



Activity Report 2018

## **Project-Team LIFEWARE**

Computational systems biology and  
optimization

RESEARCH CENTER  
Saclay - Île-de-France

THEME  
Computational Biology



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## Project-Team LIFEWARE

*Creation of the Team: 2014 January 01, updated into Project-Team: 2015 April 01*

### Keywords:

#### Computer Science and Digital Science:

- A2.1.1. - Semantics of programming languages
- A2.1.5. - Constraint programming
- A2.1.10. - Domain-specific languages
- A2.2.1. - Static analysis
- A2.3.2. - Cyber-physical systems
- A2.4. - Formal method for verification, reliability, certification
  - A2.4.1. - Analysis
  - A2.4.2. - Model-checking
  - A2.4.3. - Proofs
- A3.4.2. - Unsupervised learning
- A3.4.4. - Optimization and learning
- A6.1.1. - Continuous Modeling (PDE, ODE)
- A6.1.2. - Stochastic Modeling
- A6.1.3. - Discrete Modeling (multi-agent, people centered)
- A6.1.4. - Multiscale modeling
- A6.2.4. - Statistical methods
- A6.2.6. - Optimization
- A6.3.1. - Inverse problems
- A6.3.4. - Model reduction
- A7.2. - Logic in Computer Science
- A8.1. - Discrete mathematics, combinatorics
- A8.2. - Optimization
- A8.7. - Graph theory
- A9.7. - AI algorithmics

#### Other Research Topics and Application Domains:

- B1. - Life sciences
  - B1.1.2. - Molecular and cellular biology
  - B1.1.7. - Bioinformatics
  - B1.1.8. - Mathematical biology
  - B1.1.10. - Systems and synthetic biology
- B2. - Health
  - B2.2.3. - Cancer
  - B2.4.1. - Pharmacokinetics and dynamics
  - B2.4.2. - Drug resistance
- B9. - Society and Knowledge

# 1. Team, Visitors, External Collaborators

## Research Scientists

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## 2. Overall Objectives

### 2.1. Overall Objectives

This project aims at developing formal methods and experimental settings for understanding the cell machinery and establishing computational paradigms in cell biology. It is based on the vision of **cells as machines**, **biochemical reaction networks as programs**, and on the use of concepts and tools from computer science to master the complexity of cell processes.

This project addresses fundamental research issues in computer science on the **interplay between structure and dynamics** in large interaction networks, and on **mixed analog-discrete computation**. We contribute to the theory of biochemical computation, and develop since 2002 a modelling, analysis and synthesis software, the Biochemical Abstract Machine, **BIOCHAM**. The reaction rule-based language of this system allows us to reason about biochemical reaction networks at different levels of abstraction, in the stochastic, differential, discrete, Boolean and hybrid semantics of reaction networks. We develop a variety of static analysis methods before going to simulations and dynamical analyses. We use **quantitative temporal logics** as a mean to formalise biological behaviours with imprecise data and to constrain model building or network synthesis.

A **tight integration between dry lab and wet lab efforts** is also essential for the success of the project. This is achieved through tight collaborations with biologists and experimentalists. Furthermore, half of Lifeware is in the **InBio group** at Institut Pasteur headed by Grégory Batt who develops an experimental platform for the closed-loop control of intracellular processes. This platform combines hardware (microfluidic device and microscope), software (cell tracking and model-based predictive control algorithms) and liveware (genetically modified living cells). The originality of this project thus also deals with the recourse to advanced microscopy and synthetic biology technologies to perform accurate observations, modifications and **real-time control** at both **single cell and cell population levels**.

For this to work, collaborations with top international leaders of these techniques have been established, and consolidated with student exchange programs, especially in the framework of the Doctorate School **FIRE, Frontiers in Life Sciences, FdV** to which we are affiliated, in addition to the Doctorate School **Sciences et technologies de l'information et de la communication** (STIC).

Because of the importance of optimization techniques in our research, we keep some activity purely dedicated to optimization problems, in particular on constraint programming methods for computing with partial information systems and solving NP-hard static analysis problems, and on continuous optimization methods for dealing with continuous parameters.

## 3. Research Program

### 3.1. Computational Systems Biology

Bridging the gap between the complexity of biological systems and our capacity to model and **quantitatively predict system behaviors** is a central challenge in systems biology. We believe that a deeper understanding of the concept and theory of biochemical computation is necessary to tackle that challenge. Progress in the theory is necessary for scaling, and enabling the application of static analysis, module identification and decomposition, model reductions, parameter search, and model inference methods to large biochemical reaction systems. A measure of success on this route will be the production of better computational modeling tools for elucidating the complex dynamics of natural biological processes, designing synthetic biological circuits and biosensors, developing novel therapy strategies, and optimizing patient-tailored therapeutics.

Progress on the **coupling of models to data** is also necessary. Our approach based on quantitative temporal logics provides a powerful framework for formalizing experimental observations and using them as formal specification in model building. Key to success is a tight integration between *in vivo* and *in silico* work, and on the mixing of dry and wet experiments, enabled by novel biotechnologies. In particular, the use of microfluidic devices makes it possible to measure behaviors at both single-cell and cell population levels *in vivo*, provided innovative modeling, analysis and control methods are deployed *in silico*.

In synthetic biology, while the construction of simple intracellular circuits has shown feasible, the design of larger, **multicellular systems** is a major open issue. In engineered tissues for example, the behavior results from the subtle interplay between intracellular processes (signal transduction, gene expression) and intercellular processes (contact inhibition, gradient of diffusible molecule), and the question is how should cells be genetically modified such that the desired behavior robustly emerges from cell interactions.

### 3.2. Chemical Reaction Network (CRN) Theory

Feinberg's chemical reaction network theory and Thomas's influence network analyses provide sufficient and/or necessary structural conditions for the existence of multiple steady states and oscillations in regulatory networks. Those conditions can be verified by static analyzers without knowing kinetic parameter values nor making any simulation. In this domain, most of our work consists in analyzing the interplay between the **structure** (Petri net properties, influence graph, subgraph epimorphisms) and the **dynamics** (Boolean, CTMC, ODE, time scale separations) of biochemical reaction systems. In particular, our study of influence graphs of reaction systems, our generalization of Thomas' conditions of multi-stationarity and Soulé's proof to reaction systems<sup>1</sup>, the inference of reaction systems from ODEs<sup>2</sup>, the computation of structural invariants by constraint programming techniques, and the analysis of model reductions by subgraph epimorphisms now provide solid ground for developing static analyzers, using them on a large scale in systems biology, and elucidating modules.

### 3.3. Logical Paradigm for Systems Biology

Our group was among the first ones in 2002 to apply **model-checking** methods to systems biology in order to reason on large molecular interaction networks, such as Kohn's map of the mammalian cell cycle (800 reactions over 500 molecules)<sup>3</sup>. The logical paradigm for systems biology that we have subsequently developed for quantitative models can be summarized by the following identifications :

biological model = transition system  $K$   
 dynamical behavior specification = temporal logic formula  $\phi$   
 model validation = model-checking  $K, s \models \phi$   
 model reduction = sub-model-checking,  $K' \subset K$  s.t.  $K'?$ ,  $s \models \phi$   
 model prediction = formula enumeration,  $\phi$  s.t.  $K, s \models \phi?$   
 static experiment design = symbolic model-checking, state  $s$  s.t.  $K, s? \models \phi$   
 model synthesis = constraint solving  $K?, s \models \phi$   
 dynamic experiment design = constraint solving  $K?, s? \models \phi$

In particular, the definition of a continuous satisfaction degree for **first-order temporal logic** formulae with constraints over the reals, was the key to generalize this approach to quantitative models, opening up the field of model-checking to model optimization<sup>4</sup>. This line of research continues with the development of temporal logic patterns with efficient constraint solvers and their generalization to handle stochastic effects.

<sup>1</sup>Sylvain Soliman. A stronger necessary condition for the multistationarity of chemical reaction networks. *Bulletin of Mathematical Biology*, 75(11):2289–2303, 2013.

<sup>2</sup>François Fages, Steven Gay, Sylvain Soliman. Inferring reaction systems from ordinary differential equations. *Journal of Theoretical Computer Science (TCS)*, Elsevier, 2015, 599, pp.64–78.

<sup>3</sup>N. Chabrier-Rivier, M. Chiaverini, V. Danos, F. Fages, V. Schächter. Modeling and querying biochemical interaction networks. *Theoretical Computer Science*, 325(1):25–44, 2004.

<sup>4</sup>On a continuous degree of satisfaction of temporal logic formulae with applications to systems biology A. Rizk, G. Batt, F. Fages, S. Soliman *International Conference on Computational Methods in Systems Biology*, 251-268



### 3.4. Computer-Aided Design of CRNs for Synthetic Biology

The continuous nature of many protein interactions leads us to consider models of analog computation, and in particular, the recent results in the theory of analog computability and complexity obtained by Amaury Pouly<sup>5</sup> and Olivier Bournez, establish fundamental links with digital computation. In a paper published last year<sup>6</sup> we have derived from these results the Turing completeness result of elementary CRNs (without polymerization) under the differential semantics, closing a long-standing open problem in CRN theory. The proof of this result shows how computable function over the reals, described by Ordinary Differential Equations, namely by Polynomial Initial Value Problems (PIVP), can be compiled into elementary biochemical reactions, furthermore with a notion of analog computation complexity defined as the length of the trajectory to reach a given precision on the result. This opens a whole research avenue to analyze biochemical circuits in Systems Biology, transform behavioural specifications into biochemical reactions for Synthetic Biology, and compare artificial circuits with natural circuits acquired through evolution, from the novel point of view of analog computation and complexity.

### 3.5. Modeling of Phenotypic Heterogeneity in Cellular Processes

Since nearly two decades, a significant interest has grown for getting a quantitative understanding of the functioning of biological systems at the cellular level. Given their complexity, proposing a model accounting for the observed cell responses, or better, predicting novel behaviors, is now regarded as an essential step to validate a proposed mechanism in systems biology. Moreover, the constant improvement of stimulation and observation tools creates a strong push for the development of methods that provide predictions that are increasingly precise (single cell precision) and robust (complex stimulation profiles).

It is now fully apparent that cells do not respond identically to a same stimulation, even when they are all genetically-identical. This phenotypic heterogeneity plays a significant role in a number of problems ranging from cell resistance to anticancer drug treatments to stress adaptation and bet hedging.

Dedicated modeling frameworks, notably **stochastic** modeling frameworks, such as chemical master equations, and **statistic** modeling frameworks, such as ensemble models, are then needed to capture biological variability.

Appropriate mathematical and computational tools should then be employed for the analysis of these models and their calibration to experimental data. One can notably mention **global optimization** tools to search for appropriate parameters within large spaces, **moment closure** approaches to efficiently approximate stochastic models<sup>7</sup>, and (stochastic approximations of) the **expectation maximization** algorithm for the identification of mixed-effects models<sup>8</sup>.

### 3.6. External Control of Cell Processes

External control has been employed since many years to regulate culture growth and other physiological properties. Recently, taking inspiration from developments in synthetic biology, closed loop control has been applied to the regulation of intracellular processes. Such approaches offer unprecedented opportunities to investigate how a cell process dynamical information by maintaining it around specific operating points

<sup>5</sup>Amaury Pouly, "Continuous models of computation: from computability to complexity", PhD Thesis, Ecole Polytechnique, Nov. 2015.

<sup>6</sup>Fages, François, Le Guludec, Guillaume and Bournez, Olivier, Pouly, Amaury. Strong Turing Completeness of Continuous Chemical Reaction Networks and Compilation of Mixed Analog-Digital Programs. In CMSB'17: Proceedings of the fifteen international conference on Computational Methods in Systems Biology, pages 108–127, volume 10545 of Lecture Notes in Computer Science. Springer-Verlag, 2017.

<sup>7</sup>Moment-based inference predicts bimodality in transient gene expression, C. Zechner C, J. Ruess, P. Krenn, S. Pelet, M. Peter, J. Lygeros, and H. Koeppl, Proceedings of the National Academy of Sciences USA, 9(5):109(21):8340-5, 2012

<sup>8</sup>What population reveals about individual cell identity: estimation of single-cell models of gene expression in yeast, A. Llamasi, A.M. Gonzalez-Vargas, C. Versari, E. Cinquemani, G. Ferrari-Trecate, P. Hersen, and G. Batt, PLoS Computational Biology, 9(5): e1003056, 2015

or driving it out of its standard operating conditions. They can also be used to complement and help the development of synthetic biology through the creation of hybrid systems resulting from the interconnection of in vivo and in silico computing devices.

In collaboration with Pascal Hersen (CNRS MSC lab), we developed a platform for gene expression control that enables to control protein concentrations in yeast cells. This platform integrates microfluidic devices enabling long-term observation and rapid change of the cells environment, microscopy for single cell measurements, and software for real-time signal quantification and model based control. We demonstrated in 2012 that this platform enables controlling the level of a fluorescent protein in cells with unprecedented accuracy and for many cell generations <sup>9</sup>.

More recently, motivated by an analogy with a benchmark control problem, the stabilization of an inverted pendulum, we investigated the possibility to balance a genetic toggle switch in the vicinity of its unstable equilibrium configuration. We searched for solutions to balance an individual cell and even an entire population of heterogeneous cells, each harboring a toggle switch <sup>10</sup>.

Independently, in collaboration with colleagues from IST Austria, we investigated the problem of controlling cells, one at a time, by constructing an integrated optogenetic-enabled microscopy platform. It enables experiments that bridge individual and population behaviors. We demonstrated: (i) population structuring by independent closed-loop control of gene expression in many individual cells, (ii) cell–cell variation control during antibiotic perturbation, (iii) hybrid bio-digital circuits in single cells, and freely specifiable digital communication between individual bacteria <sup>11</sup>.

### 3.7. Constraint Solving and Optimization

Constraint solving and optimization methods are important in our research. On the one hand, static analysis of biochemical reaction networks involves solving hard combinatorial optimization problems, for which **constraint programming** techniques have shown particularly successful, often beating dedicated algorithms and allowing to solve large instances from model repositories. On the other hand, parameter search and model calibration problems involve similarly solving hard continuous optimization problems, for which **evolutionary algorithms**, and especially the covariance matrix evolution strategy (**CMA-ES**) <sup>12</sup> have been shown to provide best results in our context, for up to 100 parameters. This has been instrumental in building challenging quantitative models, gaining model-based insights, revisiting admitted assumptions, and contributing to biological knowledge <sup>13 14</sup>.

## 4. Application Domains

<sup>9</sup>Jannis Uhlendorf, Agnès Miermont, Thierry Delaveau, Gilles Charvin, François Fages, Samuel Bottani, Grégory Batt, Pascal Hersen. Long-term model predictive control of gene expression at the population and single-cell levels. *Proceedings of the National Academy of Sciences USA*, 109(35):14271–14276, 2012.

<sup>10</sup>Jean-Baptiste Lugagne, Sebastian Sosa Carrillo and Melanie Kirch, Agnes Köhler, Gregory Batt and Pascal Hersen. Balancing a genetic toggle switch by real-time feedback control and periodic forcing. *Nature Communications*, 8(1):1671, 2017.

<sup>11</sup>Remy Chait, Jakob Ruess, Tobias Bergmiller and Gavsper Tkavcik, Cvalin Guet. Shaping bacterial population behavior through computer-interfaced control of individual cells. *Nature Communications*, 8(1):1535, 2017.

<sup>12</sup>N. Hansen, A. Ostermeier (2001). Completely derandomized self-adaptation in evolution strategies. *Evolutionary Computation*, 9(2) pp. 159–195.

<sup>13</sup>Domitille Heitzler, Guillaume Durand, Nathalie Gallay, Aurélien Rizk, Seungkirl Ahn, Jihee Kim, Jonathan D. Violin, Laurence Dupuy, Christophe Gauthier, Vincent Piketty, Pascale Crépieux, Anne Poupon, Frédérique Clément, François Fages, Robert J. Lefkowitz, Eric Reiter. Competing G protein-coupled receptor kinases balance G protein and  $\beta$ -arrestin signaling. *Molecular Systems Biology*, 8(590), 2012.

<sup>14</sup>Pauline Traynard, Céline Feillet, Sylvain Soliman, Franck Delaunay, François Fages. Model-based Investigation of the Circadian Clock and Cell Cycle Coupling in Mouse Embryonic Fibroblasts: Prediction of RevErb-alpha Up-Regulation during Mitosis. *Biosystems*, 149:59–69, 2016.

## 4.1. Preamble

Our collaborative work on biological applications is expected to serve as a basis for groundbreaking advances in cell functioning understanding, cell monitoring and control, and novel therapy design and optimization. Our collaborations with biologists are focused on **concrete biological questions**, and on the building of predictive models of biological systems to answer them. Furthermore, one important application of our research is the development of a **modeling software** for computational systems biology.

## 4.2. Modeling software for systems biology and synthetic biology

Since 2002, we develop an open-source software environment for modeling and analyzing biochemical reaction systems. This software, called the Biochemical Abstract Machine (**BIOCHAM**), is compatible with SBML for importing and exporting models from repositories such as BioModels. It can perform a variety of static analyses, specify behaviors in Boolean or quantitative temporal logics, search parameter values satisfying temporal constraints, and make various simulations. While the primary reason of this development effort is to be able to **implement our ideas and experiment them quickly on a large scale**, BIOCHAM is used by other groups either for building models, for comparing techniques, or for teaching (see statistics in software section). BIOCHAM-WEB is a web application which makes it possible to use BIOCHAM without any installation. We plan to continue developing BIOCHAM for these different purposes and improve the software quality.

## 4.3. Coupled models of the cell cycle and the circadian clock

Recent advances in cancer chronotherapy techniques support the evidence that there exist important links between the cell cycle and the circadian clock genes. One purpose for modeling these links is to better understand how to efficiently target malignant cells depending on the phase of the day and patient characteristics. These questions are at the heart of our collaboration with Franck Delaunay (CNRS Nice) and Francis Lévi (Univ. Warwick, GB, formerly INSERM Hopital Paul Brousse, Villejuif) and of our participation in the ANR **HYCLOCK** project and in the submitted EU H2020 C2SyM proposal, following the former EU EraNet Sysbio **C5Sys** and FP6 **TEMPO** projects. In the past, we developed a coupled model of the Cell Cycle, Circadian Clock, DNA Repair System, Irinotecan Metabolism and Exposure Control under Temporal Logic Constraints<sup>15</sup>. We now focus on the bidirectional coupling between the cell cycle and the circadian clock and expect to gain fundamental insights on this complex coupling from computational modeling and single-cell experiments.

## 4.4. Biosensor design and implementation in non-living protocells

In collaboration with Franck Molina (CNRS, Sys2Diag, Montpellier) and Jie-Hong Jiang (NTU, Taiwan) we ambition to apply our techniques to the design and implementation of biosensors in non-living vesicles for medical applications. Our approach is based on purely protein computation and on our ability to compile controllers and programs in biochemical reactions. The realization will be prototyped using a microfluidic device at CNRS Sys2Diag which will allow us to precisely control the size of the vesicles and the concentrations of the injected proteins. It is worth noting that the choice of non-living chassis, in contrast to living cells in synthetic biology, is particularly appealing for security considerations and compliance to forthcoming EU regulation.

## 4.5. Functional characterization of the resistance of bacterial populations to antimicrobial treatments

Antibiotic resistance is becoming a problem of central importance at a global level. Two mechanisms are at the origin of non-susceptibility to antimicrobial treatments. The first one comes from adaptation of bacterial cells

<sup>15</sup>Elisabetta De Maria, François Fages, Aurélien Rizk, Sylvain Soliman. Design, Optimization, and Predictions of a Coupled Model of the Cell Cycle, Circadian Clock, DNA Repair System, Irinotecan Metabolism and Exposure Control under Temporal Logic Constraints. *Theoretical Computer Science*, 412(21):2108-2127, 2011.

to antibacterial treatments, notably through the modification of efflux pumps or the expression of enzymes that degrade the antibiotics. Cells are individually resistant. The second one, typically found in resistances to  $\beta$ -lactams, a broad class of antibiotics, originates from the release in the environment of the antibiotic degrading enzymes by the dead cells. This leads to population effects by which cells become collectively resilient.

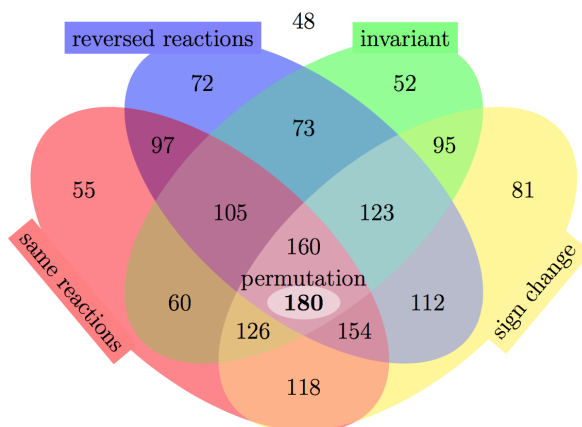
The functional characterization of these different effects is important for the best use of antibiotics (antibiotic stewardship). In collaboration with Lingchong You (Duke University) and with Philippe Glaser (Institut Pasteur), we develop experimental platforms, models, and optimal model calibration methods that gives precise estimations of individual resistance and collective resilience of bacterial populations to antibiotic treatments.

## 5. Highlights of the Year

### 5.1. Highlights of the Year

- **Multistationarity Analysis in CRNs**

The non-existence of multiple steady states in very large chemical reaction networks, out of reach of symbolic computation methods, can be predicted by a remarkably fast graph rewriting algorithm, based on Soliman 2013's theorem<sup>16</sup>. Study published in the *Journal of Theoretical Biology* [1] (graphical abstract in Fig. 1).



Number of models for which multistationarity can be ruled out by using original Thomas's positive circuit condition and Soliman's label conditions respectively among the 506 curated reaction models of BioModels.

Figure 1. Graphical abstract of [1].

- **Distinguishing resistance from resilience to prolong antibiotic potency**

<sup>16</sup>Sylvain Soliman. A stronger necessary condition for the multistationarity of chemical reaction networks. *Bulletin of Mathematical Biology*, 75(11):2289–2303, 2013.

Biomedical engineers at Duke University, in collaboration with Grégory Batt and Virgile Andréani, have shown experimentally that there is more than one flavor of antibiotic resistance and that it could – and should – be taken advantage of to keep first-line antibiotics in our medical arsenal. While an individual bacterium can be resistant to antibiotics, resilience only arises within a community. This happens when bacterial cells produce enough beta-lactamases to degrade the antibiotics, but not enough to save themselves from the initial onslaught. As some cells die and release more and more of the enzyme, the population as a whole eventually rids their environment of the antibiotic. Study published in *Science Advances* [6].

- **Biochemical Programs in Synthetic Cell-like Microreactors**

Researchers at Lab. CNRS-ALCEDIAG Sys2Diag in Montpellier, in collaboration with François Fages, have shown that an algorithm for the differential diagnosis of diabetes can be specified by three Boolean circuits and robustly implemented with real enzymes encapsulated in artificial vesicles that become fluorescent according to 5 different forms of diabetes. The robustness of the circuit was optimized in BIOCHAM by optimizing the initial concentrations of the enzymes with respect to a behavior specification in quantitative temporal logic. The protocells built with a microfluidic device were validated on a cohort of patients' urines from Montpellier's Hospital. Study published in *Molecular Systems Biology* [3] (see Fig. 2).



Figure 2. Artistic illustration by Courbet in cover page of *Molecular Systems Biology* [3].

### 5.1.1. Awards

- **La Recherche magazine 2019 Award - mention Information Sciences**

The article<sup>6</sup> “Strong Turing Completeness of Continuous Chemical Reaction Networks and Compilation of Mixed Analog-Digital Programs” by F. Fages, G. Le Guludec, O. Bournez and A. Pouly, presented and awarded Best Paper at CMSB’17 last year has received the 2019 Award of magazine “La Recherche” - in Information Sciences.

## 6. New Software and Platforms

### 6.1. BIOCHAM

*The Biochemical Abstract Machine*

KEYWORDS: Systems Biology - Bioinformatics

**FUNCTIONAL DESCRIPTION:** The Biochemical Abstract Machine (BIOCHAM) is a software environment for modeling, analyzing and synthesizing biochemical reaction networks (CRNs) with respect to a formal specification of the observed or desired behavior of a biochemical system. BIOCHAM is compatible with the Systems Biology Markup Language (SBML) and contains some unique features about formal specifications in quantitative temporal logic, sensitivity and robustness analyses and parameter search in high dimension w.r.t. behavioral specifications, static analyses, and synthesis of CRNs.

**RELEASE FUNCTIONAL DESCRIPTION:** influence networks with forces – PAC learning of influence networks from time series data – synthesis of continuous reaction networks for mathematical functions defined by polynomial differential equations – complete modular rewriting of Biocham in SWI-Prolog

- Participants: François Fages, David Coudrin, Sylvain Soliman and Thierry Martinez
- Contact: François Fages
- URL: <http://lifeware.inria.fr/biocham4/>

## 6.2. Platforms

### 6.2.1. Smart experimental platforms to automate microbiology experiments

Models play a central role in our work, either to test our understanding or to guide the design of novel systems. Model development and parameter calibration necessitate informative experiments. We develop methods to assist with the optimal design of experiments. In consequence, we have to perform, in sequence or in parallel, experiments with possibly complex input profiles. This led us to develop experimental platforms that allow for flexible and automated stimulations and measurements. Three platforms are being developed, based on (i) a microplate photometer, (ii) a bioreactor platform coupled with a flow cytometer, and (iii) a microscope equipped with microfluidic systems, respectively. In all cases, the real-time measurement and actuation capabilities allow for making reactive experiments, notably including real-time control experiments.

## 7. New Results

### 7.1. Graphical Requirements for Multistationarity in CRNs and their Verification in BioModels

**Participants:** Adrien Baudier, François Fages, Sylvain Soliman.

Thomas's necessary conditions for the existence of multiple steady states in gene networks have been proved by Soulé with high generality for dynamical systems defined by differential equations. When applied to (protein) reaction networks however, those conditions do not provide information since they are trivially satisfied as soon as there is a bimolecular or a reversible reaction. Refined graphical requirements have been proposed to deal with such cases. In [1], we present for the first time a graph rewriting algorithm for checking the refined conditions given by Soliman, and evaluate its practical performance by applying it systematically to the curated branch of the BioModels repository. This algorithm analyzes all reaction networks (of size up to 430 species) in less than 0.05 second per network, and permits to conclude to the absence of multistationarity in 160 networks over 506. The short computation times obtained in this graphical approach are in sharp contrast to the Jacobian-based symbolic computation approach. We also discuss the case of one extra graphical condition by arc rewiring that allows us to conclude on 20 more networks of this benchmark but with a high computational cost. Finally, we study with some details the case of phosphorylation cycles and MAPK signalling models which show the importance of modelling the intermediate complexations with the enzymes in order to correctly analyze the multistationarity capabilities of such biochemical reaction networks.

### 7.2. Influence Networks compared with CRNs: Semantics, Expressivity and Attractors

**Participants:** François Fages, Thierry Martinez [former member], David Rosenblueth [former member], Sylvain Soliman, Denis Thieffry.

Biochemical reaction networks are one of the most widely used formalism in systems biology to describe the molecular mechanisms of high-level cell processes. However modellers also reason with influence diagrams to represent the positive and negative influences between molecular species and may find an influence network useful in the process of building a reaction network. In [4], we introduce a formalism of influence networks with forces, and equip it with a hierarchy of Boolean, Petri net, stochastic and differential semantics, similarly to reaction networks with rates. We show that the expressive power of influence networks is the same as that of reaction networks under the differential semantics, but weaker under the discrete semantics. Furthermore, the hierarchy of semantics leads us to consider a (positive) Boolean semantics without test for absence, that we compare with the (negative) Boolean semantics with test for absence of gene regulatory networks à la Thomas. We study the monotonicity properties of the positive semantics and derive from them an algorithm to compute attractors in both the positive and negative Boolean semantics. We illustrate our results on models of the literature about the p53/Mdm2 DNA damage repair system, the circadian clock, and the influence of MAPK signaling on cell-fate decision in urinary bladder cancer.

As an application, in [11] methods are shown to add dynamics to large molecular influence maps.

### 7.3. Reducing CRNs by Tropicalization

**Participants:** Eléonore Bellot, François Fages, Aymeric Quesne, Sylvain Soliman, Elliott Suits.

We have shown in the past that model reduction relationships between CRNs can be detected on a large scale by the graph matching notion of subgraph epimorphism<sup>17</sup>, furthermore quite efficiently with constraint programming or SAT solving techniques. However this approach does not allow us to actually reduce models. In the framework of the ANR-DFG SYMBIONT project [10] we are investigating model reduction methods based on tropicalization and constraint programming techniques<sup>18</sup> together with correctness conditions based on Tikhonov theorem.

### 7.4. Compiling mathematical functions and programs in CRNs

**Participants:** Auriane Cozic, Elisabeth Degrand, François Fages, Mathieu Hemery, Wei-Chih Huang, Lena Le Quellec, Sylvain Soliman.

In a previous paper, we have proven that any computable function over the reals in the sense of computable analysis (i.e. computable with finite yet arbitrary precision by a Turing machine) is computable by a continuous CRN over a finite set of molecular species. In this approach, the real-valued molecular concentrations are the information carriers and computation can be purely analog. We have derived from the proof of this result a compiler of real functions (of either time or input concentrations) specified by polynomial initial value problems (PIVP) in elementary CRNs. This compiler makes it possible to automate the design of abstract CRNs for implementing arbitrary computable functions over the reals presented by PIVPs, in particular arithmetic, trigonometric, sigmoid and logical functions. The compilation of sequentiality, program control flows and mixed analog-digital imperative programs lead us however to consider more efficient implementations of Heavyside functions with simple CRNs that have no simple mathematical expression as input/output functions. Our goal is to develop a compiler of high-level mixed analog-digital programs in efficient abstract CRNs amenable to practical implementation with real enzymes in DNA-free vesicles, as illustrated in Section 7.8.

### 7.5. Evolving CRNs from data time series

**Participants:** Elisabeth Degrand, François Fages, Jérémy Grignard [former&future Member], Mathieu Hemery, Sylvain Soliman.

<sup>17</sup>Steven Gay, François Fages, Thierry Martinez, Sylvain Soliman, Christine Solnon. On the subgraph Epimorphism Problem. *Discrete Applied Mathematics*, 162:214–228, 2014.

<sup>18</sup>Sylvain Soliman, François Fages, Ovidiu Radulescu. A constraint solving approach to model reduction by tropical equilibration. *Algorithms for Molecular Biology*, 9(24), 2014.

Another approach to CRN design is by evolutionary algorithms. Given a function given by its graph with a finite set of points, and using the same framework based on PIVPs as above, we have designed a genetic algorithm which interleaves the evolution of a population of PIVPs with parameter optimization using CMA-ES for fitting the input curve. On the cosine function, this algorithm recovers PIVPs equivalent to the standard PIVP for cosine, while on Heavyside functions, the algorithm finds (mathematically mysterious) CRNs that are much simpler than Hill functions of high order for instance.

## 7.6. Learning CRNs from data time series

**Participants:** François Fages, Jérémy Grignard [former&future Member], Nicolas Levy, Julien Martinelli, Sylvain Soliman.

The problem of learning a mechanistic model from observed data is more difficult than learning a blackbox model fitting the data, due to the difference between causal relationships and correlations. In a biological context, learning a mechanistic model from experimental data would help understanding the underlying biological processes. To that end, considering multiple time series data generated by a hidden CRN from different initial states (by either stochastic or differential simulation), we develop a clustering-based algorithm for the inference of biological reaction networks. The output is a set of reactions which can be used to generate new traces. A model selection method is derived from these newly generated traces. We evaluate the performance of this algorithm on a range of models from Biomodels.

## 7.7. Optimizing CRN robustness

**Participants:** François Fages, Lucia Nasti, Sylvain Soliman.

In [7] we present two complementary notions of robustness of a system with respect to a property of its behaviour expressed in temporal logic: first the statistical notion of model robustness to parameter perturbations, defined as its mean functionality; and second, a metric notion of formula satisfaction robustness, defined as the penetration depth in the validity domain of the temporal logic constraints. We show how the formula robustness can be used in BIOCHAM-4 with no extra cost as an objective function in the parameter optimization procedure, to actually improve CRN robustness. We illustrate these unique features with a classical example of the hybrid systems community and provide some performance figures on a model of MAPK signalling with 37 parameters.

## 7.8. Robust biochemical programming of synthetic microreactors

**Participants:** Auriane Cozic, François Fages, Wei-Chih Huang, Lena Le Quellec, Lucia Nasti, Sylvain Soliman.

Biological systems have evolved efficient sensing and decision-making mechanisms to maximize fitness in changing molecular environments. Synthetic biologists have exploited these capabilities to engineer control on information and energy processing in living cells. While engineered organisms pose important technological and ethical challenges, de novo assembly of non-living biomolecular devices could offer promising avenues towards various real-world applications. However, assembling biochemical parts into functional information processing systems has remained challenging due to extensive multidimensional parameter spaces that must be sampled comprehensively in order to identify robust, specification compliant molecular implementations. In [3], we introduce a systematic methodology based on automated computational design and microfluidics enabling the programming of synthetic cell-like microreactors embedding biochemical logic circuits, or protosensors, to perform accurate biosensing and biocomputing operations in vitro according to temporal logic specifications. We show that proof-of-concept protosensors integrating diagnostic algorithms detect specific patterns of biomarkers in human clinical samples. Protosensors may enable novel approaches to medicine and represent a step towards autonomous micromachines capable of precise interfacing of human physiology or other complex biological environments, ecosystems or industrial bioprocesses.



## 7.9. Identification of individual cells from z-stacks of bright-field microscopy images

**Participants:** Grégory Batt, Chiara Fracassi [former Member], Jean-Baptiste Lugagne [former Member].

Obtaining single cell data from time-lapse microscopy images is critical for quantitative biology, but bottlenecks in cell identification and segmentation must be overcome. In [5], we propose a novel, versatile method that uses machine learning classifiers to identify cell morphologies from z-stack bright-field microscopy images. We show that axial information is enough to successfully classify the pixels of an image, without the need to consider in-focus morphological features. This fast, robust method can be used to identify different cell morphologies, including the features of *E. coli*, *S. cerevisiae* and epithelial cells, even in mixed cultures. Our method demonstrates the potential of acquiring and processing Z-stacks for single-layer, single-cell imaging and segmentation.

## 7.10. Applying ecological resistance and resilience to dissect bacterial antibiotic responses

**Participants:** Virgile Andreani, Grégory Batt.

An essential property of microbial communities is the ability to survive a disturbance. Survival can be achieved through resistance, the ability to absorb effects of a disturbance without a notable change, or resilience, the ability to recover after being perturbed by a disturbance. These concepts have long been applied to the analysis of ecological systems, although their interpretations are often subject to debate. In [6], we show that this framework readily lends itself to the dissection of the bacterial response to antibiotic treatment, where both terms can be unambiguously defined. The ability to tolerate the antibiotic treatment in the short term corresponds to resistance, which primarily depends on traits associated with individual cells. In contrast, the ability to recover after being perturbed by an antibiotic corresponds to resilience, which primarily depends on traits associated with the population. This framework effectively reveals the phenotypic signatures of bacterial pathogens expressing extended-spectrum  $\beta$ -lactamases when treated by a  $\beta$ -lactamase antibiotic. Our analysis has implications for optimizing treatment of these pathogens using a combination of a  $\beta$ -lactamase and a  $\beta$ -lactamase inhibitor. In particular, our results underscore the need to dynamically optimize combination treatments based on the quantitative features of the bacterial response to the antibiotic or the Bla inhibitor.

# 8. Partnerships and Cooperations

## 8.1. National Initiatives

### 8.1.1. ANR Projects

- ANR-FWF CyberCircuits (2018-2022): “Cybergenetic circuits to test composability of gene networks”, co-coordinated by C. Guet (IST Austria, Klosterneuburg, Austria) and J. Ruesch (Inria EPI Lifeware);
- ANR-DFG SYMBIONT (2018-2021) on “Symbolic Methods for Biological Systems”, coordinated by T. Sturm (CNRS, LORIA, Nancy, France) and A. Weber (Univ. Bonn, Germany) with F. Fages and F. Boulier (U. Lille), O. Radulescu (U. Montpellier), A. Schuppert (RWTH Aachen), S. Walcher (RWTH Aachen), W. Seiler (U. Kassel);
- ANR-MOST BIOPSY (2016-2020) on “Biochemical Programming System”, coordinated by F. Molina (CNRS, Sys2diag, Montpellier) and J.H. Jiang (National Taiwan University), with F. Fages;
- ANR MEMIP (2016-2020) on “Mixed-Effects Models of Intracellular Processes”, coordinated by G. Batt, with P. Hersen, (CNRS/Paris7), E. Cinquemani (Inria EPI IBIS) and M. Lavielle (Inria/CNRS/Polytechnique, EPI XPOP);

- ANR **COGEX** (2016-2019) on “Computer Aided Control of Gene Expression” coordinated by P. Hersen (MSC lab, CNRS/Paris7), with G. Batt and G. Truan (LISBP, CNRS/INSA);
- ANR Blanc **HYCLOCK** (2014-2018) on “Hybrid modeling of time for Circadian Clock Biology and Chronopharmacology”, coordinated by F. Delaunay (CNRS, Nice), with F. Lévi (INSERM Paris-Sud), G. Bernot (CNRS I3S, Nice), O. Roux (Ecole Centrale Nantes), F. Fages and S. Soliman;
- ANR Blanc **STOCH-MC** (2014-2018) on “Stochastic Models: Scalable Model Checking”, coordinated by Blaise Genest (Inria Rennes), with Grégory Batt, Wieslaw Zielonka (LIAFA), and Hugo Gimbert (LaBRI).

### 8.1.2. Inria Project Lab

- IPL **COSY** (2017-2021) “real-time control of synthetic microbial communities”, coordinated by Eugenio Cinquemani (Ibis, Inria), with Jean-Luc Gouzé (Biocore, Inria), Gregory Batt, Frédéric Bonnans (Commands, Inria), Efimov Denis (Non-A, Inria), and Hans Geiselmann (BIOP, Université Grenoble-Alpes), Beatrice Laroche (Maiaage, Inra Jouy-en-Josas).

## 8.2. European Initiatives

### 8.2.1. H2020 Projects

- H2020 FET-OPEN **COSY-BIO** (2017-2020), “Control Engineering of Biological Systems for Reliable Synthetic Biology Applications”, coordinated by Diego di Bernardo (Tigem), with Filippo Menolascina (Edinburgh U), Mario di Bernardo (Naples U), Pascal Hersen (Paris7 U), Mustafa Khammash (ETHZ), Gregory Batt, Guy-Bart Stan (Imperial College), and Lucia Marucci (Bristol U).

## 8.3. International Research Visitors

### 8.3.1. Visits of International Scientists

The following researchers have been invited for short visits

- Carlo Spaccasassi, Microsoft Research Cambridge, UK
- Debdas Paul, Univ. Stuttgart, Germany

#### 8.3.1.1. Internships

Lucia Nasti, PhD candidate at the Università di Pisa, Italy, is visiting our group for 4 months.

### 8.3.2. Visits to International Teams

#### 8.3.2.1. Research Stays Abroad

Jakob Ruess stayed at IST Austria twice a week in Feb and Nov 2018.

## 9. Dissemination

### 9.1. Promoting Scientific Activities

#### 9.1.1. Scientific Events Organisation

##### 9.1.1.1. Member of the Organizing Committees

Philippe Dague and François Fages were co-organizers of the Workshop on Computational Systems Biology for Cancer **CSBC**, Institut des Systèmes Complexes, Paris, France, 24-26 jan. 2018.

### 9.1.2. Scientific Events Selection

#### 9.1.2.1. Member of the Conference Program Committees

- Gregory Batt was member of the scientific program committee of
  - the 19th International Conference on Systems Biology (ICSB 2018), Oct 28 - Nov 1, Lyon, France
- François Fages was member of the program committee of
  - **VEMDP'18** Verification of Engineered Molecular Devices and Programs. July 19, 2018, Oxford, United Kingdom. An affiliated workshop of **CAV 2018**.
  - **CMSB'18** 16th International Conference on Computational Methods in Systems Biology. 12th-14th September 2018, Faculty of Informatics, Masaryk University, Brno, CZ.
  - **MCU'18** 8th International Conference on Machines, Computations and Universality, June 28-30, 2018, Fontainebleau, France.
  - **IJCAR'18** 9th International Joint Conference on Automated Reasoning, part of the Federated Logic Conference **FLOC'18**, July 14-17, 2018, Oxford, UK.
  - **DataMod'18** 7th International Symposium “From Data to Models and Back”, 25-26 June 2018, Toulouse, France
  - **WCB'18** 13th International Workshop on Constraint-based Methods for Bioinformatics, Oxford co-located with **FLOC'18**, July 2018.

#### 9.1.2.2. Reviewer

- Jakob Ruess has reviewed scientific articles for
  - the 57th IEEE Conference on Decision and Control (CDC 2018), Dec. 17-19, Miami, USA;
  - the 17th European Control Conference (ECC 2019), June 25-28, Naples, Italy;
  - the Bioinformatics conference, Feb 22-24 2019, Prague, Czech Republic.
- Sylvain Soliman reviewed papers for
  - IJCAR and CMSB.

### 9.1.3. Journal

#### 9.1.3.1. Member of the Editorial Boards

- François Fages is member of
  - the Editorial Board of the Computer Science area of the Royal Society Open Science journal, since 2014;
  - the Editorial Board of the journal RAIRO OR Operations Research, since 2004.

#### 9.1.3.2. Reviewer - Reviewing Activities

In addition to their Editorial Board and Program Committee duties,

- Grégory Batt reviewed journal articles for *ACS Synthetic Biology*, *Cell Systems*, *Nature Communications*, and *Current Opinion in Systems Biology*;
- François Fages reviewed journal articles for *PLOS Computational Biology*, *Interface Focus*, *Natural Computing*, *BMC Systems Biology* and the book *Automated Reasoning for Systems Biology and Medicine*;
- Jakob Ruess reviewed journal articles for *PLOS Computational Biology*, *Journal of Mathematical Biology*, *Journal of Computational Physics*, *Processes*, and *Entropy*;
- Sylvain Soliman reviewed journal articles for *Journal of Theoretical Biology* and *Transactions in Computational Biology and Bioinformatics*.

#### 9.1.4. Invited Talks

- Virgile Andreani gave an invited talk on *Modèles de résistance bactérienne aux antibiotiques to Journée apprentissage de modèles statistiques et stochastiques à partir de données biologiques*, Mar 2018, Rennes, France;
- Grégory Batt gave invited talks on
  - *Balancing a genetic toggle switch by real-time control and periodic stimulations*, IEEE Conference on Control Technology and Applications (CCTA 2018), Aug 21-24 2018, Copenhagen, Denmark
  - *Impact of phenotypic variability on commitment to apoptosis*, *Computational Systems Biology of Cancer*, Sept 2018, Paris
  - *Balancing a genetic toggle switch by real-time control and periodic stimulations*, iSSB / Genoscope seminar, Dec 2018, Evry, France
- François Fages gave invited talks on
  - *Vers une informatique de la cellule: programmes biochimiques naturels et synthétiques*, Conférence de rentrée de l'ENS Paris-Saclay (3h), Sep. 2018;
  - *Modeling and Design of Biological Systems*, Discrete Models and Formal Verification in Biology, Cambridge, UK, août 2018;
  - *Modeling and Design of Biological Systems*, Summer school on New strategies in medical diagnosis and personalized medicine, Ninh Binh, Vietnam, Jul. 2018;
  - *Computer-Aided Biochemical Programming of Synthetic Micro-reactors as Diagnostic Devices*, Groupe de Travail sur la biologie systémique symbolique, GT BIOS, Marseille, Jul. 2018;
  - *Turing Completeness of Continuous Chemical Reaction Networks, an Informatic Perspective to Systems Biology and Synthetic Biology*, MODELIFE days, Université Provence Alpes Côte d'Azur, Nice, June 2018;
  - *A Constraint-based Implementation of Tropical Equilibrations in BIOCHAM towards integrating Stability Conditions for Computing Correct Model Reductions*, Scientific Meeting, Bonn, Allemagne, March 2018;
  - *From Turing Completeness of Continuous CRN to Abstract CRN Synthesis Workflow*, Formal Methods for the Synthesis of Biomolecular Circuits, Dagstuhl, Allemagne, Feb. 2018;
  - *Coupled Models of the Cell Cycle and Circadian Clock for Chronotherapy Optimization*, Computational Systems Biology for Cancer, Paris, Jan. 2018.

Jakob Ruess gave invited talks on

- *Virtual reality for bacteria*, C3BI Seminar, Feb 2018, Paris, France.
- *Shaping bacterial population behavior through computer-interfaced control of individual cells*, Symposium on "Cybergenetics-at the interface between living and non-living regulatory systems" at the Annual Meeting of the German Association for General and Applied Microbiology (VAAM), Apr 2018, Wolfsburg, Germany.

#### 9.1.5. Leadership within the Scientific Community

- Grégory Batt is
  - co-responsible of the working group on Symbolic Systems Biology (GT Bioss), gathering >150 researchers in 25 research teams; and member of
  - the Technical Committee on Systems Biology of IEEE and CSS societies;
  - the scientific board of the French research network on Systems and Synthetic Biology (GdR BioSynSys), gathering 40 labs and 300 researchers;

- the scientific board of the French research network on Bioinformatics (GdR BIM), gathering 56 labs and several hundreds of researchers;
- the scientific committee of the Advanced Course on Computational Systems Biology summer school, Aussois, 2019;
- the scientific committee of the Curie International Course on Systems Biology of Cancer, Paris, 2018.
- François Fages is member of
  - the Steering Committee of the International Conference on Computational Methods in Systems Biology, CMSB, since 2008.
  - the **Think Tank of the structuring program MODELIFE** “Modélisation physique et Mathématique du vivant”, Université Provence Alpes Côte d’Azur.

### 9.1.6. Scientific Expertise

- Grégory Batt
  - was responsible for the Predictive Systems Biology chapter of the 2018-2022 strategic plan of Inria;
  - was reviewer and panel member of the ITMO Cancer/Inserm call on single-cell approaches to cancer;
  - and reviewer for the MIT International Science & Technology Initiatives (MISTI) Global Seed Funds and for the Leverhulme Trust.
- François Fages
  - was member of the Comité de sélection Maître de Conférence, ENS Paris-Saclay;
  - of the thesis advisory committee of one PhD student;
  - and evaluator of one European Research Council ERC Consolidator Grant;
  - one European Research Council ERC Starting Grant;
  - and one DigiCosme, Univ. Paris-Saclay project.
- Jakob Ruess has been a member of thesis advisory committee for two PhD students.

### 9.1.7. Research Administration

- François Fages is member of the “Comité des Projets du centre” Inria Saclay-IdF
- Sylvain Soliman is member of the “Commission Scientifique” of Inria Saclay-IdF

## 9.2. Teaching - Supervision - Juries

### 9.2.1. Teaching

Master: Grégory Batt (coordinator and teacher: 35h) and Jakob Ruess (25h), *Computational Biology*, M1, Master Approches Interdisciplinaires du Vivant (AIV).

Master: Grégory Batt (3h) *Synthetic Biology and Control course in Molecular and Cellular Biology* Sorbonne Université, Paris.

Master: François Fages (coordinator module 24h and teacher 12h) *C2-19 Biochemical Programming*, Master Parisien de Recherche en Informatique (MPRI), Paris.

Master: François Fages (co-coordinator module 36h and teacher 18h) and Sylvain Soliman (co-coordinator, teacher 18h) *INF555 - Constraint-based Modeling and Algorithms for Decision Making Problems Master Artificial Intelligence*, Ecole Polytechnique.

Master Modélisation en Pharmacologie Clinique et Epidémiologie Villejuif. François Fages (3h) *Méthodes de modélisation informatique des processus cellulaires: cycle cellulaire et horloge circadienne*,

Bachelor 2: Eléonore Bellot (teacher 64h) *CSE201 Object-oriented Programming in C++* TD and project supervision

Thematic Research School: Advances in systems and synthetic biology, Evry. François Fages and Sylvain Soliman (3h) *BIOCHAM Workshop*

### 9.2.2. Supervision

PhD in progress: Virgile Andreani, Calibration efficace de modèles de résistance bactérienne aux antibiotiques à l'aide d'un plan d'expériences optimal, ED STIC, Ecole Polytechnique, Sept. 2016, Gregory Batt

PhD in progress: Sebastian Sosa, "Understanding cost of protein production in yeast", ED FdV, Université Sorbonne Paris Cité, Feb. 2018, Gregory Batt

PhD in progress: Chetan Aditya, "Control of heterogenous synthetic microbial systems", ED FdV, Université Sorbonne Paris Cité, Feb. 2018, Gregory Batt

PhD in progress: Elise Weill, "Optimal control of partial differential equation systems: Application to heterogeneous cell populations", ED STIC, Ecole Polytechnique, Oct. 2018, Frédéric Bonnans and Gregory Batt

PhD in progress: Arthur Carcano, "Iterative design of single-cell experiments to learn single-cell models of biological systems", ED FdV, Université Sorbonne Paris Cité, Oct. 2018, Jakob Ruess and Gregory Batt

PhD in progress : Eléonore Bellot, "Réduction de modèles différentiels par résolution de contraintes d'algèbre tropicale (min,+)", ED STIC, Ecole Polytechnique, Sept. 2018, F. Fages & S. Soliman (50-50%)

PhD in progress : Jérémy Grignard, "Apprentissage de modèles à partir de données pour la conception d'expériences de criblage et la recherche de médicaments", ED STIC, Ecole Polytechnique, dec. 2018, F. Fages & T. Dorval, Servier (50-50%)

PhD in progress : Julien Martinelli, "Apprentissage de modèles mécanistes à partir de données temporelles, application à la personnalisation de la chronothérapie des cancers", ED STIC, Ecole Polytechnique, oct. 2018, F. Fages & A. Ballesta, Inserm (50-50%)

Master's Thesis in progress : Elisabeth Degrand, "Evolving Chemical Reaction Networks", KTH Stockholm, Sweden, F. Fages & M. Hemery (50-50%)

### 9.2.3. Juries

- Grégory Batt participated in the jurys of
  - HdR of Benjamin Pfeuty, Université de Lille, *Rapporteur*, 23/11/2018
  - PhD of Arnaud Bonnaffoux, ENS de Lyon, *Président du Jury*, 12/10/2018
  - PhD of Alexandre Deloupy, Sorbonne Université, *Rapporteur*, 14/12/2018
- François Fages participated in the jurys of
  - HDR Thi Bich Han Dao, Université d'Orléans, *Président du jury*, 14 mars 2018.
  - PhD Alexandre Rocca, Université Grenoble, *Rapporteur*, 7 mai 2018.
  - PhD Benjamin Miraglio, Université de Nice, *Rapporteur*, 16 février 2018.
- Jakob Ruess was member of the jury of the PhD thesis of Mathieu Pichené, 25/06/2018.

## 9.3. Popularization

### 9.3.1. Articles and contents

Our publication on distinguishing resistance from resilience to antibiotic treatments [6] has been the object of a press release, institutional communications (at [Inria](#) and [Institut Pasteur](#)), and some press attention, including notably by the UK daily mail journal.

### 9.3.2. Interventions

- Eléonore Bellot and Elise Weill participated at Fête de la Science in October 2018 at Inria Saclay île-de-France.
- Eléonore Bellot has received college school students for a visit to our research team on the design of logical gates in synthetic biology and their simulation in BIOCHAM.
- François Fages has received college school students for a visit to our research team with the question “Can we program any function?”, answered negatively by proving Cantor’s theorem.

### 9.3.3. Creation of media or tools for science outreach

- François Fages has created several **BIOCHAM notebooks associated to his course at MPRI** and available online.
- François Fages and Sylvain Soliman have created one **BIOCHAM tutorial notebook presented at ASSB 2018** and available online.

## 10. Bibliography

### Publications of the year

#### Articles in International Peer-Reviewed Journals

- [1] A. BAUDIER, F. FAGES, S. SOLIMAN. *Graphical Requirements for Multistationarity in Reaction Networks and their Verification in BioModels*, in "Journal of Theoretical Biology", December 2018, vol. 459, pp. 79–89, <https://arxiv.org/abs/1809.08891> [DOI : 10.1016/j.jtbi.2018.09.024], <https://hal.archives-ouvertes.fr/hal-01879735>
- [2] F. BOULIER, F. FAGES, O. RADULESCU, S. S. SAMAL, A. SCHUPPERT, W. M. SEILER, T. STURM, S. WALCHER, A. WEBER. *The SYMBIONT project: symbolic methods for biological networks*, in "ACM Communications in Computer Algebra", September 2018, vol. 52, n<sup>o</sup> 3, pp. 67-70 [DOI : 10.1145/3313880.3313885], <https://hal.inria.fr/hal-02061018>
- [3] A. COURBET, P. AMAR, F. FAGES, E. RENARD, F. MOLINA. *Computer-aided biochemical programming of synthetic microreactors as diagnostic devices*, in "Molecular Systems Biology", April 2018, vol. 14, n<sup>o</sup> 4 [DOI : 10.15252/MSB.20177845], <https://hal.inria.fr/hal-01779791>
- [4] F. FAGES, T. MARTINEZ, D. A. ROSENBLUETH, S. SOLIMAN. *Influence Networks compared with Reaction Networks: Semantics, Expressivity and Attractors*, in "IEEE/ACM Transactions on Computational Biology and Bioinformatics", 2018, vol. PP, n<sup>o</sup> 99, pp. 1-14 [DOI : 10.1109/TCBB.2018.2805686], <https://hal.inria.fr/hal-01510216>
- [5] J.-B. LUGAGNE, S. JAIN, P. IVANOVITCH, Z. BEN MERIEM, C. VULIN, C. FRACASSI, G. BATT, P. HERSEN. *Identification of individual cells from z-stacks of bright-field microscopy images*, in "Scientific Reports", July 2018, vol. 8, n<sup>o</sup> 1, 11455 p. [DOI : 10.1038/s41598-018-29647-5], <https://hal.archives-ouvertes.fr/hal-01898065>
- [6] H. MEREDITH, V. ANDREANI, H. MA, A. LOPATKIN, A. LEE, D. ANDERSON, G. BATT, L. YOU. *Applying ecological resistance and resilience to dissect bacterial antibiotic responses*, in "Science Advances", December 2018, vol. 4, n<sup>o</sup> 12, eaau1873 p. [DOI : 10.1126/SCIADV.AAU1873], <https://hal.inria.fr/hal-01950376>

### International Conferences with Proceedings

- [7] F. FAGES, S. SOLIMAN. *On Robustness Computation and Optimization in BIOCHAM-4*, in "16th Int. Conf. on Computational Methods in Systems Biology", Brno, Czech Republic, September 2018, Long version with appendices [DOI : 10.1007/978-3-319-99429-1\_18], <https://hal.inria.fr/hal-01814854>

### Scientific Books (or Scientific Book chapters)

- [8] F. FAGES, G. LE GULUDEC. *Biochemical Programs and Analog-Digital Mixed Algorithms in the Cell*, in "Life Sciences, Information Sciences", John Wiley & Sons, Inc., March 2018, pp. 201-216 [DOI : 10.1002/9781119452713.CH19], <https://hal.inria.fr/hal-01948714>

### Scientific Popularization

- [9] F. MOLINA, J.-H. JIANG, F. FAGES. *BIOPSY: Biochemical Programming System*, November 2018, Festival Scientifique France Taiwan, ANR-MOST, Poster, <https://hal.inria.fr/hal-01948702>

### Other Publications

- [10] F. BOULIER, F. FAGES, O. RADULESCU, S. S. SAMAL, A. SCHUPPERT, W. M. SEILER, T. STURM, S. WALCHER, A. WEBER. *The SYMBIONT Project: Symbolic Methods for Biological Networks*, August 2018, F1000Research 7:1341 (poster), <https://hal.inria.fr/hal-01889825>
- [11] V. SINGH, S. PANKAEW, M. OSTASZEWSKI, G. D. KALLIOLIAS, S. SOLIMAN, T. HELIKAR, A. NIARAKIS. *Executable Disease Networks: Adding dynamics to molecular maps*, September 2018, ECCB 2018, 17th European Conference on Computational Biology, Poster, <https://hal.inria.fr/hal-01875418>