Activity Report 2019

Project-Team LIFEWARE

Computational systems biology and optimization

RESEARCH CENTER
Saclay - Île-de-France

THEME
Computational Biology

Table of contents

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. - Pharmaco kinetics and dynamics
B2.4.2. - Drug resistance
B9. - Society and Knowledge

1. Team, Visitors, External Collaborators

Research Scientists

François Fages [Team leader, Inria, Senior Researcher, HDR]
Grégory Batt [Inria, Senior Researcher, HDR]
Jakob Ruess [Inria, Researcher]
Sylvain Soliman [Inria, Researcher, HDR]

Post-Doctoral Fellows

Olivier Borkowski [Inria, Post-Doctoral Fellow, from Oct 2019]
Zachary Fox [Inria, Post-Doctoral Fellow, from Jul 2019]
Mathieu Hemery [Inria, Post-Doctoral Fellow]
Davin Lunz [Inria, Post-Doctoral Fellow, from Nov 2019, with EPI Commands]

PhD Students

Chetan Aditya [Inria, PhD Student]
Virgile Andréani [École polytechnique and Inria, PhD Student]
Eléonore Bellot [Inria, PhD Student]
Arthur Carcano [Université Paris Diderot, PhD Student]
Elisabeth Degrand [Inria, PhD Student, from Oct 2019]
Eléa Greugny [Johnson&Johnson France, PhD Student, from Aug 2019, granted by CIFRE]
Jeremy Grignard [Institut de recherche Servier, PhD Student, from Mar 2019]
Julien Martinelli [INSERM, PhD Student]
Sebastian Ramon Sosa Carrillo [Inria, PhD Student]
Elise Weill Duflos [Inria, PhD Student, until Oct 2019, with EPI Commands]

Technical staff

François Bertaux [Institut Pasteur, Engineer]
David Coudrin [Inria, Engineer, until Jul 2019]
Steven Fletcher [Inria, Engineer]
Andjela Davidovic [Institut Pasteur, Engineer, part time]

Interns and Apprentices

Orianne Bargain [Inria, from Mar 2019 until Sep 2019]
Auriane Cozic [École polytechnique, until Mar 2019]
Elisabeth Degrand [Inria, until Jan 2019]
Achille Fraisse [École Normale Supérieure de Lyon, from Sep 2019]
Constance Le Gac [Ecole Polytechnique, from Sep 2019]
Léna Le Quellec [École polytechnique, until Mar 2019]
Eva Philippe [Inria, from Mar 2019 until Aug 2019]
Paul Remondeau [Inria, from Apr 2019 until Aug 2019]
Mariela Rocio Furstenheim Milerud [Institut Pasteur, from Feb 2019 until Jul 2019]
Albin Salazar [Inria, from Apr 2019 until Aug 2019]

Administrative Assistants

Natalia Alves [Inria, Administrative Assistant]
Adeline Lochet [Inria, InBio Administrative Assistant, until May 2019]
Emmanuelle Perrot [Inria, InBio Administrative Assistant, from May 2019]

Visiting Scientists

Claudia Lopez Zazueta [NTNU, Norway, Oct 2019]
Lucia Nasti [Univ. Pisa, Italy, until Mar 2019]

External Collaborator

Denis Thieffry [École Normale Supérieure de Paris, HDR]

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**.

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 [1], the inference of reaction systems from ODEs [2], 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) [3]. The logical paradigm for systems biology that we have subsequently developed for quantitative models can be summarized by the following identifications :

$$\text{biological model = transition system } K$$

$$\text{dynamical behavior specification = temporal logic formula } \phi$$

$$\text{model validation = model-checking } \quad K,\ s \models ?\ \phi$$

$$\text{model reduction = sub-model-checking, } K' \subset K \text{ s.t. } K'?,\ s \models \phi$$

$$\text{model prediction = formula enumeration, } \phi \text{ s.t. } K,\ s \models \phi?$$

$$\text{static experiment design = symbolic model-checking, state } s \text{ s.t. } K,\ s? \models \phi$$

$$\text{model synthesis = constraint solving } \quad K?,\ s \models \phi$$

$$\text{dynamic experiment design = constraint solving } \quad K?,\ s? \models \phi$$

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 [4] This line of research continues with the development of temporal logic patterns with efficient constraint solvers and their generalization to handle stochastic effects.

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

[2] 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.

[3] 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.

[4] 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 [5] and Olivier Bournez, establish fundamental links with digital computation. In a paper published last year [6] 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 [7], and (stochastic approximations of) the **expectation maximization** algorithm for the identification of mixed-effects models [8].

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

[5] Amaury Pouly, "Continuous models of computation: from computability to complexity", PhD Thesis, Ecole Polytechnique, Nov. 2015.

[6] 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 fiveteen international conference on Computational Methods in Systems Biology, pages 108–127, volume 10545 of Lecture Notes in Computer Science. Springer-Verlag, 2017.

[7] 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

[8] What population reveals about individual cell identity: estimation of single-cell models of gene expression in yeast, A. Llamosi, 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 [9].

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 [10].

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 [11].

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) [12] 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 [13] [14].

4. Application Domains

[9] Jannis Uhlendorf, Agnés 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.

[10] 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.

[11] 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.

[12] N. Hansen, A. Ostermeier (2001). Completely derandomized self-adaptation in evolution strategies. Evolutionary Computation, 9(2) pp. 159–195.

[13] 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 β-arrestin signaling. Molecular Systems Biology, 8(590), 2012.

[14] 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 characterictics. 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 [15]. 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 high-level functions in non-living vesicles for medical applications, such as biosensors for medical diagnosis [16]. 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.

[15] 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.

[16] Alexis Courbet, Patrick Amar, François Fages, Eric Renard, Franck Molina. Computer-aided biochemical programming of synthetic microreactors as diagnostic devices. Molecular Systems Biology, 14(4), 2018.

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 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 β-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

Figure 1. La Recherche magazine award 2019 ceremony.

- **Creation of a new team at Inria Paris**

  At the end of 2019, the Lifeware team gave birth to a new Inria team, called InBio and affiliated to the Inria Paris research centre. So far, InBio was a Pasteur research unit that was hosting a fraction of the members of the Lifeware team on the campus of Institut Pasteur. So in 2020, InBio becomes a new Common Project-Team between Inria Paris and Pasteur Institute. This demonstrates that Inria is actively supporting research in the computational systems biology field.
- **Launching of Inria Exploratory Action GRAM on chemical programming of artificial vesicles**

Chemical reaction networks are a computation paradigm used by natural cells to process information, take decisions and control their vital processes. The synthesis of artificial vesicles without DNA nor RNA but containing precise quantities of enzymes allows us today to implement high-level functions in proto-cells with numerous potential applications in health and the environment. Based on previous work of the Lifeware project-team on chemical analog computation theory and programming, of the CNRS-Alcediag Sys2diag laboratory on the synthesis of biosensors in artificial vesicles, and on the expertise of the Roscoff Biological Station on membrane transporters, we explore an original approach to analog chemical circuit design applied to the programming of high-level functions in chemical analog computers.

5.1.1. Awards

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

  The ceremony for awards La Recherche magazine 2019 at University Paris-Dauphine was a great occasion to present our article "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, Best Paper award at CMSB 2017, recipient of La Recherche magazine 2019 Award - 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: – notebooks of Master classes – graphical user interface on top of Jupyter – synthesis of at most binary reactions with minimisation of the variables for negative values – detection of model reduction by subgraph epimorphism – option for partial tropical equilibrations

- Participants: François Fages, David Coudrin, Sylvain Soliman and Mathieu Hemery
- Contact: François Fages
- URL: http://lifeware.inria.fr/biocham4/

6.2. casq

CellDesigner as SBML-Qual

KEYWORDS: SBML - Logical Framework - Knowledge representation

FUNCTIONAL DESCRIPTION: CaSQ transforms a big knowledge map encoded as an SBGN-compliant SBML file in CellDesigner into an executable Logical Model in SBML-Qual

- Authors: Sylvain Soliman and Anna Niarakis
- Partner: Université d'Evry-Val d'Essonne
- Contact: Sylvain Soliman
- URL: https://gitlab.inria.fr/soliman/casq/

6.3. Platforms

6.3.1. 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. CRN design by program compilation

Participants: Elisabeth Degrand, François Fages, Mathieu Hemery, Wei-Chih Huang [NTU Taiwan], Sylvain Soliman.

One goal of synthetic biology is to implement useful functions with biochemical reactions, either by reprogramming living cells or programming artificial vesicles. In this perspective, we consider Chemical Reaction Networks (CRN) as a programming language, and investigate the CRN program synthesis problem. Recent work has shown that CRN interpreted by differential equations are Turing-complete and can be seen as analog computers where the molecular concentrations play the role of information carriers. Any real function that is computable by a Turing machine in arbitrary precision can thus be computed by a CRN over a finite set of molecular species. The proof of this result gives a numerical method to generate a finite CRN for implementing a real function presented as the solution of a Polynomial Initial Values Problem (PIVP).

The compilation of high-level imperative programming languages in CRN requires however an efficient implementation of program control flows using threshold functions. The biochemical threshold function is also a crucial component in the biosensor circuits to be deployed in living cells or synthetic vesicles for disease diagnosis. In [5], based on the zero-order ultrasensitivity, we propose an economic biochemical implementation of threshold functions with reconfigurable threshold values. We show that the so-constructed threshold function module well approximates the unit step function and allows robust composition with other function modules for complex computation tasks. This is now implemented in BIOCHAM-4 for the compilation of sequentiality and conditionals in CRNs.

7.2. CRN design by artificial evolution

Participants: Elisabeth Degrand, François Fages, Mathieu Hemery, Sylvain Soliman.

In [4], [12], we study an alternative method based on artificial evolution to build a CRN that approximates a real function given on finite sets of input values. We present a nested search algorithm that evolves the structure of the CRN and optimizes the kinetic parameters at each generation. We evaluate this algorithm on the Heaviside and Cosine functions both as functions of time and functions of input molecular species. We then compare the CRN obtained by artificial evolution both to the CRN generated by the numerical method from a PIVP definition of the function, and to the natural CRN found in the BioModels repository for switches and oscillators.

On a Heaviside function of time, the results obtained by artificial evolution lead to a remarkably simple CRN of 3 molecular species and 5 reactions with double catalysts which provide a very stiff transition although using mass action law kinetics. This solution is more economical than the CRN generated by the PIVP method for sigmoid functions. On a Heaviside function of input, the CRN found by evolution are slightly more complicated than the bistable switch found in cell cycle CRN for instance, but much less complex than the MAPK signaling network that plays a similar role.

On the cosine function of time, the best CRN found by evolution contains an annihilation reaction similar to the CRN generated by the numerical method for positive and negative variables, but one less reaction thanks to an intriguing non symmetric use of the two variables which preserves the limit cycle. Interestingly, the evolved and the PIVP generated structures could be compared to prokaryote and eukaryote models of the circadian clock found in BioModels.

On the cosine function of input, a CRN surprisingly emerges with the structure of the CRN for cosine function of time, using the same trick as for PIVP compilation to stop time at the desired input value.

In [2], we use a genetic algorithm to evolve biochemical networks displaying a direct logarithmic response. Numerous biological systems are known to harbour a form of logarithmic behaviour, from Weber's law to bacterial chemotaxis. Working on a logarithmic scale allows the organism to respond appropriately to large variations in a given input at a modest cost in terms of metabolism. Interestingly, a quasi-perfect log-response implemented by the same simple core network evolves in a convergent way across our different replications. The best network is able to fit a logarithm over 4 order of magnitude with an accuracy of the order of 1%. At the heart of this network, we show that a logarithmic approximation may be implemented with one single non-linear interaction, that can be interpreted either as a phosphorylation or as a ligand induced multimerization and provide an analytical explanation of the effect. Biological log-response might thus be easier to implement than usually assumed.

7.3. CRN learning from data time series

Participants: François Fages, Jeremy Grignard, Julien Martinelli, Sylvain Soliman.

With the automation of biological experiments and the increase of quality of single cell data that can now be obtained by phosphoproteomic and time lapse videomicroscopy, automating the building of mechanistic models from these data time series becomes conceivable and a necessity for many new applications. While learning numerical parameters to fit a given model structure to observed data is now a quite well understood subject, learning the structure of the model is a more challenging problem that previous attempts failed to solve without relying quite heavily on prior knowledge about that structure. In [8], [7], we consider mechanistic models based on chemical reaction networks (CRN) with their continuous dynamics based on ordinary differential equations, and finite time series about the time evolution of concentration of molecular species for a given time horizon and a finite set of perturbed initial conditions. We present a greedy heuristics unsupervised statistical learning algorithm to infer reactions with a time complexity for inferring one reaction in $O(t.n^2)$where n is the number of species and t the number of observed transitions in the traces. We evaluate this algorithm both on simulated data from hidden CRNs, and on real videomicroscopy single cell data about the circadian clock and cell cycle progression of NIH3T3 embryonic fibroblasts. In all cases, our algorithm is able to infer meaningful reactions, though generally not a complete set for instance in presence of multiple time scales or highly variable traces.

7.4. CRN reductions

Participants: Orianne Bargain, Eléonore Bellot, François Fages, Eva Philippe, Sylvain Soliman.

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 [17], furthermore quite efficiently using constraint programming or SAT solving techniques. Nevertheless, establishing whether two models are linked through a SEPI is an NP-complete problem which can be computationally expensive in some practical cases. Furthermore, the number of SEPIs can be huge, and some of them may not have a biological interpretation. In [11], we have improved the SEPI framework in this respect in three ways: by introducing optimization criteria to restrict the set of solutions, by restricting merge operations to some notion of neighborhood, and by preprocessing the CRN graphs in normal form in order to eliminate some common model reduction patterns.

[17] Steven Gay, François Fages, Thierry Martinez, Sylvain Soliman, Christine Solnon. On the subgraph Epimorphism Problem. Discrete Applied Mathematics, 162:214–228, 2014.

Furthermore, in the framework of the ANR-DFG SYMBIONT project we investigate mathematical justification of SEPI reductions based on Tikhonov's theorem and their computation using tropical algebra methods and constraint programming techniques [18].

7.5. CRN modeling of biological systems

Participants: Auriane Cozic, Elisabeth Degrand, François Fages, Eléa Greugny, Jeremy Grignard, Constance Le Gac, Léna Le Quellec, Paul Remondeau, Sylvain Soliman.

This year, beyond implementation work on hybrid simulations in BIOCHAM and on antithetic feedback control in CRNs, we have started the computational modelling of three biological systems with important potential applications in biomedicine.

The first is about erythrocytes (i.e. red blood cells). Their most obvious function concerns the respiratory system since erythrocytes allow gas exchanges at the level of the organism by transporting dioxygen and carbon dioxide between the lungs and the tissues. However, red blood cells also have an important buffer function in the blood, which is necessary to keep blood pH in the physiological range. Modelling the red blood cells with CRNs using BIOCHAM gives us insight as to which biological objects are necessary to allow the cell to process its functions correctly. At the level of Systems Biology, it also allows us to understand the links between the different biological functions of erythrocytes.

The second concerns microtubules and their post-translational modifications involved in major cellular processes such as: mitosis, cardiomyocyte contraction, and neuronal differentiation. More precisely, in neurons, the post-translational modifications of detyrosination and tyrosination are crucial for neuronal plasticity, axon regeneration, recruitment and transports of proteins and correct neuronal wiring. We hypothesize that the decrease of density and length of microtubules and the loss of neuronal structures such as synapses, dendritic spine and growth cone which are correlated with the progressive cognitive decline [9,10] may be the consequence of the dysregulation of the cycle detyrosination/tyrosination in neurodegenerative disorder. This hypothesis is investigated in collaboration with Servier by combining experimental approaches with mathematical modelling.

The third concerns inflammation processes in skin. Skin protects the body against external agents, for instance pathogens, irritants, or UV radiation, that can trigger inflammation. Inflammation is a complex phenomenon that is classified in two main types, acute and chronic. They are distinguished by different parameters such as the duration, the underlying mechanisms, the components involved like the type of immune cells, and the nature and intensity of the associated clinical signs. The computational models developed in collaboration with Johnson&Johnson France, combine mathematical and multi-agent modelling using BIOCHAM and EPISIM modelling tools.

7.6. Automated Inference of Boolean models from molecular interaction maps

Participant: Sylvain Soliman.

Molecular interaction maps have emerged as a meaningful way of representing biological mechanisms in a comprehensive and systematic manner. However, their static nature provides limited insights to the emerging behavior of the described biological system under different conditions. Computational modeling provides the means to study dynamic properties through *in silico* simulations and perturbations.

In collaboration with Anna Niarakis (Université d'Évry, GenHotel) we have started developing the CaSQ Python package, by defining simplification rules and logical formulas for the inferred Boolean models according to the topology and the annotations of the starting molecular interaction maps. We used CaSQ to produce executable files of existing molecular maps notably a big map of the Rheumatoid Arthritis that is at the core of Évry team's work.

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

A publication on the inference process has already been submitted to Bioinformatics but work continues on the applications side to fine-tune the automatically generated model and analyze its dynamical properties.

7.7. Optimal control of an artificial microbial differentiation system for protein bioproduction

Participants: Élise Weill Duflos, Virgile Andréani, Chetan Aditya, Pierre Martinon [EPI Commands], Jakob Ruess, Grégory Batt, J. Frédéric Bonnans [EPI Commands].

The growth of microorganisms is controlled by strategies for the dynamical allocation of available resources over different cellular functions. Synthetic biology approaches are considered nowadays to artificially modify these strategies and turn microbial populations into biotechnological factories for the production of metabolites of interest. In our recent work, we have studied dynamics of microbial resource allocation and growth in terms of coarse-grained self-replicator models described by ordinary differential equations, and proposed artificial control strategies for the optimization of metabolite production based on the reengineering of resource allocation. In this contribution, we elaborated on our earlier results and further investigate synthetic resource allocation control strategies [9]. Using numerical simulation, we studied the effect on growth and bioproduction of the (biological or technological) costs associated with discontinuous control strategies, and of the time allotted to optimal substrate utilization. Results provided novel insight into the most favorable synthetic control strategies.

7.8. Can optimal experimental design serve as a tool to characterize highly non-linear synthetic circuits?

Participants: Maxim Kryukov [Pasteur Institute], Arthur Carcano, Grégory Batt, Jakob Ruess.

One of the most crippling problems in quantitative and synthetic biology is that models aiming to describe the real mechanisms of biochemical processes inside cells typically contain too many unknown parameters to be reliably inferable from available experimental data. Recent years, however, have seen immense progress in the development of experimental platforms that allow not only to measure biological systems more precisely but also to administer external control inputs to the cells. Optimal experimental design has been identified as a tool that can be used to decide how to best choose these control inputs so as to excite the systems in ways that are particularly useful for learning the biochemical rate constants from the corresponding data. Unfortunately, the experiment that is best to learn the parameters of a system depends on the precise values of these parameters, which are naturally unknown at the time at which experiments need to be designed. Here, we used a recently constructed genetic toggle switch as a case study to investigate how close to the best possible experiment we can hope to get with the most widely used optimal design approaches in the field. We found that, for strongly nonlinear systems such as the toggle switch, reliably predicting the information that can be gained from a priori fixed experiments can be difficult if the system parameters are not known very precisely [6]. This suggests that a better strategy to guarantee informative experiments might be to use feedback control and to adjust the experimental plan in real time.

7.9. Molecular noise of innate immunity shapes bacteria-phage ecologies

Participant: Jakob Ruess.

Mathematical models have been used successfully at diverse scales of biological organization, ranging from ecology and population dynamics to stochastic reaction events occurring between individual molecules in single cells. Generally, many biological processes unfold across multiple scales, with mutations being the best studied example of how stochasticity at the molecular scale can influence outcomes at the population scale. In many other contexts, however, an analogous link between micro-and macro-scale remains elusive, primarily due to the challenges involved in setting up and analyzing multi-scale models. In [3], we employed such a model to investigate how stochasticity propagates from individual biochemical reaction events in the bacterial innate immune system to the ecology of bacteria and bacterial viruses. We showed analytically how

the dynamics of bacterial populations are shaped by the activities of immunity-conferring enzymes in single cells and how the ecological consequences imply optimal bacterial defense strategies against viruses. Our results suggest that bacterial populations in the presence of viruses can either optimize their initial growth rate or their population size, with the first strategy favoring simple immunity featuring a single restriction modification system and the second strategy favoring complex bacterial innate immunity featuring several simultaneously active restriction modification systems.

7.10. Estimating information in time-varying signals

Participant: Jakob Ruess.

Across diverse biological systems - ranging from neural networks to intracellular signaling and genetic regulatory networks - the information about changes in the environment is frequently encoded in the full temporal dynamics of the network nodes. A pressing data-analysis challenge has thus been to efficiently estimate the amount of information that these dynamics convey from experimental data. In [1], we developed and evaluated decoding-based estimation methods to lower bound the mutual information about a finite set of inputs, encoded in single-cell high-dimensional time series data. For biological reaction networks governed by the chemical Master equation, we derived model-based information approximations and analytical upper bounds, against which we benchmarked our proposed model-free decoding estimators. In contrast to the frequently-used k-nearest-neighbor estimator, decoding-based estimators robustly extract a large fraction of the available information from high-dimensional trajectories with a realistic number of data samples. We applied these estimators to previously published data on Erk and Ca^{2+} signaling in mammalian cells and to yeast stress-response, and found that substantial amount of information about environmental state can be encoded by non-trivial response statistics even in stationary signals. We argued that these single-cell, decoding-based information estimates, rather than the commonly-used tests for significant differences between selected population response statistics, provide a proper and unbiased measure for the performance of biological signaling networks.

8. Bilateral Contracts and Grants with Industry

8.1. Bilateral Contract with Institut de recherche Servier

In the framework of the Cifre PhD thesis of Jeremy Grignard at Servier, we work on the coupling between computational modeling and biological experiment design, and on chemical reaction network inference methods from data time series.

8.2. Bilateral Grant with Johnson&Johnson France

In the framework of the Cifre PhD thesis of Eléa Greugny at Johnson&Johnson Santé Beauté France, we work on the computational modeling of inflammatory process in the skin, using multi-scale modeling and multi-agent simulation.

9. Partnerships and Cooperations

9.1. National Initiatives

9.1.1. 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. Ruess (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);

9.1.2. Inria Project Lab

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

9.2. European Initiatives

9.2.1. FP7 & H2020 Projects

- H2020 FET-OPEN COSY-BIO (2017-2020), on “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), Grégory Batt, Guy-Bart Stan (Imperial College), and Lucia Marucci (Bristol U).

9.3. International Research Visitors

9.3.1. Visits of International Scientists

The following researchers have been invited for short visits:

- Jean-Louis Lassez, retired IBM Yorktown, USA
- Lucia Nasti, Univ. Pisa, Italy
- Claudia Lopez Zazueta, NTNU, Norway

9.3.1.1. Internships

- Orianne Bargain (TU Dresden Germany)
- Elisabeth Degrand (KTH, Stockholm Sweden)

10. Dissemination

10.1. Promoting Scientific Activities

10.1.1. Scientific Events: Organisation

10.1.1.1. General Chair, Scientific Chair

Chetan Aditya was the co-organiser of a thematic workshop entitled "From Bench to Bedside" at the Center for Interdisciplinary research, Paris, April 2019.

Grégory Batt was a co-organizer of the thematic session entitled "Predictive approaches for biological systems engineering" at the JOBIM conference, Nantes, July 2019.

François Fages and Sylvain Soliman were scientific co-chairs of

- the Constraints and Life Sciences Track of CP'2019 25th International Conference on Principles and Practice of Constraint Programming, Stamford, CT, USA, September 30 to October 4, 2019.

François Fages was scientific co-chair of

- Workshop on Computational Systems Biology for Complex Disease CSBCD, ENS Paris-Saclay, Cachan, France, 28-29 Nov. 2019.
- Formal methods for the synthesis of biomolecular circuits Shonan Village, Japan, 2-6 Sep 2019.
- France-Taiwan Summer School on New Strategies in Medical Diagnosis and Precision Medicine, NTU, Taipei, Taiwan, 9-10 July 2019.

10.1.2. Scientific Events: Selection

10.1.2.1. Member of the Conference Program Committees

- Grégory Batt was a member of the program committee of the IFAC Conference on Foundations of Systems Biology in Engineering (FOSBE 2019), Valencia, Spain, and of the scientific committee of Advanced Lecture Course on Computational Systems Biology summer school, Aussois, France.
- François Fages was PC member of
  - CIBCB'19 16th IEEE International Conference on Computational Intelligence in Bioinformatics and Computational Biology – Certosa di Pontignano, Siena - Tuscany, Italy, July 9-11, 2019
  - CMSB'19 17th International Conference on Computational Methods in Systems Biology. Trieste, Italy, September 2019.
  - HSB'19, 6th International Workshop on Hybrid Systems and Biology, will be held at the Charles University, Prague (CZ) on the 6th and 7th April 2019, and is colocated with ETAPS 2019.
  - BIOINFORMATICS'19 10th International Conference on Bioinformatics Models, Methods and Algorithms" co-located with BIOSTEC'19, Prague, Czech republic, Feb 2019.
- Jakob Ruess was a PC member of CMSB'19, the 17th International Conference on Computational Methods in Systems Biology. Trieste, Italy, September 2019.
- Sylvain Soliman was a PC member of CSBio 2019 10th International Conference on Computational Systems-Biology and Bioinformatics — Nice (France), December 4–7, 2019

10.1.2.2. Reviewer

Jakob Ruess was a reviewer for two conferences: *European Control Conference (ECC)* and *Bioinformatics 2020*.

10.1.3. Journal

10.1.3.1. 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.

10.1.3.2. Reviewer - Reviewing Activities

Grégory Batt was a reviewer for *Bioinformatics* and *ACS Synthetic Biology* journals.

François Fages made reviews for the journals *Biosystems* and *Transactions on Computational Biology and Bioinformatics*.

Mathieu Hemery made 5 reviews for the journal *Physical Review E*, and was a reviewer for the journals *Physical Review Letters* and *Royal Society Open Science*.

Jakob Ruess was a reviewer for the journals *Entropy* and *Scientific Reports*.

Sylvain Soliman was a reviewer for *Briefings in Bioinformatics*, and for the *Journal of Theoretical Biology*.

10.1.4. Invited Talks

- Chetan Aditya gave a presentation entitled "Synthetic optogenetic differentiation system for bioproduction in budding yeast" at the EMBL workshop "Creating is Understanding: Synthetic Biology Masters Complexity", September 2019, Heidelberg, Germany
- Grégory Batt gave the following invited talks:
  - Driving cellular processes with quantitative accuracy using real-time control approaches, 6th Cross Disciplinary Genomics Meeting of Sorbonne University, Nov 2019, Paris, France
  - Driving cellular processes with quantitative accuracy using real-time control approaches, SynGen Series UK, Nov 2019, London, UK
  - An experimental platform for automated calibration of antimicrobial resistance models, APHP/IP AMR STORM, Oct 2019, Paris, France
  - Balancing a genetic toggle switch by real-time control and periodic stimulations, International Workshop on Control Engineering and Synthetic Biology, Sept 2019, Oxford, UK
  - Experimental and computational methods for modeling cellular processes, Shonan meeting on Formal methods for the synthesis of biomolecular circuits, Sept 2019, Shonan, Japan
  - Driving cellular processes with quantitative accuracy using real-time control approaches, Seminar of Unité Physico Chimie Curie, Jan 2019, Paris, France
- François Bertaux gave an invited talk at the JOBIM thematic session entitled "Methods for single-cell omics data analysis", Nantes, July 2019.
- Andjela Davidovic gave a presentation entitled "Calibration of stochastic biochemical models using single-cell video-microscopy experiments" at the Stochastic models for biology conference (BioHasard 2019), August 2019, Rennes, France
- François Fages gave invited talk to
  - Turing-completeness of Chemical Reaction Networks in Natural cells and Artificial vesicles (keynote CSBio 2019) The 10th International Conference on Computational Systems-Biology and Bioinformatics. Nice, France. December 4 to 7, 2019
  - Cells as Analog Chemical Computers: Turing Completeness and Synthesis Formal methods for the synthesis of biomolecular circuits Shonan Village, Japan, 2-6 Sep 2019.
  - Modeling and Design of Biological Systems, France-Taiwan Summer School on New Strategies in Medical Diagnosis and Precision Medicin, NTU, Taipei, Taiwan, 9-10 July 2019.
  - La Cellule un Calculateur Chimique Journées scientifiques Inria, Lyon, 5-7 juin 2019.
  - La Cellule un Calculateur Chimique Dassault-Systèmes, Vélizy, June 4th 2019.
  - Calculs Analogiques dans les Programmes Biochimiques Naturels et Synthétiques Colloque d'ouverture 50 ans du Laboratoire Jacques-Louis Lions, Roscoff, France, 4-8 mars 2019.
  - Calculs analogiques dans les réseaux biochimiques naturels et synthétiques Prix La recherche - 15th edition. Université Paris Dauphine, 4 Feb. 2019.
- Jakob Ruess gave an invited talk at the SYMBIONT meeting entitled "Molecular noise shapes bacteria-phage ecologies", Paris, April 2019.

10.1.5. Leadership within the Scientific Community

- Grégory Batt is co-animator of the working group on Symbolic Systems Biology (GT Bioss).
- Grégory Batt is a member of
  - the Technical Committee on Systems Biology of IEEE and CSS societies
  - the scientific board of the French research network on Bioinformatics (GdR BIM)
  - the scientific committee of the Advanced Course on Computational Systems Biology summer school, in Aussois
- François Fages is member of
  - the Steering Committee of the International Conference on Computational Methods in Systems Biology, CMSB, since 2008.
  - the Scientific Committee of the Doctorate School ED 474 FIRE, ex Frontiers in Life Sciences, FdV
  - the Scientific Committee of the Summer School Modélisation Formelle de Réseaux de Régulation Biologique Ile de Porquerolles du 23 au 28 juin 2019 .

10.1.6. Scientific Expertise

François Fages was

- member of the Jury for Inria Awards 2019
- evaluator of two DFG grant proposals
- reviewer of a program proposal from Institut Pascal - Paris Saclay
- reviewer of professorship application, Indraprastha Institute of Information Technology - Delhi, India.

10.1.7. Research Administration

- Grégory Batt is the deputy director of the department of Computational Biology at Institut Pasteur
- François Fages is member of the “Comité des Projets du centre” Inria Saclay-IdF, and Inria representative for Doctorate School Institut Polytechnique de Paris
- Sylvain Soliman is member of the “Commission Scientifique” of Inria Saclay-IdF

10.2. Teaching - Supervision - Juries

10.2.1. Teaching

Summer school: François Fages (teacher 6h) Modélisation Formelle de Réseaux de Régulation Biologique, Ile de Porquerolles.

Summer school: Jakob Ruess (teacher 6h) Blackboard course on Optimally learning dynamical models from data at the "Advanced lecture course on computational systems biology" in Aussois, France, April 2019.

Master: Grégory Batt (coordinator and teacher: 35h) and Jakob Ruess (25h), *Computational Biology*, M1, Interdisciplinary Approaches to Life Sciences (AIRE-LiSc).

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

Master: François Bertaux (co-coordinator and teacher: 20h), *Systems Biology*, M1, Master Interdisciplinary Approaches to Life Sciences (AIRE-LiSc).

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.

Bachelor 3: François Fages (co-coordinator module and teacher 4h30) and Sylvain Soliman (co-coordinator module, teacher 4h30) and Mathieu Hemery (TD 10h) CSE 301b: Constraint Logic Programming.

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

L1: Julien Martinelli (teacher 64h) Mathematics and Calculus, Univ. Paris Descartes

10.2.2. Supervision

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

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

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

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 Grégory Batt

PhD in progress : Elisabeth Degrand, "Chemical Programming in Non-living Vesicles", ED IPP, Ecole Polytechnique, Oct. 2019, 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 IPP, Ecole Polytechnique, dec. 2018, F. Fages & T. Dorval, Servier (50-50%)

PhD in progress : Eléa Greugny, "Development and Implementation of a Mathematical Model of Inflammation in the Human Skin", ED IPP, Ecole Polytechnique, Aug. 2019, F. Fages & J. Bensaci & G. Stamatas, Johnson&Johnson Santé Beauté France (1/3-2/3))

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

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

Master's Thesis: Orianne Bargain, "Graph matching, theory and SAT implementation", TU Dresden, Germany, Sep. 2019, F. Fages & S. Soliman (50-50%)

Master's Thesis: Elisabeth Degrand, "Evolving Chemical Reaction Networks", KTH Stockholm, Sweden, June 2019, F. Fages & M. Hemery (50-50%)

10.2.3. Juries

- Grégory Batt participated in the jurys of
  - PhD Antoine Barizien, Ecole Potytechnique and Institut Pasteur, *Rapporteur*, Paris, May 2019
  - PhD Mathilde Koch, Ecole Polytechnique and INRA, *Examinateur*, Paris, November 2019
- François Fages participated in the jurys of
  - HDR Sabine Pérès, Université Paris-Saclay, *Reviewer*, 18 Nov. 2019

- HDR David Safranek, Mazaryk Univ., Czech Republic *Reviewer*, July 2019
- HDR Sriram Krishnamachary, Indraprastha Institute of Information Technology, Delhi, India, *Reviewer*, April 2019
- PhD Adrien Husson, Université Paris-Diderot, *Rapporteur*, 16 Dec. 2019
- PhD Jorgelindo da Viega Moreira, Université Paris-Saclay, *Rapporteur*, 18 April 2019
- Master Thesis, Orianne Bargain, Graph matching, theory and SAT implementation, Technische Universität Dresden, Germany, *Superviser* October 2019
- Master Thesis, Zi-Jun Lin, National Taiwan University, *Reviewer*, July 2019
- Master Thesis, Wei-Chih Huang, National Taiwan University, *Reviewer*, July 2019
- Master Thesis, Elisabeth Degrand, Evolving Chemical Reaction Networks, Kungliga tekniska ho¨gskolan, Stockholm, Sweden, *Superviser* April 2019,

10.3. Popularization

10.3.1. Articles and contents

- For a Festschrift in honor of Catuscia Palamidessi, François Fages was unexpectedly inspired to compose a small music score and write a short essay on information leakage in music scores [10].
- Jakob Ruess gave an interview for the Swiss Institute of Bioinformatics (SIB) as a past laureate of the SIB Award.

10.3.2. Interventions

- Eléonore Bellot, Mathieu Hemery and Elise Weill Duflos participated at Fête de la Science at Inria SIF, Oct. 2019
- 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.
- Mathieu Hemery has animated debates with college school students on Science and Ethics at Inria and Association Arbre des Connaissance, Oct. 2019.

10.3.3. Creation of media or tools for science outreach

François Fages has created several BIOCHAM interactive notebooks that are integrated in the current release of BIOCAHM-4 to illustrate and exercise the main concepts of analog chemical computer and chemical programming, as teached in his Master course at MPRI.

11. Bibliography

Publications of the year

Articles in International Peer-Reviewed Journals

[1] S. A. CEPEDA-HUMEREZ, J. RUESS, G. TKAČIK. *Estimating information in time-varying signals*, in "PLoS Computational Biology", September 2019, vol. 15, n^{o} 9, e1007290 p. [*DOI :* 10.1371/JOURNAL.PCBI.1007290], https://hal.inria.fr/hal-02304413

[2] M. HEMERY, P. FRANÇOIS. *Convergent evolution in silico of biochemical log-response*, in "Journal of Chemical Physics", February 2019, https://hal.inria.fr/hal-02389284

[3] J. RUESS, M. PLEŠKA, C. C. GUET, G. TKAČIK. *Molecular noise of innate immunity shapes bacteria-phage ecologies*, in "PLoS Computational Biology", July 2019 [*DOI :* 10.1371/JOURNAL.PCBI.1007168], https://hal.archives-ouvertes.fr/hal-02229803

International Conferences with Proceedings

[4] E. DEGRAND, M. HEMERY, F. FAGES. *On Chemical Reaction Network Design by a Nested Evolution Algorithm*, in "CMSB 2019 - 17th International Conference on Computational Methods in Systems Biology", Trieste, Italy, LNCS, Springer-Verlag, September 2019, n° 11773, https://hal.inria.fr/hal-02173682

[5] W.-C. HUANG, J.-H. JIANG, F. FAGES, F. MOLINA. *Biochemical Threshold Function Implementation with Zero-Order Ultrasensitivity*, in "BioCAS 2019 - IEEE Biomedical Circuits and Systems Conference", Nara, Japan, IEEE, October 2019, pp. 1-4 [*DOI :* 10.1109/BIOCAS.2019.8919176], https://hal.inria.fr/hal-02425761

[6] M. KRYUKOV, A. CARCANO, G. BATT, J. RUESS. *Can optimal experimental design serve as a tool to characterize highly non-linear synthetic circuits?*, in "ECC 2019 - European Control Conference", Naples, Italy, June 2019, https://hal.inria.fr/hal-02304425

[7] J. MARTINELLI, J. GRIGNARD, S. SOLIMAN, F. FAGES. *A Statistical Unsupervised Learning Algorithm for Inferring Reaction Networks from Time Series Data*, in "ICML 2019 - Workshop on Computational Biology", Long Beach, CA, United States, June 2019, https://hal.inria.fr/hal-02163862

[8] J. MARTINELLI, J. GRIGNARD, S. SOLIMAN, F. FAGES. *On Inferring Reactions from Data Time Series by a Statistical Learning Greedy Heuristics*, in "CMSB 2019 - 17th Computational Methods in Systems Biology", Trieste, Italy, L. BORTOLUSSI, G. SANGUINETTI (editors), LNCS, Springer-Verlag, September 2019, n° 11773, https://hal.inria.fr/hal-02173721

[9] E. WEILL, V. ANDREANI, C. ADITYA, P. MARTINON, J. RUESS, G. BATT, F. BONNANS. *Optimal control of an artificial microbial differentiation system for protein bioproductioń*, in "ECC 2019 - European Control Conference", Naples, Italy, June 2019, https://hal.inria.fr/hal-02429963

Scientific Popularization

[10] F. FAGES. *Information Leakage in a Music Score*, in "The Art of Modelling Computational Systems - A Journey from Logic and Concurrency to Security and Privacy - Essays Dedicated to Catuscia Palamidessi on the Occasion of Her 60th Birthday", Lecture Notes in Computer Science, Springer-Verlag, October 2019, vol. Festschrift - LNCS, n° 11760 [*DOI :* 10.1007/978-3-030-31175-9], https://hal.inria.fr/hal-02365478

Other Publications

[11] O. BARGAIN. *Graph matching, theory and SAT implementation*, Technische Universität Dresden, October 2019, https://hal.inria.fr/hal-02339907

[12] E. DEGRAND. *Evolving Chemical Reaction Networks*, Master's Thesis, Kungliga tekniska högskolan (Stockholm), April 2019, pp. 1-70, https://hal.inria.fr/hal-02333691