INRIA

INSTITUT NATIONAL DE RECHERCHE EN INFORMATIQUE ET EN AUTOMATIQUE

Team IBIS

Modeling, simulation, measurement, and control of bacterial regulatory networks

Grenoble - Rhône-Alpes

THEME BIO

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2. Overall Objectives

2.1. Overview

Bacteria provide fascinating examples of the survival strategies developed by single-cell organisms to respond to environmental stresses. The stress responses of bacteria are controlled by large and complex networks of molecular interactions that involve genes, mRNAs, proteins, small effector molecules, and metabolites. The study of bacterial stress response networks requires experimental tools for characterizing the interactions making up the networks and measuring the dynamics of cellular processes on the molecular level. In addition, when dealing with systems of this size and complexity, we need mathematical modeling and computer simulation to integrate available biological data, and understand and predict the dynamics of the system under various environmental and physiological conditions. The analysis of living systems through the combined application of experimental and computational methods has gathered momentum in recent years under the name of systems biology.

The first aim of the IBIS team is the unravelling of bacterial survival strategies through a systems-biology approach, making use of both models and experiments. In particular, we focus on the enterobacterium *Escherichia coli*, for which enormous amounts of genomic, genetic, biochemical and physiological data have been accumulated over the past decades. A better understanding of the survival strategies of *E. coli* in situations of nutritional stress is a necessary prerequisite for interfering with these strategies by specific perturbations or by even rewiring the underlying regulatory networks. This is the second and most ambitious aim of the project. It does not only spawn fundamental research on the control of living matter, but which may ultimately acquire medical relevance since *E. coli* serves as a model for many pathogenic bacteria.

The aims of IBIS raise four main challenges that generate new problems on the interface of (experimental) biology, applied mathematics, and computer science. In particular, the success of the project critically depends on (1) the modeling of large and complex bacterial regulatory networks, (2) the computer analysis and simulation of the network dynamics by means of these models, (3) high-precision and real-time measurements of gene expression to validate the models, and (4) the control and re-engineering of bacterial regulatory networks. While the first three items have been active research topics over the past few years, the control of regulatory networks is a novel challenge for IBIS that will be developed in the coming years.

The challenges of the research programme of the IBIS team require a wide range of competences on the interface of (experimental) biology, applied mathematics, and computer science. Since no single person can be expected to possess all of these competences, the international trend in systems biology is to join researchers from different disciplines into a single group. In line with this development, the IBIS team is a merger of an experimental biology group on the one hand, and a bioinformatics and biological modeling group on the other hand. In particular, the IBIS team is composed of members of the group of Hans Geiselmann at the Laboratoire Adaptation et Pathogénicité des Microorganismes of the Université Joseph Fourier (UJF, CNRS UMR 5163), and the network modeling and simulation group formerly part of the HELIX project-team at INRIA Grenoble-Rhône-Alpes, a group coordinated by Hidde de Jong. Both groups include researchers and technicians from other institutes, such as CNRS and the Université Pierre Mendès France (UPMF). The two groups have established a fruitful collaboration, which has resulted in more than 40 peer-reviewed publications in journals, conferences, and books since 2000.[1]

Hidde de Jong is the head of the IBIS team and Hans Geiselmann its co-director. The experimental biology component of IBIS also remains part of the Laboratoire Adaptation et Pathogénicité des Microorganismes, and Hans Geiselmann continues to represent this group in the interactions with the laboratory and university administration.

2.2. Highlights of the year

The IBIS team officially started on 1 January, 2008. Its new web site, developed by Metamorphoz, went online last November (http://ibis.inrialpes.fr).

A paper by Pedro Monteiro was accepted for presentation at the *European Conference of Computational Biology* and published in a special issue of *Bioinformatics*. The acceptation rate was about 9%.

Organization of the second annual meeting of the FP-6 Pathfinder project COBIOS in Grenoble on February 11-12.

Samuel Drulhe defended his PhD thesis in December 2008. The thesis is based on collaborative work with Riccardo Porreca and Giancarlo Ferrari-Trecate (university of Pavia, Italy) and has given rise to two journal publications this year: *IEEE Transactions on Automatic Control* and *Journal of Computational Biology*.

3. Scientific Foundations

[1] See http://ibis.inrialpes.fr for a complete list.

Figure 1. The ibis was an object of religious veneration in ancient Egypt, particularly associated with the god Thoth. Thoth was seen, among other things, as a god of the ***measurement and regulation of events****. Here Thoth is shown in human form with the face of an ibis. (Sources: http://en.wikipedia.org/wiki/Ibis, http://en.wikipedia.org/wiki/Thoth, and http://www.shoarns.com).*

3.1. Models: Development and reduction of models of bacterial regulatory networks

Keywords: *Kinetic modeling*, *genetic regulatory networks*, *global regulators*, *metabolic networks*, *model reduction*.

Participants: Delphine Ropers [Correspondent], Hidde de Jong, Johannes Geiselmann, Yan Cao, Yves Markowicz, Sara Berthoumieux, Valentina Baldazzi, Jérôme Izard.

The adaptation of bacteria to changes in their environment is controlled on the molecular level by large and complex interaction networks involving genes, mRNAs, proteins, and metabolites (Figure 2). The elucidation of the structure of these networks has much progressed as a result of decades of work in genetics, biochemistry, and molecular biology. Most of the time, however, it is not well understood how the response of a bacterium to a particular environmental stress emerges from the interactions between the molecular components of the network. This has called forth an increasing interest in the mathematical modeling of the dynamics of biological networks, in the context of a broader movement called systems biology.

In theory, it is possible to write down mathematical models of biochemical networks, and study these by means of classical analysis and simulation tools. In practice, this is not easy to achieve though, as quantitative data on kinetic parameters are usually absent for most systems of biological interest. Moreover, the models include a large number of variables, are strongly nonlinear and include different time-scales, which make them difficult to handle both mathematically and computationally. A possible approach to this problem has been to use approximate models that preserve essential dynamical properties of the networks. Different approaches have been proposed in the literature, such as the use of approximations of the typical response functions found in gene and metabolic regulation and the reduction of the model dimension by decomposing the system into fast and slow variables. These reductions and approximations result in simplified models that are easier to analyze mathematically and for which parameter values can be more reliably estimated from the available experimental data.

Model reduction approaches are exploited in IBIS to gain a better understanding of the ability of the *E. coli* to adapt to a various nutritional and other environmental stresses, such as carbon, phosophate, and nitrogen starvation. We are particularly interested in gaining a better understanding of the role of the so-called global regulators of gene expression in shaping the survival strategies of the bacteria. Moreover, we study the

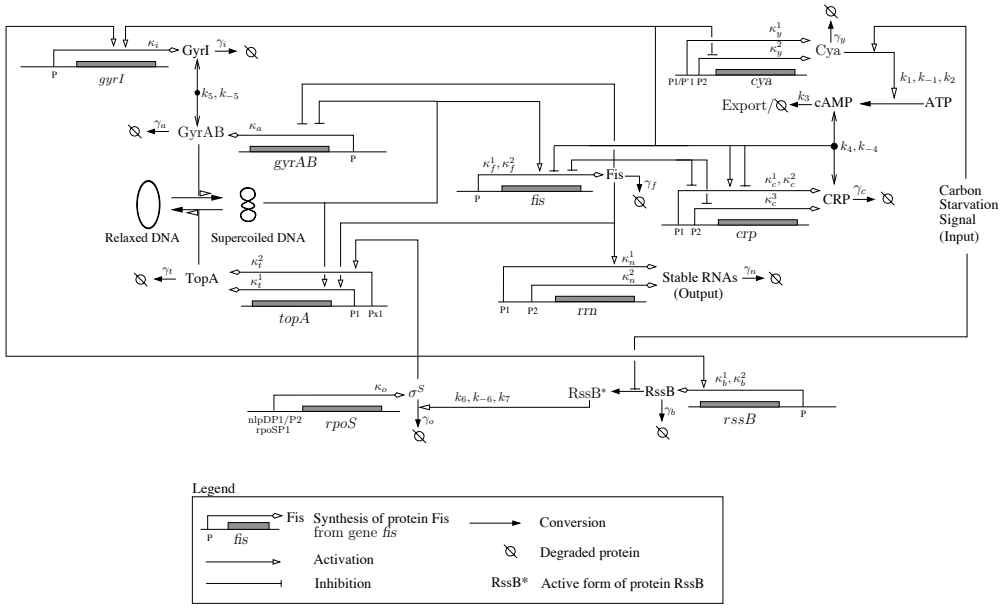


Figure 2. Network of key genes, proteins, and regulatory interactions involved in the nutritional stress network in E. coli (figure adapted from Monteiro et al., Bioinformatics, 24(16):i227-i233, 2008).

interactions between metabolism and gene expression in the adaptation of *E. coli* to changes in available carbon sources. These topics are studied in collaboration with the HELIX and COMORE project-teams at INRIA.

3.2. Methods: Computer analysis and simulation of bacterial regulatory networks

Keywords: *Genetic Network Analyzer*, *Genetic regulatory networks*, *ODE models*, *PLDE models*, *formal verification*, *metabolic networks*, *model checking*, *numerical simulation*, *qualitative simulation*, *system identification*, *systems biology*.

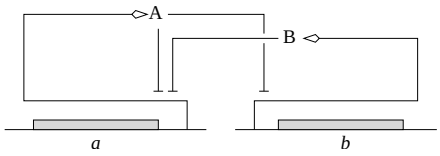
Participants: Valentina Baldazzi, Bruno Besson, Hidde de Jong [Correspondent], Estelle Dumas, Johannes Geiselmann, Pedro Monteiro, Michel Page, François Rechenmann, Delphine Ropers, Sara Berthoumieux, Woeh-Fu Wang.

Computer simulation is a powerful tool for explaining the capability of bacteria to adapt to sudden changes in their environment in terms of structural features of the underlying regulatory network, such as interlocked positive and negative feedback loops. Moreover, computer simulation allows the prediction of unexpected or otherwise interesting phenomena that call for experimental verification. The use of simplified models of the stress response networks makes simulation easier in two respects. In the first place, model reduction restricts the class of models to a form that is usually easier to treat mathematically, in particular when quantitative information on the model parameters is absent or unreliable. Second, in situations where quantitative precision is necessary, the estimation of parameter values from available experimental data is easier to achieve when using models with a reduced number of parameters.

Over the past few years, we have developed in collaboration with the COMORE project-team a qualitative simulation method adapted to a class of piecewise-linear (PL) differential equation models of genetic regulatory networks. The PL models, originally introduced by Leon Glass and Stuart Kauffman, provide a coarse-grained picture of the dynamics of genetic regulatory networks. They associate a protein or mRNA concentration variable to each of the genes in the network, and capture the switch-like character of gene regulation by means of step functions that change their value at a threshold concentration of the proteins. The advantage of using PL models is that the qualitative dynamics of the high-dimensional systems are relatively simple to analyze, using inequality constraints on the parameters rather than exact numerical values. The qualitative dynamics of genetic regulatory networks can be conveniently analyzed by means of discrete abstractions that transform the PL model into so-called state transition graphs.

The development and analysis of PL models of genetic regulatory network has been implemented in the qualitative simulation tool GENETIC NETWORK ANALYZER (GNA) (Section 4.1). GNA has been used for the analysis of several bacterial regulatory networks, such as the initiation of sporulation in *B. subtilis*, quorum sensing in *P. aeruginosa*, the carbon starvation response in *E. coli*, and the onset of virulence in *E. chrysanthemi*. GNA is currently distributed by the Genostar company, but remains freely available for academic research. The analysis of realistic models of bacterial regulatory networks by means of GNA leads to large state transition graphs, which makes manual verification of properties of interest practically infeasible. This has motivated the coupling of GNA to formal verification tools, in particular model checkers that allow properties formulated in temporal logic to be verified on state transition graphs. This is part of on-going collaborations with the POP-ART and VASY project-teams at INRIA Grenoble - Rhône-Alpes.

The validation of the models may lead to the conclusion that the latter do not account for the experimental data and need to be revised. That is, the model may for instance not be able to reproduce the observed order of activation of certain genes. The model checker returns diagnostics which can be analyzed for clues for revision, such as the addition or modification of interactions. Another way to proceed is by inferring the model, and more particularly the model structure, from time-series data that are increasingly becoming available (see Section 3.3). Initial work in IBIS has focused on linear and piecewise-linear models of the network of global regulators in bacteria. In collaboration with colleagues from the University of Pavia (Italy), these structural identification approaches will be further developed and completed with approaches appropriate for the more general classes of reduced models of regulatory networks discussed in Section 3.1.



(a)

$$
\begin{aligned}
\dot{x}_a &= \kappa_a \, s^-(x_a, \theta_a^2) \, s^-(x_b, \theta_b) - \gamma_a \, x_a \\
\dot{x}_b &= \kappa_b \, s^-(x_a, \theta_a^1) - \gamma_b \, x_b \\
s^+(x, \theta) &= \begin{cases} 1, & \text{if } x > \theta \\ 0, & \text{if } x < \theta \end{cases} \\
s^-(x, \theta) &= 1 - s^+(x, \theta)
\end{aligned}
\tag{2}
$$

(b)

Figure 3. (a) Example of a genetic regulatory network of two genes (a and b), each coding for a regulatory protein (A and B). Protein B inhibits the expression of gene a, while protein A inhibits the expression of gene b and its own gene. (b) PLDE model corresponding to the network in (a). Protein A is synthesized at a rate κ_a, if and only if the concentration of protein A is below its threshold θ_a^2 ($x_a < \theta_a^2$) and the concentration of protein B below its threshold θ_b ($x_b < \theta_b$). The degradation of protein A occurs at a rate proportional to the concentration of the protein itself ($\gamma_a \, x_a$).

3.3. Data: High-precision measurements of gene expression in bacteria

Keywords: *Molecular biology*, *bacteriology*, *biotechnology*, *gene expression*, *gene regulation*, *genomics*, *metabolims*, *proteomics*, *reporter genes*.

Participants: Corinne Pinel, Hidde de Jong, Johannes Geiselmann [Correspondent], Delphine Ropers, Sara Berthoumieux, Caroline Ranquet-Brazzolotto, Stephan Lacour, Yves Markowicz, Antoine Frenoy, Jérôme Izard, Guillaume Baptist.

The goals of a model are to describe the functioning of bacterial regulatory networks in a way that helps to understand the underlying mechanisms and to predict the behavior of the system in new situations. In order to achieve these goals, we have to confront model predictions with experimental observations. This requires the availability of high-precision measurements of gene expression and other key processes in the cell.

Since their emergence in the mid-1990s, DNA microarrays have become a method of choice for measuring the dynamical response of a genetic regulatory network. This is in large part due to their unparalleled capacity of providing snapshots of gene expression across the whole genome of an organism. The use of DNA microarrays is less appropriate for our purpose though. The precision of the measurements is generally low and the costs to monitor the dynamics of gene expression at a high temporal resolution quickly become prohibitive. We have therefore resorted to the measurement of fluorescent and luminescent reporter genes, which allow monitoring the expression of only a few dozens of regulators in parallel, but with the precision and temporal resolution needed for the validation of our models. More specifically, we have constructed transcriptional and translational fusions of key regulatory genes of *E. coli* to fluorescent and luminescent reporter genes (Figure 4). The variation of the expression of these reporter genes are measured *in vivo* and in real time by an automated, thermostated microplate reader.

While reporter gene systems allow the dynamics of gene expression to be measured with high precision and temporal resolution on the level of cell populations, they do not provide information on all variables of interest though. Additional technologies may complement those that we have developed in our laboratory, such as mass-spectrometry tools in proteomics and metabolomics that are able to quantify the amounts of proteins and metabolites, respectively, in the cells at a given time-point. In addition, for many purposes it is also important to be able to characterize gene expression on the level of single cells instead of cell populations. This requires experimental platforms that measure the expression of reporter genes in isolated cells by means of fluorescence and luminescence microscopy. IBIS has access to these technologies through collaborations with other groups

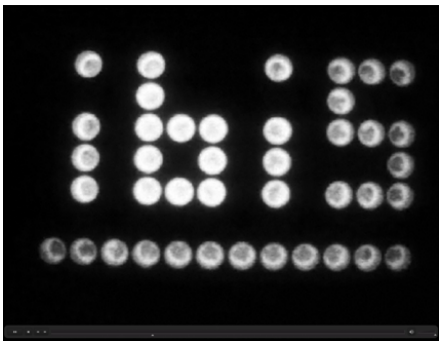


Figure 4. Playful illustration of the principle of reporter genes (see http://ibis.inrialpes.fr for the corresponding movie). A microplate containing a minimal medium (with glucose and acetate) is filmed during 36 hours. Wells contain E. coli bacteria which are transformed with a reporter plasmid containing the luciferase operon (luxCDABE) under control of the acs promoter. This promoter is positively regulated by the CRP-cAMP complex. When bacteria have metabolized all the glucose, the cAMP concentration increases quickly and activates the global regulator CRP which turns on the transcription of the luciferase operon producing the light. The glucose concentration increases from left to right on the microplate, so its consumption takes more time when going up the gradient and the letters appear one after the other. The luciferase protein needs reductive power (FMNH2) to produce light. At the end, when acetate has been depleted, there is no more carbon source in the wells. As a consequence, the reductive power falls and the "bacterial billboard" switches off. Source: Guillaume Baptist.

on the local and national level, such as the INSA de Toulouse and the Laboratoire de Spectrométrie Physique at the Université Joseph Fourier.

4. Software

4.1. Genetic Network Analyzer (GNA)

Keywords: *Genetic regulatory networks*, *qualitative simulation*.

Participants: Bruno Besson, François Rechenmann, Estelle Dumas, Hidde de Jong [Correspondent], Pedro Monteiro, Michel Page, Delphine Ropers.

GENETIC NETWORK ANALYZER (GNA) is the implementation of a method for the qualitative modeling and simulation of genetic regulatory networks developed in the HELIX project. The input of GNA consists of a model of the regulatory network in the form of a system of piecewise-linear differential equations, supplemented by inequality constraints on the parameters and initial conditions. From this information, GNA generates a state transition graph summarizing the qualitative dynamics of the system. GNA is currently distributed by the company Genostar, but remains freely available for academic research purposes. For more information, see http://www-helix.inrialpes.fr/gna.

4.2. ISee

Keywords: *Bioinformatics*, *e-learning*.

Participant: François Rechenmann [Correspondent].

The aim of ISEE (IN SILICO BIOLOGY E-LEARNING ENVIRONMENT) is to explain the principles of the main bioinformatics algorithms through interactive graphical user interfaces and to illustrate the application of the algorithms to real genomic data. Written in Java, ISEE defines a generic framework for combining algorithms with courses. More precisely, the environment implements the metaphor of a lab notebook: the left pages present and explain the experiments to be carried out by the student, whereas the right pages display the progress of these experiments, *i.e.*, the execution of the associated algorithms. In its present state, the environment offers different algorithmic modules structured into three main chapters: sequence comparison, statistical analysis of DNA sequences for the identification of coding regions, and basic pattern-matching algorithms including the use of regular expressions. These and other algorithms have been integrated in two original practical courses. The first one is an introduction to the statistical analysis of genetic sequences and leads the student to the identification of the origin of replication within bacterial genomes. The second one shows the student how to identify coding regions in bacterial genomes and to characterize their products. The latter course was developed in collaboration with the CCSTI ("Centre de Culture Scientifique Technique et Industrielle") in Grenoble, which used ISEE for its "École de l'ADN". For more information, see: http://ibis.inrialpes.fr/article124.html.

4.3. WellReader

Keywords: *Gene expression*, *reporter gene data*.

Participants: Bruno Besson, Johannes Geiselmann, Hidde de Jong [Correspondent], Delphine Ropers, Guillaume Baptist, Jérôme Izard.

WELLREADER is a program for the analysis of gene expression data obtained by means of fluorescent and luminescent reporter genes. WELLREADER reads data files in an XML format or in a format produced by microplate readers, and allows the user to detect outliers, perform background corrections and spline fits, and compute promoter activities and protein concentrations. WELLREADER has been written in MATLAB and will be made available to the academic research community in 2009.

5. New Results

5.1. Models: Development and reduction of models of bacterial regulatory networks

The new results in 2008 concern (i) the extension and validation of previously-developed models of the *E. coli* carbon starvation response and (ii) the reduction of these models to simplified nonlinear models.

5.1.1. Development and validation of models of the carbon starvation response in E. coli

In the bacterium *E. coli*, the adaptation to the carbon-source availability is controlled by a complex network involving signaling cascades, metabolic reactions, gene expression, and the cellular machinery. The different modes of regulation are interwoven to such an extent that it is difficult to understand how they coordinate the response of *E. coli* cells to changes in nutrient conditions. To address this question we have continued the development of kinetic models of the functioning of this complex regulatory network, using standard approaches in biochemistry. These models, taking the form of large systems of nonlinear ODEs, describe the rate of change of the concentrations of the network components.

First, Delphine Ropers extended a previous model that focuses on the role played by global regulators in the reorganization of gene expression in *E. coli* following a carbon upshift or downshift. These transcription factors control the expression of many genes that have cellular functions important for the adaptation of the bacterial growth to the environmental conditions. We added new interactions and new components to the carbon starvation response network (namely, the general stress factor RpoS, the regulator of degradation RssB, and the gyrase inhibitor, GyrI). The model has been used to analyze the role played by the factor RpoS during the adaptation of *E. coli* to a carbon downshift. Contrary to the intuition, the factor is predicted to play a key role in the control of the DNA supercoiling level, but not in the growth arrest [4].

Second, in the framework of the MetaGenoReg and EC-MOAN projects (Sections 7.1 and 7.2), we analyzed the contribution of the metabolic and genetic regulations of carbon assimilation by *E. coli*. This network controls the sensing and uptake of glucose, as well as its metabolism into pyruvate through the glycolysis pathway, and the reverse reaction, neoglucogenesis. The interactions in the network involve several levels of (intricate) regulation: global regulators of transcription control the synthesis of enzymes as well as their own expression, while enzymatic activities are controlled by end products of metabolism, which also control the activity of transcription factors. The kinetic model of this network, developed by Delphine Ropers, Hans Geiselmann, Yves Markowicz, and Hidde de Jong, is composed of 39 nonlinear differential equations with 159 parameters that are mostly unknown. The reduction of the model to a more manageable form is currently being undertaken (Section 5.1.2). The resulting reduced model will be calibrated with available experimental data and used for elucidating the complicated regulatory mechanisms controlling glucose and acetate metabolism.

Recent experimental data obtained by the group of Hans Geiselmann show that the interactions between global regulators of transcription are not sufficient to account for the control of gene expression during the growth transitions of *E. coli*. The analysis of the patterns of expression of the global regulators suggests an effect of the cellular gene expression machinery, which controls the global regulators at the transcriptional, translational, and stability levels. Moreover, the activity of the cellular gene expression machinery is known to vary with the environmental conditions. To verify this hypothesis, Cao Yan and Delphine Ropers are in the process of developing a kinetic model of the global control of gene expression by the cellular transcription and translation machinery.

5.1.2. Reduction of models of the carbon starvation response

The kinetic models described above are large systems of nonlinear ODEs, spanning different time scales, with parameter values that are mostly unknown within a range of several orders of magnitude. This has motivated the application of model reduction approaches, which makes possible the analysis of the dynamic properties of the systems (Section 3.1). Over the past few years we have settled on a reduction strategy widely used in the modeling of biochemical processes, the quasi-steady-state approximation. This approximation allows the fast variables to be eliminated from the system of ODEs, by expressing them in terms of the slow variables through algebraic equations. In certain cases, the models have been further simplified by applying a piecewise-linear approximation. The latter simplification allows a quick scan of the qualitative dynamics of the system, without numerical information on the parameters.

First, Delphine Ropers and Valentina Baldazzi tested whether the model reduction strategy based on quasi-steady-state and piecewise-linear approximations preserve essential dynamical properties of the system, over a wide range of parameter values. To this end, the classical nonlinear and piecewise-linear models of the carbon starvation response in *E. coli* were systematically compared, using an ensemble approach. The results showed that, in comparison with the nonlinear models, the piecewise-linear approximations generally preserve the dynamics of the carbon starvation response network. An analysis of why the approximations work in the case of the *E. coli* network has allowed the identification of some conditions for the successful application of the model reduction strategy based on quasi-steady-state and piecewise-linear approximations. Two papers based on this work have been submitted for publication.

Second, following the above reduction strategy, Valentina Baldazzi and Hidde de Jong started to work on the reduction of the model of the carbon assimilation described in Section 5.1.1, which includes both metabolic and genetic regulatory processes. Although the general model reduction strategy outlined above still applies, the use of the quasi-steady-state approximation leads to large systems of nonlinear algebraic equations that are difficult to solve. We are therefore working on approaches that combine the quasi-steady state approximation with sensitivity criteria from metabolic control analysis to uncover the direct and indirect interactions between the slow variables of the system. A paper describing the method is in preparation.

5.2. Methods: Computer analysis and simulation of bacterial regulatory networks

Our efforts focused on several on-going projects around the development of methods and tools for the analysis of bacterial regulatory networks: (i) the extension of the tool GNA with a network editor, (ii) the connection of the modeling and simulation approach underlying GNA with formal verification tools, and (iii) the identification of genetic regulatory networks.

5.2.1. Editor of bacterial regulatory networks

IBIS and Genostar together participate in the European project COBIOS (Section 7.2), where their main role is the development of a computational infrastructure for synthetic network modeling and design. In order to achieve this, we reuse, adapt, and integrate existing and well-tested tools in bioinformatics and systems biology, rather than develop new tools from scratch.

INRIA and Genostar jointly developed a conceptual model to represent bacterial regulatory networks. The model has been implemented into a library called IOGMANETWORK, using AROM, the underlying entity-relationship data and knowledge model of Genostar's IOGMA platform. The use of AROM ensures that new types of entities and relationships can easily be added in order to meet new modeling requirements. Presently available items for building networks are: individual entities (coding sequences, promoters, proteins,...), assemblies of components (complexes,...), reactions (gene expression, degradation, transport, state transitions,...), and regulation (activation, inhibition). This work has notably involved Bruno Besson, Hidde de Jong, Michel Page, François Rechenmann, and engineers of Genostar.

On the one hand, the IOGMANETWORK library underlies the new NETWORKBUILDER module of the IOGMA platform, which can be connected to the other modules through the shared data and knowledge model. On the other hand, the development of GNA as a module in the IOGMA platform has been carried further by integrating the IOGMANETWORK library into GNA. This has required linking the mathematically-oriented model formalism of GNA with the biologically-oriented concepts underlying the network editor. We have specified and implemented an algorithm for automatically translating networks constructed by means of the editor to reduced networks that match the GNA model formalism. Moreover, the library has been extended so as to be able to represent entities specific to GNA, and customize the view in order to visualize the results of the reduction algorithm.

First steps have been undertaken to integrate the design of the interaction network with the entire process of qualitative modeling and simulation carried out by means of GNA, which requires rethinking the global architecture of the application. The new version of GNA integrating the above-mentioned work is scheduled to be available by the end of 2009.

5.2.2. Formal verification of genetic regulatory networks

Since the PhD thesis of Grégory Batt, concluded early 2006, HELIX and its follow-up team IBIS have much invested in making formal verification technology available to the analysis of bacterial regulatory networks. This notably involves the participation in the European project EC-MOAN (Section 7.2), together with Radu Mateescu of the VASY project-team.

First, Pedro Monteiro in the framework of his PhD thesis, previously defined a temporal logic named CTRL (Computation Tree Regular Logic), which is particularly useful for expressing dynamic properties of genetic regulatory networks. CTRL extends CTL with regular expressions and fairness operators. This year we completed the formal definition of the syntax and semantics of CTRL and, in collaboration with Radu Mateescu, developed a model checking procedure for CTRL by reusing as much as possible the existing verification technology available in the CADP toolbox developed by VASY. This translation, implemented by Estelle Dumas (funded by an associate engineer grant from INRIA and by Université Joseph Fourier), allowed us to obtain an on-the-fly linear-time model checking procedure for a significant fragment of CTRL, which can capture multistability and oscillation properties. This work has been presented at the International Symposium on Automated Technology for Verification and Analysis (ATVA) [13].

Second, Estelle Dumas worked on the development of a web service allowing GNA to communicate with CADP. A main advantage of the web service technology is that it provides a flexible way to exploit state-of-the-art model checking technology by bioinformatics applications, avoiding common portability and

installation problems. The architecture under development allows the user to specify temporal logic properties through the graphical user interface of GNA and send the latter for verification to the web server. The results, consisting of a verdict (true/false) and a diagnostic (a witness or a counterexample) are returned to GNA and presented to the modeler in graphical form. A prototype of the web service architecture is operational, while performance tests under a variety of user conditions are under way. In collaboration with Gregor Goessler (POP-ART) and Grégory Batt (CONTRAINTES), we have also explored implicit instead of explicit graph representations of the dynamics of genetic regulatory networks, to increase the efficiency of the interactions with the web service.

Third, to facilitate the usage of model checking within the system biology community, we adopted the use of patterns, that is, high-level query templates that capture recurring questions of biological interest and that can be automatically translated to temporal logic. Pedro Monteiro developed a set of patterns for the analysis of dynamic models of cellular regulatory networks, as described in contributions to a special issue of *Bioinformatics* on the European Conference on Computational Biology [4] and to the proceedings of the European Conference on Artificial Intelligence (ECAI) [9]. In collaboration with Ana-Teresa Freitas, invited professor in the BAMBOO team, we proposed several generic query templates, based on a review of frequently-asked questions by modelers. These templates were translated into temporal logic formulas and applied to the analysis of the qualitative dynamics of large and complex models of the *E. coli* carbon starvation response models, described in Section 3.1.

5.2.3. Identification of bacterial regulatory networks

Originally started in the framework of the European project HYGEIA, the work on the structural identification of genetic regulatory networks using PL models was completed with the PhD thesis defense of Samuel Drulhe in December 2008 [1], and the publication of papers in the *IEEE Transactions on Automatic Control* [3] and the *Journal of Computational Biology* [5]. The input of the PL identification method consists of time-series measurements of concentrations of gene products. As output it produces estimates of the modes of operation of the genetic regulatory network as well as all possible minimal combinations of threshold concentrations of the gene products accounting for switches between the modes of operation. The applicability of the PL identification method has been evaluated using simulated data obtained from a model of the carbon starvation response in *E. coli*. This has allowed us to systematically test the performance of the method under different data characteristics, notably variations in the noise level and the sampling density. Woeh-Fu Wang, in the framework of her PhD thesis, is also interested in the structural identification of genetic regulatory networks, but she focuses on the detection of latent variables and uses different types of models (linear and power-law models).

The gradual move from PL models to broader classes of nonlinear models of bacterial regulatory networks, and the increased emphasis on quantitative rather than qualitative models, has been further reinforced with the PhD project of Sara Berthoumieux. This project, funded by a CORDIS grant from INRIA, continues a MSc internship in IBIS started in the beginning of this year. It concerns methods for the reduction of large and complex models of biochemical networks, in particular the combined genetic and metabolic networks studied in the ANR project MetaGenoReg (coordinated by Daniel Kahn, co-supervisor of the thesis). It requires the formulation of reduced models of the genetic and metabolic parts of the system, the estimation of the values of the model parameters from experimental data, and the use of the reduced models for gaining a better understanding of the adaptive processes of the bacteria during a glucose-acetate diauxie.

5.3. Data: High-precision measurements of gene expression in bacteria

During this past year we spent much effort in improving the quality and extent of the experimental observations by (i) modifying the reporter gene system and validating key steps of the reporter system, (ii) constructing expression vectors that allow subtle perturbations of the regulatory system, and (iii) developing a new method for identifying the pertinent genes to be included in a model.

5.3.1. Improvements of gene expression measurements

We have improved the gene expression measurements by exploring new variants of the green fluorescent protein (GFP), by transferring some of the constructions to the chromosome and by measuring and validating intermediate steps of gene expression. This work involves Corinne Pinel and Caroline Ranquet-Brazzolotto, post-doctoral researcher in the framework of the EC-MOAN project.

We are using two types of reporter genes: bacterial luciferase and variants of the GFP. The luciferase is much more sensitive than GFP, but since it is an enzyme, its activity depends not only on gene expression, but also on the metabolic state of the cell. We therefore systematically construct an identical reporter vector using GFP. The disadvantage of GFP is the fact that the cell itself possesses an autofluorescence which produces a considerable background noise. In order to diminish this background we have begun to replace our *gfp* gene by variants that possess different fluorescence colors. The red fluorescent protein (RFP) gave the best results and we are currently replacing some of our constructs with *rfp* fusions. Another important consideration for the measurements is the half-life of the reporter protein. Rapid changes in gene expression are difficult to measure with very stable reporter proteins. We have therefore engineered GFP by adding (or not) a degradation tag in order to control its half-life.

Our reporter constructs are located on a low copy-number plasmid (about 20 copies per cell). However, we can not formally exclude that some of our experimental manipulations change the plasmid copy number. In this case, we would misinterpret signal changes as changes in gene expression when really they only reflect changes in plasmid copy number. We have therefore constructed two "platforms" on the chromosome of *E. coli* into which we can transfer our reporter constructs, assuring that there is exactly one copy per cell. We are currently adapting this new system to the constraint, particularly important for the *gfp* constructs, that a single copy decreases the signal intensity by a factor of 20.

The relevant variable in our models, described by most equations, are the promoter activity of a gene under investigation and the concentration of its protein. However, reporter genes measure the accumulation of the reporter protein after transcription of the gene into mRNA and translation of this mRNA into protein. In order to relate the signal (reporter gene activity) to the relevant variables (promoter activity, protein concentration) we have to make assumptions about the kinetics of the intermediate steps. In order to validate these assumptions we have directly measured the relevant quantities (mRNA accumulation and stability) for one model gene. We are currently analyzing these data, using programs developed by Antoine Frenoy during an internship at INRIA. An article describing the results is in preparation.

5.3.2. System perturbations

In order to probe the system properties, we have to perturb the system and observe the dynamics of the system response by means of reporter genes. Until now, most of our perturbations consisted in changing the nutrient source of the bacteria. This affects the activity of a small number of genes. In order to perturb the system more systematically, Caroline Ranquet-Brazzolotto and Corine Pinel have constructed a series of expression vectors that allow the controlled induction of any particular gene at a precise moment during the time course of the experiment. We have characterized the induction profile of our expression vector and have constructed a second vector, using a different external signal, which will allow us to externally control the expression of two different genes in the bacterium.

We have also modified some of the proteins that are expressed from these vectors by adding a degradation tag to the proteins. The second vector can then be used to express a specific protease that will destroy the target protein when the external signal is given. We are currently calibrating this system, developed by Guillaume Baptist in the framework of his PhD thesis. These constructs will allow us to perturb the system in many different and very controlled ways, in exact analogy to modifications that can be introduced into the dynamical model of the network.

Somewhat 'cruder' perturbations consist in eliminating an entire node of the network. This corresponds biologically to the deletion of the corresponding gene. We have explored many of these single mutants (see below), but we have also begun to construct multiple mutants, where two or more genes of the network are removed. This serves to further probe the system behavior and test model predictions.

5.3.3. Detecting the input function of a gene

Another important task for the modeling and the biological understanding of a process is to well delimit the system boundaries. While high-throughput methods for detecting the target of a particular regulator are now classical (typically, DNA microarrays are used to study the effect of a particular mutation on the expression of all genes of a genome, see Section 3.3), no efficient method exists to determine the regulators that affect, directly or indirectly, the expression of a gene under investigation (the 'input function').

We have developed such a technique by making use of the Keio mutant collection of *E. coli* and devising a method for efficiently transforming our reporter plasmid into more than 4000 different clones. We have optimized the different steps of the procedure: transformation, detection of different signals (luminescence or coloration), image analysis, mutant selection and dynamical measurements of gene expression in the selected mutants. We have applied the method for identifying regulators that control the expression of genes that are critical for growth on acetate by *E. coli*, and regulators that modulate the expression of genes responsible for extracellular structures, so-called curli. This work has involved all members of the experimental side of IBIS and is currently being prepared for publication.

In both cases we have confirmed known regulatory mechanisms, but we have also discovered novel inputs to the regulation of these genes. We have established collaborations with other laboratories in France and Europe, specialized in the measurement of metabolites or metabolic activities, that will complement our measurements of gene expression. This direction is paralleled on the modeling side by the inclusion of metabolic reactions in the regulatory scheme and the resulting development of coarse-grained models and methods for model reduction (Section 3.1).

6. Contracts and Grants with Industry

6.1. Genostar

Participant: François Rechenmann.

Genostar, an INRIA start-up created in 2004, is a company developing software and solutions for the management and analysis of genomic and post-genomic data. The software has been developed, from 1999 to 2004, by the Genostar consortium (INRIA, Institut Pasteur, and the two biotech companies Genome Express and Hybrigenics) and by the HELIX research team. It includes several modules originally developed by HELIX , notably GenoAnnot, GenoLink, GenoBool and GenoExpertBacteria. The modules have been integrated in the Iogma bioinformatics environment, which also includes the modeling and simulation tool GNA developped by members of IBIS. François Rechenmann is scientific consultant of the company and members of IBIS and Genostar together collaborate in the COBIOS project (7.2). For more information, see http://www.genostar.com.

7. Other Grants and Activities

7.1. National projects

Project name	MetaGenoReg – Towards an understanding of the interrelations between metabolic and gene regulation: E. coli carbon metabolism as a test case
Coordinator	D. Kahn
IBIS participants	V. Baldazzi, G. Baptist, S. Berthoumieux, J. Geiselmann, H. de Jong, Y. Markowicz, C. Pinel, C. Ranquet-Brazzolotto, D. Ropers, W.-F. Wang
Type	ANR BIOSYS (2006-2009)
Web page	Not available for now

Project name	ColAge – Lifespan control in bacteria: Natural and engineering solutions
Coordinator	H. Berry
IBIS participants	G. Baptist, J. Geiselmann, H. de Jong, J. Izard, S. Lacour, C. Pinel, D. Ropers, W.-F. Wang
Type	Action d'Envergure INRIA-INSERM (2008-2012)
Web page	not available for now

Project name	Séminaire grenoblois des systèmes complexes
Coordinators	O. François, A. Girard et D. Ropers
IBIS participants	D. Ropers
Type	Funding by Institut des Systèmes Complexes de Lyon (IXXI)
Web page	http://www.ixxi.fr/Seminaires.php

7.2. European projects

Project name	EC-MOAN: Scalable modeling and analysis techniques to study emergent cell behavior: Understanding the E. coli stress response
Coordinators	J. van der Pol
IBIS participants	V. Baldazzi, G. Baptist, E. Dumas, J. Geiselmann, H. de Jong, Y. Markowicz, P. Monteiro, M. Page, C. Pinel, C. Ranquet-Brazzolotto, D. Ropers
Type	European Commission, FP6 NEST (2006-2009)
Web page	http://wwwhome.cs.utwente.nl/~ecmoan1/

Project name	COBIOS: Engineering and Control of Biological Systems: A New Way to Tackle Complex Diseases and Biotechnological Innovation
Coordinators	D. di Bernardo
HELIX participants	B. Besson, H. de Jong, M. Page, F. Rechenmann, D. Ropers
Type	European Commission, FP6 NEST (2006-2009)
Web page	http://www.cobios.net

8. Dissemination

8.1. Editorial, organizational, and reviewing activities

Hidde de Jong

Type	Journal, conference, agency
Member Editorial Board	Journal of Mathematical Biology
Member Editorial Board	ACM/IEEE Transactions on Computational Biology and Bioinformatics
Member Program Committee	AIME 09, CMSB 08, ECAI 08, HiBi 09, JOBIM 09, QR 08
Member Evaluation Committee	Agence Nationale de Recherche (ANR), Conception et Simulation (COSINUS)
Member Evaluation Committee	Netherlands Organisation for Scientific Research (NWO), Computational Life Science
Coordinator (with S. Robin)	Working group on Transcriptome, protéome, modélisation, inférence et analyse des réseaux biologiques of GDR CNRS 3003 Bioinformatique moléculaire
Member PhD Committee	Samuel Drulhe (Université Joseph Fourier)
Member Habilitation (HDR) Committee	Hugues Berry (Université d'Orsay), Anne Siegel (Université de Rennes I)
Project reviews	ANR, ETH (Switzerland)

Hans Geiselmann

Type	Journal, conference, agency
Member Evaluation Committee	AERES
Member PhD Committee	José Vinuelas (INSA Lyon), Letizia Tagliabu (University of Milan, Italy)
Member Habilitation (HDR) Committee	Rabah Iratni (Université Joseph Fourier), Ali Hakimi (Université Joseph Fourier)
Project reviews	ANR, CEA, Welcome Trust

Delphine Ropers

Type	Journal, conference, agency
Member Program Committee	JOBIM 08
Member Organization Committee	SeMoVi (Séminaire de Modélisation du Vivant)
Project reviews	ANR

8.2. Other administrative activities

Hans Geiselmann is leader of the Control of Gene Expression group in the "Laboratoire Adaptation et Pathogénie des Microorganismes" (UMR 5163).

Hidde de Jong is correspondent of the Departments of European Partnerships and International Relations of INRIA at the Grenoble - Rhône-Alpes research centre. He also coordinated with François Taddei a working group INRIA-INSERM on the modeling of biological systems (2007-2008).

François Rechenmann is head of the editorial committee of the Interstices website (http://interstices.info).

Delphine Ropers represents INRIA Grenoble - Rhône-Alpes in the scientific board of IXXI, the Complex Systems Institute in Lyon (http://www.ixxi.fr).

8.3. Seminars and PhD thesis defenses

Valentina Baldazzi

Title	Event and location	Date
Reduction of models of E. coli stress response: An evaluation study	Seminar of the group "Biologie des Systèmes et Modélisation Cellulaire" (BSMC), INSA de Lyon	Jan. 2008
A hybrid model of the early immune response inside a lymphnode	Seminar of the Laboratoire de Physique et Modélisation de Milieux Condensés (LPMMC), Grenoble	May 2008
Qualitative simulation of carbon starvation response in E. coli	European Conference on Mathematical and Theoretical Biology (ECMTB), Edimburgh (Scotland)	Jul. 2008
Modeling biological systems at the cellular and molecular levels: lymph node and carbon starvation response in E. coli	Seminar MAGMA, CPT , Marseille	Oct. 2008
Qualitative simulation of carbon starvation response in E.coli	Workshop MaReBio (Modéles discrets de réseaux biologiques : de la structure à la dynamique), CIRM, Marseille	Nov. 2008

Guillaume Baptist

Title	Event and location	Date
Mesure d'une dynamique d'expression génique	Seminar Laboratoire Adaptation et Pathogénicité des Microorganismes (LAPM), Grenoble	Jan. 2008

Hidde de Jong

Title	Event and location	Date
Qualitative modeling and simulation of bacterial regulatory networks	Seminar CEA, Saclay	Mar. 2008
Modeling and simulation of bacterial regulatory networks	Atelier INSERM "Modélisation et analyse statistique des réseaux biologiques"	May 2008
Qualitative modeling and simulation of bacterial regulatory networks	DISC Summer School Cells and Systems, Woudschoten (the Netherlands)	Jun. 2008
Qualitative modeling and simulation of bacterial regulatory networks	Invited talk during 5th Workshop on Computational Systems Biology (WCSB 2008), Leipzig (Germany)	Jun. 2008
Modeling of genetic regulatory networks: Simple models for complex systems	Invited talk ECBB Workshop on "Networks: Theory and Practice", Cagliari (Italy)	Sep. 2008
Qualitative modeling and simulation of genetic regulatory networks in bacteria	Seminar at Microsoft Research - University of Trento Centre for Computational and Systems Biology (COSBI)	Sep. 2008
Qualitative modeling and simulation of genetic regulatory networks in bacteria	Invited talk during Journées Nationales de Calcul Formel (JNCF 2008), Marseille	Oct. 2008
Modeling of genetic regulatory networks: Simple models for complex systems	Seminar Galderma, Sophia-Antipolis	Oct. 2008
Qualitative modeling and simulation of genetic regulatory networks in bacteria	Invited talk at 6th Conference on Computational Methods in Systems Biology (CMSB 2008), Rostock (Germany)	Oct. 2008
Some examples of systems biology projects at INRIA	Life Science Computing Conference, Paris	Nov. 2008
Qualitative simulation of genetic regulatory networks in bacteria	Invited talk European American Innovation Day, Boston (USA)	Dec. 2008

Samuel Drulhe

Title	Event and location	Date
Identification de modèles affines par morceaux de réseaux de régulation génique à partir des données expérimentales	PhD thesis defense, Université Joseph Fourier, Grenoble	Dec. 2008

Hans Geiselmann

Title	Event and location	Date
From gene expression to genetic regulatory networks	Département de Biologie Moléculaire, Université Libre de Bruxelles (Belgium)	Jan. 2008
Systems biology: Interaction networks and the dynamics of gene expression	Ecole Thématique de Microbiologie Moléculaire, Carry-le-Rouet	Oct. 2008
Deciphering bacterial regulatory networks by a combination of genomics and modeling	Department of Biomolecular Sciences and Biotechnology, University of Milano (Italy)	Oct. 2008
Topology and dynamics of genetic regulatory networks: Measurements and models	Conference “Physique de la cellule au tissu”, Sète	Nov. 2008

Pedro T. Monteiro

Title	Event and location	Date
Temporal logic patterns for querying qualitative models of genetic regulatory networks	22nd International Qualitative Reasoning workshop (QR’O8), Boulder (USA)	Jun. 2008
Temporal logic patterns for querying dynamic models of cellular interaction networks	Journées Ouvertes Biologie Informatique Mathématiques (JOBIM 2008), Lille	Jul. 2008
Temporal logic patterns for querying qualitative models of genetic regulatory networks	18th European Conference on Artificial Intelligence (ECAI 2008), Patras (Greece)	Jul. 2008
Temporal logic patterns for querying dynamic models of cellular interaction networks	European Conference on Computational Biology (ECCB 2008), Cagliari (Italy)	Sep. 2008
Computation Tree Regular Logic for Genetic Regulatory Networks	6th International Symposium on Automated Technology for Verification and Analysis (ATVA 2008), Seoul (South Korea)	Oct. 2008

Delphine Ropers

Title	Event and location	Date
Qualitative simulation of the carbon starvation response in Escherichia coli	seminar INRA, Avignon	Feb. 2008
Qualitative simulation of the carbon starvation response in Escherichia coli	Ecole doctorale "Structure et Fonctions des Macromolécules Biologiques, Bioinformatique et Modélisation", Université Libre de Bruxelles (Belgium)	May 2008

8.4. Popular science writing

The members of IBIS are actively involved in the dissemination of research results in systems biology and bioinformatics to a wider, non-specialist audience.

François Rechenmann is a regular contributor to *La Recherche* and coordinated in 2008 a special issue of *DocSciences* (http://www.docsciences.fr/). He is also involved in the development of an In Silico biology e-learning environment (ISee), which explains the principles of the main bioinformatics algorithms and illustrates their use on real data (Section 4.2). He is a member of the editorial committee of the Interstices website (http://interstices.info). Interstices offers pedagogic presentations of research themes and activities in the computer science domain.

Hidde de Jong coordinated an issue of the "Cahiers de l'INRIA" in *La Recherche*, on the subject of bioinformatics and systems biology. It contains an article on the EC-MOAN project written by IBIS and VASY members [19].

8.5. Teaching

Four members of the IBIS team are either full professor, associate professor or assistant professors at the Université Joseph Fourier or the Université Pierre Mendès-France in Grenoble. They therefore have a full teaching service (at least 192 hours per year) and administrative duties related to the organization and evaluation of the university course programs on all levels (from BSc to PhD). Besides the full-time academic staff in IBIS, the following people have contributed to courses last year.

Guillaume Baptist

Subject	Year	Location	Hours
Prokaryotic genetics	1-4	Department of Biology, Université Joseph Fourier	73

Bruno Besson

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	4	INSA, Lyon	4

Hidde de Jong

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	4	INSA, Lyon	14
Modeling and simulation of genetic regulatory networks	5	Instituto Gulbenkian de Cienca, Lisbon, Portugal	7

Pedro Monteiro

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	5	Instituto Gulbenkian de Cienca, Lisbon, Portugal	4

Delphine Ropers

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	5	Instituto Gulbenkian de Cienca, Lisbon, Portugal	7
Modeling and simulation of genetic regulatory networks	5+	Université Joseph Fourier and Doctoral School in Pharmacy, Universities of Geneva and Lausanne (Switzerland)	3

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Year Publications

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Articles in International Peer-Reviewed Journal

[2] G. BATT, H. DE JONG, M. PAGE, J. GEISELMANN. *Symbolic reachability analysis of genetic regulatory networks using discrete abstractions*, in "Automatica", vol. 44, n^{o} 4, 2008, p. 982-989.

[3] S. DRULHE, G. FERRARI-TRECATE, H. DE JONG. *Reconstruction of switching thresholds in piecewise-affine models of genetic regulatory networks*, in "IEEE Transactions on Automatic Control & IEEE Transactions on Circuits and Systems I", vol. 53, n^{o} 1, 2008, p. 153-165.

[4] P. MONTEIRO, D. ROPERS, R. MATEESCU, A. FREITAS, H. DE JONG. *Temporal logic patterns for querying dynamic models of cellular interaction networks*, in "Bioinformatics", vol. 24, n^{o} 16, 2008, p. i227-i233.

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International Peer-Reviewed Conference/Proceedings

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[9] P. MONTEIRO, D. ROPERS, R. MATEESCU, A. FREITAS, H. DE JONG. *Temporal logic patterns for querying dynamic models of cellular interaction networks*, in "Proceedings of 18th European Conference on Artifical Intelligence (ECAI'08), Amsterdam", M. GHALLAB, C. SPYROPOULOS, N. FAKOTAKIS, N. AVOURIS (editors), IOS Press, 2008, p. 229-233.

[10] M. MUSTERS, H. DE JONG, P. VAN DEN BOSCH, N. VAN RIEL. *Kinetic modeling and analysis of nonlinear biochemical networks with no quantitative information*, in "Book of abstracts of 9th International Conference on Systems Biology (ICSB'08), Göteborg, Sweden", 2008, 27.

[11] P. PACI, F. CASTIGLIONE, M. BERNASCHI, V. BALDAZZI. *A discrete/continuous model of anti-HIV response and therapy*, in "Proceedings of the Tenth International Conference on Computer Modeling and Simulation (UKSIM'08), Washington, DC, USA", IEEE Computer Society, 2008, p. 481-486.

National Peer-Reviewed Conference/Proceedings

[12] P. MONTEIRO, D. ROPERS, R. MATEESCU, A. FREITAS, H. DE JONG. *Temporal logic patterns for querying dynamic models of cellular interaction networks*, in "Working notes of the Journées Ouvertes Biologie, Informatique et Mathématiques (JOBIM'08), Lille, France", J. VAN HELDEN, Y. MOREAU (editors), 2008.

Scientific Books (or Scientific Book chapters)

[13] R. MATEESCU, P. MONTEIRO, E. DUMAS, H. DE JONG. *Computation tree regular logic for genetic regulatory networks*, in "Proceedings of the 6th International Symposium on Automated Technology for Verification and Analysis (ATVA'08), Berlin", S. CHA, J.-Y. CHOI, M. KIM, I. LEE, M. VISWANATHAN (editors), Lecture Notes in Computer Science, vol. 5311, Springer-Verlag, 2008, p. 48-63.

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Scientific Popularization

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