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 : Computational Biology and Bioinformatics

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# 2. Overall Objectives

## 2.1. Overview

When confronted with changing environmental conditions, bacteria and other single-cell organisms have a remarkably capacity to rapidly adapt their functioning. 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 mapping the interaction structure of 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 adaptive capacities of *E. coli* in situations of nutritional stress is a necessary prerequisite for interfering with the cellular responses 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 may ultimately also lead to practical applications. Because *E. coli* is easy to manipulate in the laboratory, it serves as a model for many pathogenic bacteria and is widely used in biotechnology, for such diverse applications as the development of vaccines, the mass production of enzymes and other (heterologous) proteins, and the production of biofuels.

The aims of IBIS raise four main challenges that generate new problems on the interface of 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 a microbiology and molecular genetics 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]

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

Hidde de Jong is the head of the IBIS team and Hans Geiselmann its co-director. The experimental 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.

*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).*

## 2.2. Highlights of the year

The publication of a method for the systematic derivation of a gene regulatory network from the underlying system of biochemical reactions, applied to the carbon assimilation network in *E. coli*. The paper was published in the June 2010 issue of *PLoS Computational Biology*.

The start of the ANR project GeMCo, on the modeling and control of the gene expression machinery in *E. coli*, in December 2010.

# 3. Scientific Foundations

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

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

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.

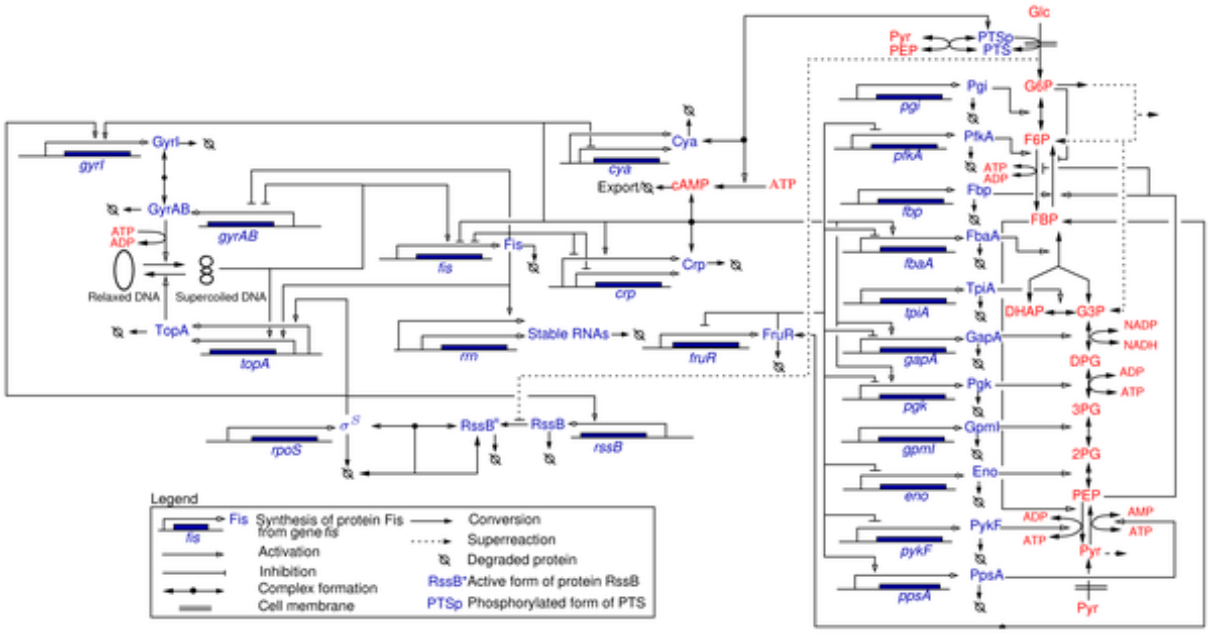



*Figure 2. Network of key genes, proteins, and regulatory interactions involved in the carbon assimilation network in E. coli (Baldazzi et al., PLoS Computational Biology, 6(6):e1000812, 2010 ). The metabolic part includes the glycolysis/gluconeogenesis pathways as well as a simplified description of the PTS system, via the phosphorylated and non-phosphorylated form of its enzymes (represented by PTSp and PTS, respectively). The pentose-phosphate pathway (PPP) is not explicitly described but we take into account that a small pool of G6P escapes the upper part of glycolysis. At the level of the global regulators the network includes the control of the DNA supercoiling level, the accumulation of the sigma factor RpoS and the Crp·cAMP complex, and the regulatory role exerted by the fructose repressor FruR.*

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 subsystems. 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 *E. coli* to adapt to a various nutritional and other environmental stresses, such as carbon, phosphate, 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 adaptive response of the bacteria. Moreover, we study the 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 BAMBOO and COMORE project-teams at INRIA.

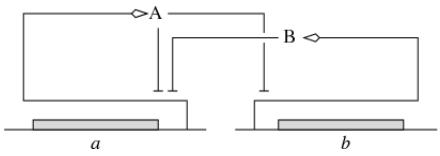
## 3.2. Methods: Analysis, simulation, and identification of bacterial regulatory networks

**Participants:** Sara Berthoumieux, Eugenio Cinquemani, Johannes Geiselmann, Hidde de Jong [Correspondent], Pedro Monteiro, Michel Page, François Rechenmann, Delphine Ropers, Diana Stefan, 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 models of actual 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




(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 $\kappa_a$, if and only if the concentration of protein A is below its threshold $\theta_a^2$ ($x_a < \theta_a^2$) and the concentration of protein B below its threshold $\theta_b$ ($x_b < \theta_b$). The degradation of protein A occurs at a rate proportional to the concentration of the protein itself ($\gamma_a \, x_a$).*

properties formulated in temporal logic to be verified on state transition graphs. This has been the subject of collaborations with the POP-ART and VASY project-teams at INRIA Grenoble - Rhône-Alpes.

Recent advances in experimental techniques have led to approaches for measuring cellular processes in real-time on the molecular level, both in single cells and populations of bacteria (Section 3.3). The data sources that are becoming available by means of these techniques contain a wealth of information for the quantification of the interactions in the regulatory networks in the cell. This has stimulated a broadening of the methodological scope of IBIS, from qualitative to quantitative models, and from PL models to nonlinear ODE models. The group has notably started to work on what is the bottleneck in the practical use of these models, the structural and parametric identification of bacterial regulatory networks from time-series data, in collaboration colleagues from the University of Pavia (Italy) and ETH Zürich (Switzerland). This raises difficult problems related to identifiability, measurement noise, heterogeneity of data sources, and the design of informative experiments that are becoming increasingly prominent in the systems biology literature.

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

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

The aim of a model is to describe the functioning of bacterial regulatory networks so as to gain a better understanding of the molecular mechanisms that control cellular responses and to predict the behavior of the system in new situations. In order to achieve these goals, we have to calibrate the model so that it reproduces available experimental data and confront model predictions with the results of new experiments. This presupposes the availability of high-precision measurements of gene expression and other key processes in the cell.

We have resorted to the measurement of fluorescent and luminescent reporter genes, which allow monitoring the expression of a few dozens of regulators in parallel, 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 signals of these reporter genes are measured *in vivo* by an automated, thermostated microplate reader. This makes it possible to monitor in real time the variation in the expression of a few dozens of genes in response to an

external perturbation. We have developed an experimental pipeline that resolves most technical difficulties in the generation of reproducible time-series measurements. The pipeline comes with data analysis software that converts the measurements into representations of the time-course of promoter activities that can be compared with model predictions (Section 4.2). In order to obtain rich information about the network dynamics, we have begun to measure the expression dynamics in both wild-type and mutant cells, using an existing *E. coli* mutant collection. Moreover, we have developed tools for the perturbation of the system, such as expression vectors for the controlled induction of particular genes.

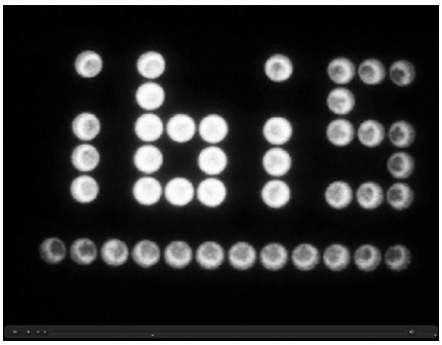


*Figure 4. Playful illustration of the principle of reporter genes (see http://ibis.inrialpes.fr for the corresponding movie). A microplate containing a minimal medium (with glucose and acetate) is filmed during 36 hours. Wells contain E. coli bacteria which are transformed with a reporter plasmid containing 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.*

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 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)

**Participants:** Hidde de Jong [Correspondent], Pedro Monteiro, Michel Page, François Rechenmann, 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 IBIS 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. In order to analyze large graphs, GNA allows the user to specify properties of the qualitative dynamics of a network in temporal logic, using high-level query templates, and to verify these properties on the state transition graph by means of standard model-checking tools, either locally installed or accessible through a remote web server. GNA is currently distributed by the company Genostar, but remains freely available for academic research purposes. The current version is GNA 8.1. In comparison with the previously distributed versions, GNA 8.1 has the following additional functionalities. First, it supports the editing and visualization of regulatory networks, in an SBGN-compatible format, and second it semi-automatically generates a prototype model from the network structure, thus accelerating the modeling process. For more information, see http://www-helix.inrialpes.fr/gna.

## 4.2. WellReader

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

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, compute promoter activities and protein concentrations, and compare expression profiles across different conditions. WELLREADER has been written in MATLAB and is available under an LGPL licence, both as source code (M files) and compiled code (platform-specific binary files). For more information, see http://ibis.inrialpes.fr/article957.html.

# 5. New Results

## 5.1. Integrated environment for qualitative modeling, simulation, analysis, and verification of genetic regulatory networks

Within the framework of two European projects that finished in 2010, COBIOS and EC-MOAN (Section 7.2), IBIS has completed version 8 of GENETIC NETWORK ANALYZER (GNA). GNA is a tool for the qualitative modeling and simulation of the dynamics of genetic regulatory networks by means of PL models, as described in Section 4.1. Version 8 of GNA has been deposited at the Agence pour la Protection des Programmes (APP). In comparison with version 7, it includes several novel functionalities and its graphical user interface has been adapted to Java 6.

In the EC-MOAN project, in collaboration with Radu Mateescu of the VASY project-team, we developed a formal verification module that allows the user to specify dynamic properties of genetic regulatory networks by means of so-called patterns, high-level query templates that capture recurring questions of biological interest. The patterns can be automatically translated to temporal logic, for instance the CTRL (Computation Tree Regular Logic) that Pedro Monteiro developed during his PhD thesis, defended in Lisbon in May 2010 [1]. A paper on CTRL has been accepted for *Theoretical Computer Science* this year [8], for a special issue associated with the conference Computational Methods in System (CMSB), held in Rostock in 2008. The formal verification module allows the user of GNA to have access to formal verification tools through a service-oriented architecture, in order to verify network properties on the state transition graphs representing the qualitative network dynamics. This architecture, which has been completely implemented by Estelle Dumas, Pedro Monteiro, and Michel Page, integrates modeling and simulation clients like GNA to model-checker servers, via an intermediate request manager. In particular, the client can perform formal verification requests through the web, which the request manager dispatches to an appropriate model-checker server. When the

model-checker server has answered the request, the results are sent back to the client for display and further analysis in the graphical user interface of the tool. The service-oriented architecture is modular and general, and has the advantage of reusing existing formal verification technology as much as possible. Developed in collaboration with Grégory Batt (CONTRAINTES), version 8 of GNA proposes an efficient, implicit encoding of the state transition graphs, so as to optimize the interactions between GNA and the model-checker servers in the case of large networks. This implicit encoding has been exploited for the development of methods for the verification of incompletely specified PL models of genetic regulatory networks (Section 5.6).

In the COBIOS project, IBIS and Genostar have jointly developed a conceptual model to represent bacterial regulatory networks. The model has been implemented into a library called IOGMANETWORK, using the underlying entity-relationship data and knowledge model of Genostar's IOGMA platform. This work has notably involved Bruno Besson, Hidde de Jong, Michel Page, François Rechenmann, and engineers of Genostar. Version 8 of GNA offers an editor for the graphical definition of regulatory network based on the IogmaNetwork library. The aim of this extension is to support, within a single tool, the entire modeling process from the structural definition or design of networks to their simulation, analysis, and verification within a single environment. The integration has involved a complete reorganization of the architecture of GNA and the development of a new graphical user interface. This allows the modeler to flexibly move back and forth between the definition of a network, the reduction of this network to a form compatible with the PL models supported by GNA, and the semi-automatic translation of the network structure into a model. A book chapter describing version 8 of GNA, and illustrating the features of the network editor on a model of the carbon starvation response in *E. coli*, will be published in a forthcoming volume on the modeling of bacterial molecular networks [20].

Several collaborations with other groups using GNA have been started or continued this year. Delphine Ropers has worked with groups at IST Lisbon on the modeling of the FLR1 network in yeast, resulting in a conference abstract [16] and a paper submitted for publication this year. Hidde de Jong is contributing to the modeling of the TOL system in *Pseudomonas putida*, carried out at the Spanish National Biotechnology Center (CNB).

## 5.2. Platform for measuring gene expression using reporter genes

The use of reporter genes allows real-time monitoring of gene expression, both at the level of individual cells and cell populations (Section 3.3). In order to meaningfully interpret these data, we need to assess what exactly reporter gene measurements can teach us about the actual processes going on in the cell. Mathematical models are crucial for inferring biologically relevant quantities from reporter gene data. Most approaches present ways to infer the promoter activity from the primary data. By genetic construction, the measured promoter activity of a reporter gene carries over to any host gene that is under the control of the same promoter. Another approach is to reconstruct (relative) measures of the reporter mRNA and protein concentrations from the data and use these as estimates of the corresponding products of the host gene. This approach is intuitively attractive, as it allows a straightforward read-out of the expression of any gene whose regulatory sequences are cloned into a reporter construct. However, it poses the question of the accuracy of the estimates, because the kinetics of host and reporter gene expression may be different.

Within the framework of the EC-MOAN project, we have systematically investigated this question by means of a combination of models and experiments, in a paper published in *BMC Systems Biology* this year [11]. Our specific contributions are the experimental validation of the latter approach, by comparing the quantities reconstructed from reporter gene data on the population level, obtained using an automated microplate reader, with direct measurements of the accumulation of mRNA and protein, obtained using Northern and Western blots, respectively. Moreover, we have used models of the reporter systems to pinpoint potential systematic biases arising from the folding time of fluorescent reporter proteins, and from differences in the half-lives of the products of host and reporter genes. This allows us to correct for the resulting systematic errors in the measurements and obtain a more accurate estimate of synthesis rates and concentrations of the host protein.

To illustrate the interest of this approach for the analysis of gene expression in bacteria, we have constructed fluorescent and luminescent reporter systems of the gene *fis* of *E. coli*. The *E. coli* host gene codes for the protein Fis, a global regulator of transcription that plays a central role in, among other things, the control

of metabolism and the coupling of the DNA topology to cellular physiology. A first interesting finding is that the relative mRNA and protein concentrations obtained from the reporter gene data are in good overall correspondence with the Northern and Western blot measurements, respectively. This suggests that the use of fluorescent and luminescent reporter genes in combination with automated microplate readers may yield reasonably accurate estimates of the expression profile of the products of the host gene. Second, we show that corrections for systematic biases due to differences in the half-lives of reporter and host mRNAs have mostly negligible effects, whereas corrections for differences in the half-lives of reporter and host proteins further improve the agreement between the inferred Fis concentration profiles and the Western blots. This conclusion, corroborated by simulation studies, suggests that the latter differences may need to be taken into account when using reporter gene data for the reconstruction of regulatory networks (Section 5.3).

In a typical microplate experiment, 96 cultures are followed in parallel, over several hours, resulting in 10,000-100,000 measurements of absorbance and fluorescence and luminescence intensities. Computer tools are therefore essential for extracting relevant information from these large amounts of data. To our knowledge, no user-friendly computer tools for analyzing population-level fluorescence and luminescence reporter gene data exist in the public domain. The program WELLREADER aims at filling this gap, thus facilitating the exploitation of the technology for the monitoring of gene expression in microorganisms (Section 4.2). We released version 3 of WELLREADER in 2009, and deposited the software at the APP. An application note on WELLREADER has been published in *Bioinformatics* this year [4].

Several improvements of the platform for measuring gene expression are the subject of ongoing work, including a novel method for efficiently cloning reporter gene constructions on the chromosome of *E. coli*, and the development by Caroline Ranquet-Brazzolotto and Corine Pinel of 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. These tools are actually used in a series of studies, such as the validation of a model of the network in global regulators in *E. coli* by Sara Berthoumieux and Hidde de Jong, and the analysis of the network involved in motility and sessility by Omaya Dudin and Stephan Lacour.

## 5.3. Structural identification of genetic regulatory networks

In general, structural identification of genetic regulatory networks involves fitting appropriate network structures and parameters to the data. While modern measurement techniques such as gene reporter systems provide data of ever-increasing quality, the problem remains challenging because exploring all possible network structures in the search of the best fitting model is prohibitive, and no satisfactory solution is available in the literature.

In order to address the structural identification problem, Eugenio Cinquemani proposed in collaboration with the Automatic Control Lab at ETH Zurich (Switzerland) and the Computer Engineering & Systems Science Department of the University of Pavia (Italy), an ODE modelling framework which we refer to as models with unate-like structure. In Boolean network modelling, unate functions are argued to capture virtually all observable interactions in genetic regulatory networks. In our quantitative framework, unate logics are encoded in the structure of the nonlinear synthesis rates of the network proteins. This framework allows us to integrate *a-priori* information on the most likely network structures, and the models enjoy monotonicity properties that can be exploited to ameliorate the identification task.

Based on unate logics, we have devised a procedure for model identification from simultaneous protein concentration and synthesis rate measurements organized in two steps. The first step isolates a family of model structures compatible with the data based on ordering relationships among the observed data points. This reduces the whole family of ODE models to a small subset of candidate model structures organized in a hierarchical fashion. The second step explores this family, starting from the simplest model compatible with the data, and adding complexity at every iteration, until a model is found that fits the data in a statistically acceptable way. In general, the procedure returns a small pool of models (structure and parameters) that fit the data well, providing equally plausible biological hypotheses and indications for further identification experiments. The procedure has been tested with success on challenging data from the literature, the IRMA synthetic network in yeast, and on simulated data for the *E. coli* carbon starvation response network. This work

was published in *Bioinformatics* [9] and an extension presented at the 2010 IEEE Conference on Decision and Control (CDC) [18]. We are now in the process of testing the method on real *E.coli* data from the Laboratoire Adaptation et Pathogénie des Microorganismes (work done by Diana Stefan in the framework of her recently started PhD thesis).

A different, experimental approach to identify the regulators of a gene has been explored in parallel. 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), no efficient method exists to determine the regulators that affect, directly or indirectly, the expression of a gene under investigation. 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 a publication is in preparation.

## 5.4. Analysis of metabolic coupling in genetic regulatory networks

The regulation of gene expression is tightly interwoven with metabolism and signal transduction. A realistic view of genetic regulatory networks should therefore not only include direct interactions resulting from transcription regulation, but also indirect regulatory interactions mediated by metabolic effectors and signaling molecules. We called these indirect interactions mediated by metabolism metabolic coupling. Ignoring metabolic coupling during the analysis of the network dynamics may lead crucial feedback loops to be missed.

In the framework of the MetaGenoReg project, we have developed a method for systematically deriving indirect interactions from a model of the underlying biochemical reaction network, using weak time-scale assumptions in combination with sensitivity criteria from metabolic control analysis. We have applied our approach to a model of the upper part of the carbon assimilation network in *E. coli*, consisting of the glycolysis and gluconeogenesis pathways and their genetic and metabolic regulation. The analysis of the derived gene regulatory network has lead to three new insights. First, contrary to what is often assumed, the network is densely connected due to numerous feedback loops resulting from indirect interactions. This additional complexity is an important issue for the correct interpretation of data from genome-wide transcriptome studies. Second, the derived gene regulatory network for carbon assimilation in *E. coli* is sign-determined, in the sense that the signs of interactions are essentially fixed by weak information on flux directions of biochemical reactions, without explicit specification of kinetic rate laws or parameter values. Therefore the feedback structure is robust to changes in kinetic properties of enzymes and other biochemical reactions species. Third, a change in environmental conditions may invert fluxes, and thus the signs of indirect interactions, resulting in a dynamic rewiring of the regulatory network. This work has been published in *PLoS Computational Biology* this year [2] and presented at the national bioinformatics conference JOBIM 2010 [13].

It remains an open question, however, to which extent the indirect interactions induced by metabolic coupling affect the dynamics of the system. This is a key issue for understanding the relative contributions of the regulation of gene expression and metabolism during the adaptation of the cell to changes in its environment. In collaboration with Valentina Baldazzi, formerly post-doctoral fellow in IBIS and now research scientist at INRA (Avignon), we have carried out a dynamic analysis by developing a qualitative PL model of the genetic regulatory network, including both the direct and indirect interactions. This work has been submitted for publication. We have previously shown, in another paper to appear in the *IEEE/ACM Transactions on Computational Biology and Bioinformatics*, that PL models provide a good approximation of the direct and indirect interactions occurring in gene regulation [10].

## 5.5. Parameter estimation for kinetic models of carbon metabolism in E. coli

Kinetic models capture the dynamics of the large and complex networks of biochemical reactions that endow bacteria with the capacity to adapt their functioning to changes in the environment. In comparison with the qualitative PL models described in Section 5.1, these more general classes of ODE models are intended to provide a quantitative description of the network dynamics, both on the genetic and metabolic level. New experimental techniques have led to the accumulation of large amounts of data, such as time-course measurements of metabolite, mRNA and protein concentrations and measurements of metabolic fluxes under different growth conditions. However, the estimation of parameter values in the kinetic models from these data remains particularly challenging in biology, mostly because of incomplete knowledge of the molecular mechanisms, noisy, indirect, heterogeneous, and partial observations, and the large size of the systems, with dynamics on different time-scales. We have addressed parameter estimation in the framework of the MetaGenoReg project (Section 7.1), concerned with the analysis of the interactions between metabolism and gene expression in carbon metabolism in *E. coli*.

In collaboration with Matteo Brilli and Daniel Kahn (BAMBOO), we have developed an approximate model of central metabolism of *E. coli*, using so-called linlog functions to approximately describe the rates of the enzymatic reactions. More precisely, linlog models describe metabolic kinetics by means of a linear model of the logarithms of metabolite concentrations. We have used metabolome and transcriptome data sets from the literature to estimate the parameters of the linlog models, a task in principle greatly simplified by the mathematical form of the latter. However, a major problem encountered during parameter estimation was the occurrence of missing data, due to experimental problems or instrument failures. In the framework of her PhD thesis, Sara Berthoumieux has addressed the missing-data problem by developing an iterative parameter estimation approach based on an Expectation-Maximization (EM) procedure. This approach adapted from the statistical literature has the advantage of being well-defined analytically and applicable to other kinds of linear regression problems with missing data. It has been tested on simulations experiments with missing data and performs well compared to basic and advanced regression methods. The method and its application to the linlog model of central metabolism in *E. coli* are the subject of a paper submitted for publication.

A second line of work is based on the use of classical kinetic models that are, in comparison with the above-mentioned linlog models, much reduced in scope (the focus is on the metabolic and genetic regulation of the glycolysis pathway) and granularity (individual reactions are lumped together). The models, developed by Delphine Ropers, have been calibrated using experimental data from the MetaGenoReg partners, notably the experimental part of the IBIS group for the gene expression measurements and the group of Jean-Charles Portais at INSA in Toulouse for the measurements of metabolism. The model with the estimated parameter values is currently being further tested and used to understand some key mechanisms in the adaptation of *E. coli* to the exhaustion of glucose.

## 5.6. Control of regulatory networks

While systems biology is primarily concerned with natural systems shaped by evolution, synthetic biology opens up a new generation of fundamental research by trying to redesign natural systems or create novel systems from scratch. Mathematical modeling and analysis are essential components of synthetic biology, as they help understanding the consequences of (changes in) the network of interactions on the dynamical behavior of the system. More specifically, a model can be a powerful tool for the control and regulation of the system towards a desired goal.

In collaboration with Grégory Batt (CONTRAINTES) and Gregor Goessler (POP-ART), and Irene Cantone formerly at TIGEM (Naples, Italy) and now at Imperial College (London, UK), we cast the problem of the control of genetic regulatory networks into a parameter search problem for qualitative PL models (Section 5.1). In qualitative models the number of different parametrizations is finite and the number of possible values for each parameter usually rather low. This makes parameter search easier to handle than in quantitative models, where exhaustive search of the continuous parameter space is in general not feasible. Nevertheless, the parametrization of qualitative models remains a complex problem. For most models of networks of biological interest the state and parameter spaces are too large to exhaustively test all combinations of parameter values. The aim of our work was therefore to address this search problem for PL models by treating it in the context

of formal verification and model checking. More specifically, we developed a method based on symbolic model checking that avoids enumerating all possible parametrizations, and show that this method performs well on real biological problems, using the IRMA synthetic network in yeast and benchmark data sets. We have tested the consistency between the PL model of IRMA and time-series expression profiles, and searched for parameter modifications that would make the external control of the system behavior more robust. This work has been presented at the 9th European Conference on Computational Biology and published in a special issue of *Bioinformatics* [3].

Within the projects ColAge and GeMCo (Section 5.1), we attempt to control one of the fundamental physiological properties of bacterial cells, their growth rate. In particular, in order to control the growth rate, we propose to focus on the gene expression machinery of *E. coli*, whose activity is controlled by a complex regulatory network with many components and intertwined feedback loops. We are developing models of this network and Jérome Izard, in the context of his PhD thesis, is rewiring part of the network to enable control of the network dynamics. These preliminary results will be further developed in the next year.

# 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 project-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 developed by members of IBIS (Section 4.1). François Rechenmann is scientific consultant of the company and members of IBIS and Genostar together collaborate in the COBIOS project (Section 7.2). For more information, see http://www.genostar.com.

# 7. Other Grants and Activities

## 7.1. National projects

| Project name | ColAge – Lifespan control in bacteria: Natural and engineering solutions |
|---|---|
| Coordinator | H. Berry |
| IBIS participants | G. Baptist, E. Cinquemani, J. Geiselmann, H. de Jong, J. Izard, S. Lacour, C. Pinel, D. Ropers |
| Type | Action d'Envergure INRIA-INSERM (2008-2012) |
| Web page | http://colage.saclay.inria.fr |

| 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 | G. Baptist, S. Berthoumieux, E. Cinquemani, J. Geiselmann, H. de Jong, Y. Markowicz, C. Pinel, C. Ranquet-Brazzolotto, D. Ropers, W.-F. Wang |
| Type | ANR BIOSYS (2006-2010) |
| Web page | Not available |

| Project name | GeMCo – Model reduction, experimental validation, and control for the gene expression machinery in *E. coli* |
|---|---|
| Coordinator<br>IBIS participants<br><br>Type<br>Web page | M. Chaves<br>G. Baptist, E. Cinquemani, J. Geiselmann, H. de Jong, J. Izard, S. Lacour, C. Pinel, D. Ropers<br>ANR Blanc (2010-2013)<br>http://www-sop.inria.fr/members/Madalena.Chaves/ANR-GeMCo/main.html |

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

| Project name | Identification structurelle et paramétrique des réseaux de régulation bactériens |
|---|---|
| Coordinator<br>IBIS participants<br>Type<br>Web page | E. Cinquemani<br>E. Cinquemani, J. Geiselmann, H. de Jong, D. Stefan<br>Funding PhD grant, Cluster ISLE, Région Rhône-Alpes<br>http://cluster-isle.grenoble-inp.fr/ |

## 7.2. European projects

| Project name | EC-MOAN: Scalable modeling and analysis techniques to study emergent cell behavior: Understanding the E. coli stress response |
|---|---|
| Coordinator<br>IBIS participants<br><br>Type<br>Web page | J. van der Pol<br>G. Baptist, J. Geiselmann, H. de Jong, Y. Markowicz, P. Monteiro, M. Page, C. Pinel, C. Ranquet-Brazzolotto, D. Ropers<br>European Commission, FP6 NEST (2007-2010)<br>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 |
|---|---|
| Coordinator<br>IBIS participants<br>Type<br>Web page | D. di Bernardo<br>H. de Jong, M. Page, F. Rechenmann, D. Ropers<br>European Commission, FP6 NEST (2007-2010)<br>http://www.synbiosafe.eu/index.php?page=cobios |

## 7.3. International projects

| Project name | French bioinformatics contribution to ICGC |
|---|---|
| Coordinator<br>IBIS participants<br>Type<br>Web page | G. Thomas<br>F. Rechenmann<br>International Cancer Genome Consortium (ICGC)<br>http://www.icgc.org/ |

The goal of ICGC (International Cancer Genome Consortium) is to obtain a comprehensive description of genomic, transcriptomic and epigenomic changes in 50 different cancer types. In France, INCa (French National Cancer Institute) contributes to this project and focuses on two types of cancer. The main idea

is to sequence the human genome of normal and tumoral cells of a large set of patients and to compare these genomic sequences to identify the mutations which may explain the development of the cancers. Bioinformatics is clearly involved in the management, the analysis and the visualization of the huge sets of data and results. Bioinformatics of the French contribution is carried out at Lyon, in the context of the Synergie Lyon Cancer Foundation. François Rechenmann has joined this bioinformatics team and contributes to the organization of the data management and analysis workflow, under the leadership of prof. Gilles Thomas.

## 7.4. International collaborations

IBIS has a strong collaboration with the group of Giancarlo Ferrari-Trecate at the Computer Engineering & Systems Science Department of the University of Pavia (Italy) and the group of John Lygeros at the Automatic Control Lab at ETH Zürich (Switzerland). This collaboration started with the FP6 project Hygeia, in which the above groups and IBIS (then HELIX) participated. Over the years, it has resulted in a dozen of co-authored papers and the co-supervision of a PhD thesis by Hidde de Jong and Giancarlo Ferrari-Trecate. Eugenio Cinquemani was a post-doctoral fellow at ETH in the framework of the Hygeia project, and joined the IBIS group as a research scientist in the fall of 2009.

# 8. Dissemination

## 8.1. Editorial, organizational, and reviewing activities

**Eugenio Cinquemani**

| Type | Journal, conference, agency |
|---|---|
| Member Organization Committee | Workshop on Identification and Control of Biological Interaction Networks, INRIA Grenoble - Rhône-Alpes, February 2011 |

**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 Editorial Board | Biosystems |
| Member Program Committee | AIME 11, ECCB 10, HiBi 10, JOBIM 10, QR 10 |
| Member Recruitment Committee | Université Montpellier, assistant professor Université Montpellier/INRA |
| 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 | Antoine Coulon (INSA de Lyon), Ibrahima Ndiaye (Université Nice Sophia-Antipolis), Thanassis Polynikis (University of Bristol, UK), Mattia Zampieri (SISSA, Trieste, Italy) |
| Member HdR Committee | Béatrice Laroche (Université Paris Sud), Denis Mestivier (Université Paris Diderot) |
| Member Organization Committee | Journée biologie synthétique et micro et nanotechnologies, OMNT, CEA Grenoble, March 2010 |
| Member Organization Committee | Journée satellite JOBIM : Modélisation dynamique et simulation des réseaux biologiques, September 2010 |
| Advisor | Grenoble team for iGEM 2011 competition |
| Project reviews | ANR, Région Acquitaine, Région Ile-de-France, FP7, CNRS |

**Hans Geiselmann**

| Type | Journal, conference, agency |
|---|---|
| Member PhD Committee | Anna Brückner (Université Joseph Fourier), Lilia Brinza (INSA de Lyon), Alexandre Dias (Université Joseph Fourier) |
| President Recruitment Committee | Université Joseph Fourier, assistant professor cellular biology |
| President Recruitment Committee | Université Joseph Fourier, assistant professor metabolism and physiology |
| Member Recruitment Committee | Université Joseph Fourier, assistant professor biophysics |
| Member Organization Committee | Journée biologie synthétique et micro et nanotechnologies, OMNT, CEA Grenoble |
| Advisor | Grenoble team for iGEM 2011 competition |
| Project reviews | ANR, Swiss National Science Foundation |

**Delphine Ropers**

| Type | Journal, conference, agency |
|---|---|
| Member Program Committee | JOBIM 10 |
| Member Organization Committee | SeMoVi (Séminaire de Modélisation du Vivant) |
| Member Organization Committee | Satellite meeting of JOBIM 2010 |
| Member Organization Committee | Workshop on Identification and Control of Biological Interaction Networks, INRIA Grenoble - Rhône-Alpes, February 2011 |

## 8.2. Other administrative activities

Hans Geiselmann is head of the Control of Gene Expression group in the Laboratoire Adaptation et Pathogénie des Microorganismes (UMR 5163) and director of the laboratory starting in January 2011.

Yves Markowicz is a national representative of the UNSA trade union.

Hidde de Jong is local representative of the Department of International Relations of INRIA at the Grenoble - Rhône-Alpes research center.

François Rechenmann is leader 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

**Guillaume Baptist**

| Title | Event and location | Date |
|---|---|---|
| Réseaux de régulation chez E. coli | Seminar Laboratoire Adaptation et Pathogénicité des Microorganismes (LAPM), Grenoble | Apr. 2010 |

**Sara Berthoumieux**

| Title | Event and location | Date |
|---|---|---|
| Developpement of an algorithm for parameter estimation in the case of missing data and its application to a model of central metabolism in E. coli | JOBIM 2010 satellite meeting, Montpellier | Sep. 2010 |

**Eugenio Cinquemani**

| Title | Event and location | Date |
|---|---|---|
| Stochastic hybrid approaches to modelling, simulation and identification of cellular processes | Du désordre moléculaire aux programmes cellulaires : microscopie et modélisation, Atelier Institut de Recherche Interdisciplinaire (IRI), Villeneuve d'Ascq, | Apr. 2010 |
| Modelling and identification of genetic network dynamics: a stochastic hybrid approach | Séminaires de Statistique et Probabilités Appliquées de Grenoble, Laboratoire Jean Kuntzmann (LJK), Grenoble | Jun. 2010 |
| Structural identification of unate-like genetic network models from time-lapse protein concentration measurements | 49th IEEE Conference on Decision and Control, Atlanta (USA) | Dec. 2010 |
| Stochastic receding horizon control with output feedback and bounded control inputs | 49th IEEE Conference on Decision and Control, Atlanta (USA) | Dec. 2010 |

**Hidde de Jong**

| Title | Event and location | Date |
|---|---|---|
| Piecewise-linear differential equation models of bacterial regulatory networks | Seminar at University of Bristol (UK) | Feb. 2010 |
| Modeling of regulatory networks in bacteria | Invited talk at ISCAS 2010 workshop on Nano-Electronics and Nano-Biotechnologies, Paris | May 2010 |
| From qualitative to quantitative models of bacterial regulatory networks | Plenary talk at 19th International Symposium on Mathematical Theory of Networks and Systems (MTNS 2010), Budapest (Hungary) | Jul. 2010 |
| The carbon assimilation network in Escherichia coli is densely connected and largely sign-determined by directions of metabolic fluxes | Journées Ouvertes Biologie, Informatique et Mathématiques (JOBIM 2010), Montpellier | Sep. 2010 |
| Gene regulatory networks in bacteria: From structure to dynamics | Keynote at second annual symposium of Netherlands Consortium for Systems Biology | Oct. 2010 |

**Pedro Monteiro**

| Title | Event and location | Date |
|---|---|---|
| Towards an integrative approach for the modeling and formal verification of biological regulatory networks | PhD thesis defense, IST Lisbon | May 2010 |

**François Rechenmann**

| Title | Event and location | Date |
|---|---|---|
| Génome et cancérologie | Une Heure Ensemble, Montbonnot | Apr. 2010 |
| Informatique et génomes | Fête de la Science, Montbonnot | Oct. 2010 |

**Delphine Ropers**

| Title | Event and location | Date |
|---|---|---|
| Quantitative modeling of carbon assimilation in Escherichia coli: from data to models and back | seminar IXXI, Lyon | May 2010 |
| The use of fluorescent and luminescent reporter genes for analysing the regulatory network controlling the growth adaptation of the bacterium Escherichia coli | NGS Workshop, Zvenigorod (Russia) | June 2010 |
| Quantitative modeling of carbon assimilation in Escherichia coli: from data to models and back | JOBIM satellite meeting, Montpellier | Sep. 2010 |

## 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 leader of the editorial committee of the Interstices (http://interstices.info). Interstices offers pedagogic presentations of research themes and activities in the computer science domain, including at its interface with life sciences. In 2010, François Rechenmann coordinated a computer science special issue of TDC (Textes et Documents pour la Classe, http://www.cndp.fr/tdc/, June 2010), a journal which is widely read by secondary school teachers.

## 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.

**Sara Berthoumieux**

| Subject | Year | Location | Hours |
|---|---|---|---|
| Linear algebra and multivariate functions | 2 | Université Joseph Fourier | 36 |

**Eugenio Cinquemani**

| Subject | Year | Location | Hours |
|---|---|---|---|
| Identification of dynamical models of genetic networks | 5 | INSA de Lyon | 2 |

**Jérome Izard**

| Subject | Year | Location | Hours |
|---|---|---|---|
| Génétique procaryote | 2 | Université Joseph Fourier | 40 |

**Hidde de Jong**

| Subject | Year | Location | Hours |
|---|---|---|---|
| Modeling and simulation of genetic regulatory networks | 5 | INSA de Lyon | 16 |
| Modeling and simulation of genetic regulatory networks | 5 | ENS, Paris | 8 |

**Delphine Ropers**

| Subject | Year | Location | Hours |
|---|---|---|---|
| Modeling and simulation of genetic regulatory networks | 4 | Université Joseph Fourier | 7.5 |

Hidde de Jong organized with Daniel Kahn a module on the modeling of genetic and metabolic networks at INSA de Lyon.

# 9. Bibliography

## Publications of the year

### Doctoral Dissertations and Habilitation Theses

[1] P. MONTEIRO. *Towards an integrative approach for the modeling and formal verification of biological regulatory networks*, IST Lisbon/Université Claude Bernard Lyon, Lisbon/Lyon, 2010.

### Articles in International Peer-Reviewed Journal

[2] V. BALDAZZI, D. ROPERS, Y. MARKOWICZ, D. KAHN, J. GEISELMANN, H. DE JONG. *The carbon assimilation network in Escherichia coli is densely connected and largely sign-determined by directions of metabolic fluxes*, in "PloS Computational Biology", 2010, vol. 6, n$^{o}$ 6, e1000812.

[3] G. BATT, M. PAGE, I. CANTONE, G. GOESSLER, P. MONTEIRO, H. DE JONG. *Efficient parameter search for qualitative models of regulatory networks using symbolic model checking*, in "Bioinformatics", 2010, vol. 26, n$^{o}$ 18, p. i603-i610.

[4] F. BOYER, B. BESSON, G. BAPTIST, J. IZARD, C. PINEL, D. ROPERS, J. GEISELMANN, H. DE JONG. *A MATLAB program for the analysis of fluorescence and luminescence reporter gene data*, in "Bioinformatics", 2010, vol. 26, n$^{o}$ 9, p. 1262-1263.

[5] D. CHATTERJEE, E. CINQUEMANI, J. LYGEROS. *Maximizing the probability of attaining a target prior to extinction*, in "Nonlinear Analysis: Hybrid Systems", 2010, In press.

[6] E. CINQUEMANI, M. AGARWAL, D. CHATTERJEE, J. LYGEROS. *Convexity and convex approximations of discrete-time stochastic control problems with constraints*, in "Automatica", 2010, In press.

[7] P. HOKAYEM, E. CINQUEMANI, D. CHATTERJEE, F. RAMPONI, J. LYGEROS. *Stochastic receding horizon control with output feedback and bounded control inputs*, in "Automatica", 2010, In press.

[8] R. MATEESCU, P. MONTEIRO, E. DUMAS, H. DE JONG. *CTRL: Extension of CTL with regular expressions and fairness operators to verify genetic regulatory networks*, in "Theoretical Computer Science", 2010, In press.

[9] R. PORRECA, E. CINQUEMANI, J. LYGEROS, G. FERRARI-TRECATE. *Identification of genetic network dynamics with unate structure*, in "Bioinformatics", 2010, vol. 26, n$^{o}$ 9, p. 1239-1245.

[10] D. ROPERS, V. BALDAZZI, H. DE JONG. *Model reduction using piecewise-linear approximations preserves dynamic properties of the carbon starvation response in Escherichia coli*, in "IEEE/ACM Transactions on Computational Biology and Bioinformatics", 2010, In press.

[11] H. DE JONG, C. RANQUET, D. ROPERS, C. PINEL, J. GEISELMANN. *Experimental and computational validation of models of fluorescent and luminescent reporter genes in bacteria*, in "BMC Systems Biology", 2010, vol. 4, 55.

### Invited Conferences

[12] H. DE JONG. *From qualitative to quantitative models of gene regulatory networks in bacteria*, in "Proceedings of 19th International Symposium on Mathematical Theory of Networks and Systems (MTNS 2010)", Budapest, Hungary, 2010.

### International Peer-Reviewed Conference/Proceedings

[13] V. BALDAZZI, D. ROPERS, Y. MARKOWICZ, D. KAHN, J. GEISELMANN, H. DE JONG. *The carbon assimilation network in Escherichia coli is densely connected and largely sign-determined by directions of metabolic fluxes*, in "Actes des Journées Ouvertes Biologie, Informatique et Mathématiques (JOBIM 2010)", Montpellier, France, 2010, p. 11-12.

[14] P. HOKAYEM, E. CINQUEMANI, D. CHATTERJEE, F. RAMPONI, J. LYGEROS. *Stochastic MPC with imperfect state information and bounded controls*, in "Proceedings of the UKACC International Conference on Control", Coventry, UK, 2010.

[15] P. HOKAYEM, E. CINQUEMANI, D. CHATTERJEE, F. RAMPONI, J. LYGEROS. *Stochastic receding horizon control with output feedback and bounded control inputs*, in "Proceedings of the 49th IEEE Conference on Decision and Control", Atlanta, GA, USA, 2010, p. 6095-6100.

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[18] R. PORRECA, E. CINQUEMANI, J. LYGEROS, G. FERRARI-TRECATE. *Structural identification of unate-like genetic network models from time-lapse protein concentration measurements*, in "Proceedings of the 49th IEEE Conference on Decision and Control (CDC 2010", Atlanta, GA, USA, 2010, p. 2529-2534.

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[19] V. BALDAZZI, P. MONTEIRO, M. PAGE, D. ROPERS, J. GEISELMANN, H. DE JONG. *Qualitative analysis of genetic regulatory networks in bacteria*, in "Understanding the Dynamics of Biological Systems: Lessons Learned from Integrative Systems Biology", W. DUBITZKY, J. SOUTHGATE, H. FUSS (editors), Springer, 2010, In press.

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