Large number of HIV infected individuals develop signs of accelerated vascular aging that progress to atherosclerosis (AS). The use of combined antiretroviral therapy (cART) has been thought to contribute to AS by exacerbating multiple cellular processes and underlying signaling mechanisms that are often triggered by HIV infection. However, mechanisms of dysregulation that leads to the development of AS especially in HIV+ subjects are largely unknown.

In order to study signaling networks dysregulated in HIV + subjects on cART we used two pronged approach of integrating logical modeling with experimental validation and exploration. In the first approach, levels of plasma soluble factors from HIV+ and HIV- subjects were measured and signaling networks regulated by these factors were assembled from literature using informatics pipeline developed in python. This network was simulated to study regulation of P-Selectin glycoprotein ligand-1 (PSGL-1) in monocytes since differentiation of monocytes have been associated with development of AS. In the second explorative approach, we recruited HIV+/- subjects with and without AS, aged 50 years or more who are at increased risk of developing measurable changes in markers of AS. We measured a broad panel of pro and anti-inflammatory serum cytokines by Luminex, performed a genome-wide small-RNA and mRNA sequencing to identify micro-RNAs (miRNAs) and mRNA from PBMCs that might be involved in the gene dysregulation. Regulatory network was developed inferring interactions between cytokines, transcription factors and miRNAs using mutual information based metric. A novel algorithm called Boolean Omics Network Invariant-time Analysis (BONITA) was used to investigate dysregulated mechanisms (1). Validations were performed using *in-vitro* molecular biology assays.

The expression of PSGL-1, which has been implicated in adhesion and transmigration during AS was elevated in CD14+ and CD14+CD16+ monocytes following HIV infection and was driven by a combined action of glutamate and sCD40L. Signaling network regulated by glutamate and sCD40L consisted of 155 nodes and 341 edges with an average node degree of 4.4, node clustering coefficient of 0.2, and node betweenness centrality of 0.012. The discrete dynamic model was developed and simulated using asynchronous update algorithm. The simulations were validated using known activity profiles of sCD40L induced signaling molecules. The network was then used to investigate differential activities of signaling molecules upon joint sCD40L and glutamate treatment to identify c-Myc as a potential regulator. The differential activation of c-Myc was validated in *ex vivo* experiments indicating higher activity of c-Myc in HIV+ subjects on cART (2, 3).

In the second approach, the regulatory undirected network was constructed integrating the miRNA, transcription factor activities inferred from mRNA profiling and serum cytokine measurement from 40 subjects. The network was used as an input for BONITA to infer logic rules (1, 4). BONITA is based on variance model that captures cellular heterogeneity leading to higher accuracies in the prediction of logic rules from cross-sectional data. The

analysis identified three miRNAs as key regulators that are known biomarkers of CVD. Additionally, TNF $\alpha$  mediated transcriptional network was revealed to be dysregulated in HIV+AS+ subjects. TNF $\alpha$  has been linked to the pathogenesis of atherosclerosis and differentiation of monocytes. Interestingly also, several micro-RNAs were found to be combinatorically regulated by IL8. Thus the study reveals novel mechanisms of dysregulation of AS in HIV+ subjects.

In conclusion, logic rules based modeling in conjunction with systems serology and clinical genomics approaches have revealed networks that are dysregulated in HIV+ subjects on long-term cART therapy, which might lead to vascular aging. In future, we will validate additional findings, specifically role of IL8 and TNF $\alpha$  mediated signaling in monocytes.

## References

1. Palli, R. and Thakar, J (in preparation). BONITA: Boolean Omics Network Invariant-time Analysis for rule inference and pathway analysis.
2. Connor R, Jones LD, Qiu X, Thakar J\*, Maggirwar SB\* (2017). Frontline Science: c-Myc regulates P-selectin glycoprotein ligand-1 expression in monocytes during HIV-1 infection. *Journal of leukocyte biology* 102(4): 953-64.
3. Anderson CS, DeDiego ML, Topham DJ, Thakar J (2016). Boolean Modeling of Cellular and Molecular Pathways Involved in Influenza Infection. *Computational and Mathematical Methods in Medicine* 2016:7686081.
4. Albert R, Thakar J (2014). Boolean modeling: a logic-based dynamic approach for understanding signaling and regulatory networks and for making useful predictions. *Wiley interdisciplinary reviews. Systems biology and medicine* 6(5):353-69.

## Learning Boolean regulations of a metabolic network: a case-study.

A. Cornet (Irisa, Univ Rennes), C. Barouck (Inra, Toulouse), A. Bockmayr (Frei Berlin Univ), L. CoFret (Inra, Toulouse), L. Paulevé (LRI, Univ Paris Sud), A. Siegel (Irisa, Univ Rennes).

Several technologies (CellNopt [1], Caspo [2,3], Trempi [4]) have been developed to infer Boolean regulations from a prior knowledge network and experimental data such as phosphoproteomics data describing the response of a biological network to several combinations of perturbations of stimuli and inhibitors.

An important feature for the application of these methods is to be able to clamp the values of the stimuli and inhibitors to a fixed value (activated or inactivated) during the total duration of the considered experiment. This is a main limitation for the application of such technics to learn Boolean rules which control the adaptation of metabolic networks to external stimuli, such as observed in a diauxic shift [5].

In this work, we will introduce a prototype of pipeline which could lead to the inference of such Boolean rules from experimental data describing the quantities of metabolites and fluxes in a metabolic network in response to different external metabolic stimuli.

By focusing on a very simplified case-study of the diauxic shift model [4], we show that the regulatory control of the metabolic system can be accurately recovered by combining the resolution of a combinatorial optimization problem and the filtering of candidate solutions with dynamic FBA-based approaches [6]. Compared to the case of signaling networks identified from phosphoproteomics data by minimizing a MSE metric between data and predictions, the study of regulations of metabolic networks requires to reformulate the optimization problem by replacing the MSE metric by a metric taking into account the quantitative disruptions of the metabolic compounds measurements.

We will present the scheme of a generic pipeline allowing to solve this issue by combining and adapting the Caspo time-series software (initially developed for the learning of Boolean rules of signaling networks) [2] and the FlexFlux software (developed for the dynamic-FBA based simulaCon of regulated metabolic network) [7].

## References

1. C Terfve et al. (2012). CellNOptR: a flexible toolkit to train protein signaling networks to data using multiple logic formalisms. *BMC Systems Biology*.
2. Ostrowski, et al. (2016). Boolean network identification from perturbation time series data combining dynamics Abstraction and logic programming. *Biosystems* 149.
3. Videla, S., et al. (2017). Caspo: a toolbox for automated reasoning on the response of logical signaling networks families. *Bioinformatics* 33.
4. Streck A et al. (2016). Data-driven optimization for model checking of multi-valued regulatory networks. *Biosystems*.
5. Covert et al. (2001). Regulation of gene expression in flux balance models of metabolism. *J Theor Biol* 213.
6. Waldherr S et al. (2015). Dynamic optimization of metabolic networks coupled with gene expression *J Theor Biol*.
7. Marmiesse et al. (2015). FlexFlux: combining metabolic flux and regulatory network analyses. *BMC Sys. Biol*.





09h00-09h10	A Niarakis	<i>Welcome and introduction to the workshop</i>
09h10-10h40	C Chaouiya (Oieras) – Chair	<b>Session I: Methods and tools (1)</b>
09h10-09h40	L Tournier (Jouy en Josas )	<i>Analyzing interconnections of asynchronous Boolean networks with biological applications</i>
09h40-10h00	E Gjerga et al (Aachen)	<i>Advances in computational methods for the modelling of signalling networks</i>
10h00-10h20	L van der Zee et al (Amsterdam)	<i>Deciphering yeast physiology by a multi- scale framework integrating cell cycle and metabolism</i>
10h20-10h40	T Chatain et al (Saclay)	<i>Boolean Networks: Beyond Generalized Asynchronicity</i>
10h40-11h10	<b>Coffee/tea break</b>	
11h10-12h40	I Xenarios (Lausanne) – Chair	<b>Session II: Methods and tools (2)</b>
11h10-11h30	L Sun et al (Berlin)	<i>Logical modelling: Inferring structure from dynamics</i>
11h30-11h50	S Pankaew et al (Evry)	<i>Automated pipeline for the inference of Boolean models from molecular interaction maps</i>
11h50-12h10	C Réda & B Wilczynski (Warsow)	<i>Automated Inference of Gene Regulatory Networks Using Explicit Regulatory Modules</i>
12h10-13h30	<b>Lunch break</b>	
13h30-15h00	M Barberis (Amsterdam) – Chair	<b>Session III: Applications (1)</b>
13h30-14h00	I Koch (Frankfort)	<i>Concepts for functional analysis of signaling pathways in complex networks based on Manatee invariants</i>
14h00-14h20	C Hernandez et al (Paris)	<i>Dynamical modelling of T cell co-inhibitory pathways to predict anti-tumour responses to checkpoint inhibitors</i>
14h20-14h40	J Dorier et al (Lausanne)	<i>Integrative logical and experimental modeling: application to macrophage phenotype transition</i>
14h40-15h00	G Selvaggio et al (Oieras)	<i>Logical modelling and analysis of cell adhesion properties along Epithelial to Mesenchymal Transition</i>
15h00-15h20	<b>Coffee/tea break</b>	
15h20-17h00	A Niarakis (Evry) – Chair	<b>Session IV: Applications (2)</b>
15h20-15h40	FM Khan (Rostock)	<i>Identification of Diagnostic and Therapeutic Markers in Tumor Invasion using Logic-based Modeling</i>
15h40-16h00	A Montagud et al (Paris)	<i>Patient-specific prostate logical models allow clinical stratification of patients and personalized drug treatment</i>
16h00-16h20	L Benoodt et al (Rochester)	<i>Dysregulated signaling networks implicated in vascular aging of HIV+ subjects investigated by logical modeling</i>
16h20-16h40	A Cornet et al (Rennes)	<i>Learning Boolean regulations of a metabolic network: a case-study</i>
16h40-	<b>Conclusions, discussion &amp; drinks</b>	