
IN PARTNERSHIP WITH:
**Université Joseph Fourier (Grenoble)**

Activity Report 2012

# Project-Team IBIS

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

IN COLLABORATION WITH: Laboratoire Adaptation et Pathogénie des Microorganismes (LAPM)

RESEARCH CENTER
**Grenoble - Rhône-Alpes**

THEME
**Computational Biology and Bioinformatics**

# Table of contents


**1. Members** ..... **1**
**2. Overall Objectives** ..... **2**
2.1. Overview 2
2.2. Highlights of the Year 3
**3. Scientific Foundations** ..... **3**
3.1. Modeling of bacterial regulatory networks 3
3.2. Analysis, simulation, and identification of bacterial regulatory networks 5
3.3. High-precision measurements of gene expression in bacteria 6
**4. Software** ..... **7**
4.1. Genetic Network Analyzer (GNA) 7
4.2. WellReader 8
**5. New Results** ..... **8**
5.1. Analysis of gene regulatory networks by means of piecewise-linear (PL) models 8
5.2. Experimental mapping of gene regulatory networks in bacteria 8
5.3. Analysis of metabolic coupling in gene regulatory networks 9
5.4. Parameter estimation for kinetic models of carbon metabolism in bacteria 10
5.5. Structural identification of gene regulatory networks 11
5.6. Stochastic modeling and identification of gene regulatory networks in bacteria 11
5.7. Control of regulatory networks in bacteria 12
5.8. Shared control of gene expression by global physiological effects and specific regulators 12
**6. Bilateral Contracts and Grants with Industry** ..... **12**
6.1. Genostar 12
6.2. BGene 13
**7. Partnerships and Cooperations** ..... **13**
7.1. Regional initiatives 13
7.2. National initiatives 14
7.3. International projects 14
7.4. International collaborations 15
7.5. International research visitors 15
**8. Dissemination** ..... **15**
8.1. Editorial, animation, and reviewing activities 15
8.2. Other administrative activities 16
8.3. Seminars, presentations, and PhD thesis defenses 17
8.4. Popular science writing 19
8.5. Teaching 19
**9. Bibliography** ..... **20**

## Project-Team IBIS

**Keywords:** Computational Biology, Systems Biology, Microbiology, Regulatory Networks, System Analysis And Control

*IBIS is bilocated at the Inria Grenoble - Rhône-Alpes research center in Montbonnot and the Laboratoire Adaptation et Pathogénie des Microorganismes (UMR 5163) at the Institut Jean Roget in La Tronche.*

*Creation of the Project-Team:* January 01, 2009 .

# 1. Members

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Hidde de Jong [Team leader, senior research scientist (DR), Inria, HdR]
François Rechenmann [Senior research scientist (DR), Inria, HdR]
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Sara Berthoumieux [PhD student, Inria/Université Claude Bernard, Lyon, until June 2012. Supervisors: Hidde de Jong and Daniel Kahn (Inria, BAMBOO)]
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# 2. Overall Objectives

## 2.1. Overview

When confronted with changing environmental conditions, bacteria and other single-cell organisms have a remarkable capacity to rapidly adapt their functioning. The stress responses of bacteria are controlled on the molecular level by large and complex networks of 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. 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 physiological and genetic perturbations. 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 mathematical biology group on the other hand. In particular, the IBIS team is composed of members of the group of Johannes 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 Johannes Geiselmann its co-director. The experimental component of IBIS also remains part of the Laboratoire Adaptation et Pathogénicité des Microorganismes, and Johannes 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

Three students defended their PhD thesis this year: Guillaume Baptist [1], Sara Berthoumieux [2], and Jérôme Izard [3]. One of the papers derived from the work of Sara Berthoumieux was accepted for *Molecular Systems Biology* [7].

The collaborative project RESET was accepted in the Bioinformatics call of the Investissements d'Avenir program. RESET joins seven partners, including the company Metabolic Explorer SA, and runs until 2016. RESET studies the gene expression machinery in bacteria, by means of models and experiments, and develops biotechnological applications based on the control of the gene expression machinery.

Former IBIS member Caroline Ranquet and Johannes Geiselmann created, with Marie-Gabrielle Jouan (Floralis, Université Joseph Fourier), the start-up company BGene, active in the field of DNA engineering.

# 3. Scientific Foundations

## 3.1. Modeling of bacterial regulatory networks

**Participants:** Sara Berthoumieux, Eugenio Cinquemani, Johannes Geiselmann, Nils Giordano, Edith Grac, Hidde de Jong, Stéphane Pinhal, Delphine Ropers [Correspondent], Valentin Zulkower.

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

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.

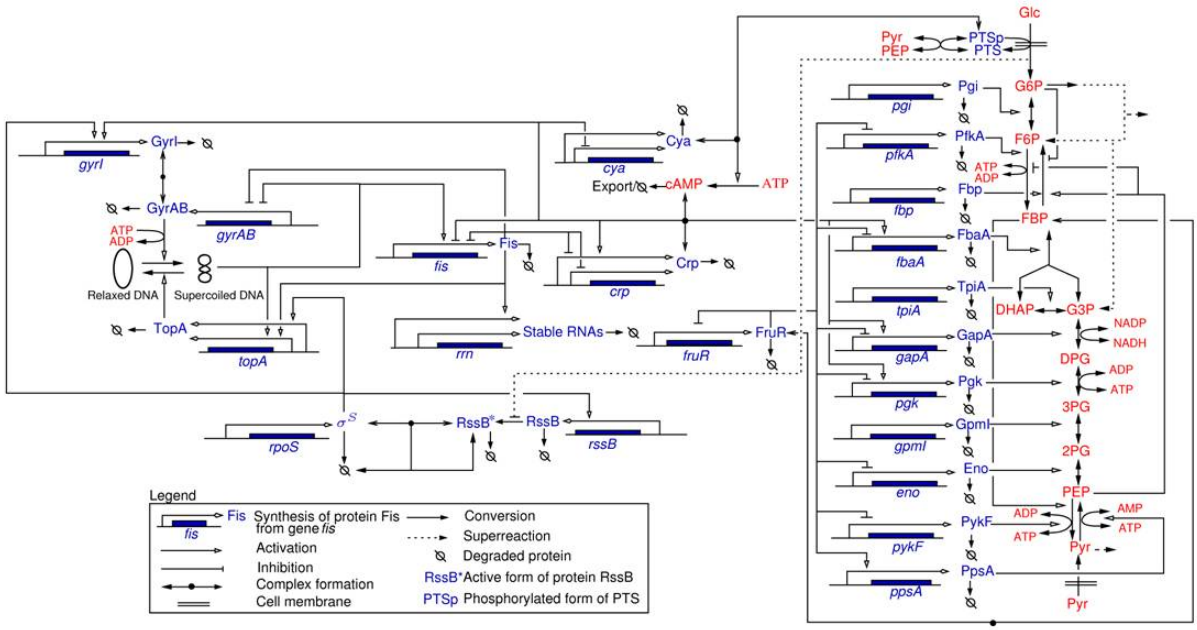



*Figure 2. Network of key genes, proteins, and regulatory interactions involved in the carbon assimilation network in E. coli (Baldazzi et al., PLoS Computational Biology, 6(6):e1000812, 2010 ). The metabolic part includes the glycolysis/gluconeogenesis pathways as well as a simplified description of 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.

Several modeling 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 the role of networks of global regulators in shaping the adaptive response of bacteria. Moreover, we study the interactions of these networks with metabolism and the gene expression machinery. These topics involve collaborations with the BEAGLE, COMORE, and CONTRAINTES project-teams at Inria.

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

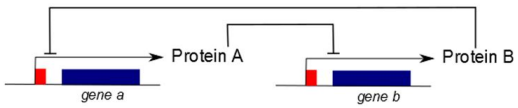
**Participants:** Sara Berthoumieux, Eugenio Cinquemani, Johannes Geiselmann, Nils Giordano, Hidde de Jong [Correspondent], Michel Page, François Rechenmann, Delphine Ropers, Diana Stefan, Valentin Zulkower.

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 gene regulatory networks. The PL models, originally introduced by Leon Glass and Stuart Kauffman, provide a coarse-grained picture of the dynamics of gene 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 gene 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 gene 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 onset of virulence in *E. chrysanthemi*, and environmental biodegradation by *P. putida* mt-2. 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 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 and even stochastic 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 INRA, the University of Pavia (Italy) and ETH Zürich (Switzerland). This raises




(a)

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

(b)

*Figure 3. (a) Example of a gene 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 ($\gamma_a \, x_a$).*

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. High-precision measurements of gene expression in bacteria

**Participants:** Guillaume Baptist, Sara Berthoumieux, Julien Demol, Johannes Geiselmann [Correspondent], Edith Grac, Jérôme Izard, Hidde de Jong, Stephan Lacour, Yves Markowicz, Corinne Pinel, Stéphane Pinhal, Delphine Ropers, Claire Villiers, Valentin Zulkower.

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 notably 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.

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,

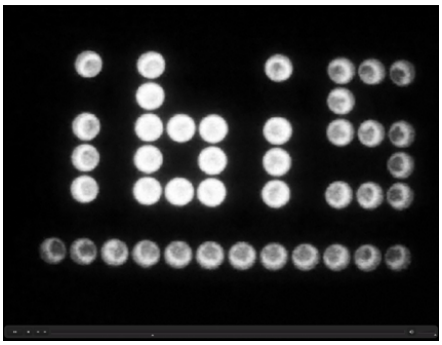


*Figure 4. Playful illustration of the principle of reporter genes (see http://ibis.inrialpes.fr for the corresponding movie). A microplate containing a minimal medium (with glucose and acetate) is filmed during 36 hours. Wells contain E. coli bacteria which are transformed with a reporter plasmid containing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

such as the tools from transcriptomics, proteomics, and metabolomics that are able to quantify the amounts of mRNAs, 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 Interdisciplinaire de Physique at the Université Joseph Fourier.

# 4. Software

## 4.1. Genetic Network Analyzer (GNA)

**Participants:** Hidde de Jong [Correspondent], Michel Page, François Rechenmann, Delphine Ropers.

Gene regulatory networks, qualitative simulation, model checking

GENETIC NETWORK ANALYZER (GNA) is the implementation of a method for the qualitative modeling and simulation of gene 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 8.3. In comparison with the previously distributed versions, GNA 8.3 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.

Gene expression, reporter gene data

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. Analysis of gene regulatory networks by means of piecewise-linear (PL) models

GENETIC NETWORK ANALYZER (GNA) is a tool for the qualitative modeling and simulation of the dynamics of gene regulatory networks by means of PL models, as described in Section 4.1. GNA has been integrated with the other bioinformatics tools distributed by Genostar (http://www.genostar.com/). Version 8.4 of GNA was released by IBIS and Genostar this year. This version is an update of version 8.0, deposited at the Agence pour la Protection des Programmes (APP). Some bugs have been corrected in the new version and the program has been adapted to the latest versions of Java and the software platform of Genostar. A book chapter describing the current version of GNA has been published in a volume on the modeling of bacterial molecular networks [13]. The chapter is a tutorial illustrating the practical use of recent functionalities of GNA like the network editor and the formal verification module by means of an example network in *E. coli*.

The predictions obtained with the help of GNA are purely qualitative, describing the dynamics of the network by means of a state transition graph. While a qualitative analysis is appropriate for certain problems, the absence of precise quantitative predictions may not be desirable in others, such as the analysis of a limit cycle or the design of a controller for a synthetic network. The quantitative study of PL models of gene regulatory networks is hindered by the fact that the step functions describing the logic of regulatory interactions lead to discontinuities in the right-hand side of the PL models (Section 3.2). This has motivated extensions of the PL models based on differential inclusions and Filippov solutions. As of now, no numerical simulation tool for the simulation of these Filippov extensions is available. In collaboration with the BIPOP project-team, we have shown how tools developed for the simulation of nonsmooth mechanical, electrical and control systems can be adapted for this purpose. A paper describing these results is being prepared for submission.

## 5.2. Experimental mapping of gene regulatory networks in bacteria

The use of fluorescent and luminescent 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 fully exploit this technology, we need methods to rapidly construct reporter genes, both on plasmids and on the chromosome, mathematical models to infer biologically relevant quantities from the primary data, and computer tools to achieve this in an efficient and user-friendly manner. For instance, 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. Over the past few years, we put into place an experimental platform and data analysis software, notably the WELLREADER program (Section 4.2), to allow biologists to make the most of the information contained in reporter gene expression data. 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*.

These tools have been used in a series of studies directed at the experimental mapping of gene regulatory networks in *E. coli*. One example, carried out in the framework of the PhD thesis of Guillaume Baptist, is the development of a new screening methodology for identifying all genes that control the expression of a target gene through genetic or metabolic interactions. The screen combines mutant libraries with luciferase reporter constructs. Instead of a static picture of gene expression, this method allows dynamical monitoring in different environmental conditions. Mutants with interesting phenotypes can thus be selected based on multiple criteria, and the expression dynamics of the target gene can be extensively characterized. The method has been applied to the identification of the direct and indirect regulators of the gene *acs* in *Escherichia coli*. We confirmed known genetic regulators of the gene and identified new regulatory influences, many of which involve metabolic intermediates or metabolic sensing. An analysis of mutants involved in glycolysis and glucose transport demonstrates that the classical model of catabolite repression in *E. coli* needs to be amended. A paper describing the above work is currently under revision.

Other examples of on-going work are the analysis of the network involved in motility and sessility and the modulation of the RpoS regulon in *E. coli* by Stephan Lacour, the analysis of the regulation of cAMP levels in the bacterial cell by Claire Villiers, and the analysis of various aspects of the regulation of carbon metabolism by Valentin Zulkower and Stéphane Pinhal.

## 5.3. Analysis of metabolic coupling in gene 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 coined the term metabolic coupling to denote these indirect interactions mediated by metabolism. Ignoring metabolic coupling during the analysis of the network dynamics may lead crucial feedback loops to be missed.

In previous work, published in *PLoS Computational Biology* in 2010, we showed how indirect interactions arising from metabolic coupling can be derived from a model of the underlying biochemical reaction network. We applied this approach to the carbon assimilation network in *Escherichia coli* investigating how the structural properties of the network are modified by the inclusion of metabolic interactions. Our results showed that the derived gene regulatory network is densely connected, contrary to what is usually assumed. Moreover, we found that the signs of the indirect interactions are largely fixed by the direction of metabolic fluxes, independently of specific parameter values and rate laws, and that a change in flux direction may invert the sign of indirect interactions. This leads to a feedback structure that is at the same time robust to changes in the kinetic properties of enzymes and that has the flexibility to accommodate radical changes in the environment.

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 gene regulatory network, including both the direct and indirect interactions.

In order to obtain a clearer view of the dynamic role of metabolic coupling in the adaptation of gene expression, we developed several qualitative models corresponding to a network topology including all, some, or none of the indirect interactions. The dynamical properties of the models were analyzed and compared with available experimental data using the computer tool GNA (Section 4.1). In particular, we compared the steady-state concentrations of enzymes and transcription regulators during growth on glucose and acetate, as well as

the dynamic response of gene expression to the exhaustion of glucose and the subsequent assimilation of acetate. We find significant differences between the dynamics of the system in the absence and presence of metabolic coupling. This confirms that indirect interactions are essential for correctly reproducing the observed adaptation of gene expression to a change in carbon source. Our work thus underlines the importance of metabolic coupling in gene regulatory networks, and shows that such indirect interactions cannot be neglected when studying the adaptation of an organism to changes in its environment. A paper describing these results has been published in the *Journal of Theoretical Biology* [5]. Another publication, reviewing the applicability of these and other ideas for multi-scale modeling in plants, has appeared in *Trends in Plant Science* [4].

## 5.4. Parameter estimation for kinetic models of carbon metabolism in bacteria

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 Sections 5.1 and 5.3, 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 context of the analysis of the interactions between metabolism and gene expression in carbon metabolism in *E. coli*.

In collaboration with Matteo Brilli and Daniel Kahn (INRA and Université Claude Bernard in Lyon), we previously developed an approximate model of central metabolism of *E. coli*, as described in an article published in *Bioinformatics* in 2011. The model was based on the use of so-called linlog functions to approximately describe the rates of enzymatic reactions. More precisely, linlog models define reactions rates as proportional to both the enzyme concentrations and a linear combination of the logarithms of metabolite concentrations. The estimation of parameters in the linlog model from metabolomics, transcriptome, proteomics data sets required the development of a new approach, adapted to the occurrence of numerous missing values in the data sets. When applied to the above-mentioned linlog model, exploiting a high-throughput dataset published in the literature, we were able to obtain reasonable estimates of the 100 parameters.

The results of the above application also revealed the fundamental role played by the identifiability of the model parameters, an issue often overlooked in systems biology. This prompted us for a thorough investigation of the concepts of structural identifiability (in presence of perfect, idealized data), practical identifiability (in presence of noisy and limited amounts of data), and the relations among the two. In addition, we looked into the implications of this analysis for the reduction of nonidentifable to identifiable models. While having a solid mathematical basis, the study was tailored to the actual experimental practice, and resulted in a practical model reduction method that improves upon our previous approach in case of large measurement noise. This study, and the results from its application to both *in-silico* case studies and state-of-the-art datasets, were reported in a paper that has been accepted for publication in the *Journal of Mathematical Biology* [6] (see also [11] for a short version with preliminary results).

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 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 tested and used to understand some key mechanisms in the adaptation of *E. coli* to the exhaustion of glucose. The PhD thesis of Manon Morin, which started at the end of this year in the framework of a collaboration supported by a Contrat Jeune Scientifique INRA-Inria, will further develop these research directions.

## 5.5. Structural identification of gene 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 reporter gene 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.

In order to address the structural identification problem, Eugenio Cinquemani developed in collaboration with the Automatic Control Lab at ETH Zürich (Switzerland) and the Computer Engineering & Systems Science Department of the University of Pavia (Italy), an ODE modelling framework based on so-called unate-like functions, and a method that exploits monotonicity properties of these functions to effectively prune models that are incompatible with the data from the family of all unate-like modelling alternatives. This model invalidation step is based on simple preprocessing of time-course protein concentration and synthesis rate profiles, assumed available, and allows one to reduce the search of the best fitting model to a small subset of viable model structures.

The method, first published in *Bioinformatics* in 2010 and demonstrated on real data from the synthetic network IRMA, allows one to integrate *a-priori* knowledge on the expected network dynamics in a natural way. Leveraging on this, in the context of the same international collaboration, the method has been further developed in particular by considering relevant subclasses of the family of unate-like models that also enjoy certain quasi-convexity properties. For this restricted class, combined use of monotonicity and quasi-convexity properties allows one to ameliorate the model invalidation step, *i.e.* retain even fewer viable model structures based on affordable data preprocessing. These developments have been presented and demonstrated *in silico* in a paper published in the 2012 special issue on System Identification for Biological Systems of the *International Journal of Robust and Nonlinear Control* [9] .

We are currently applying the above methods to actual, known or partially unknown, networks. In the framework of the PhD thesis of Diana Stefan, the network inference method has been applied to gene expression data from the network regulating motility of *E. coli*. First encouraging results have suggested further experimental and computational investigations that are currently in progress.

## 5.6. Stochastic modeling and identification of gene regulatory networks in bacteria

At the single-cell level, the processes that govern gene expression are often better described by stochastic models. Modern techniques for the real-time monitoring of gene expression in single cells enable one to apply stochastic modelling to study the origins and consequences of random noise in response to various environmental stresses, and the emergence of phenotypic variability. The potential impact of single-cell stochastic analysis and modelling is tremendous, ranging from a better comprehension of the biochemical regulatory mechanisms underlying life, to the development of new strategies for the control of cell populations and even of single cells. General modeling paradigms, such as the Chemical Master Equation, exist for the description of stochastic dynamics at the single-cell level. However, due to the complexity of the interactions, current studies have often preferred to focus on specific cases of interest by *ad-hoc* modeling and analysis. In addition, theoretical and practical challenges inherent in the inference of stochastic models from biological experimental data have limited the development of general identification approaches.

In view of the potential and the relevance of the subject, one research line of IBIS is dedicated to the probabilistic modeling of the dynamics of gene regulatory networks at the level of individual cells. Our activity is centered around two main challenges. On the one hand, we address the problem of developing methods for fitting unknown network parameters of stochastic models to experimental data. As a reference case study we consider the network regulating the inset of the arabinose uptake process in *E. coli* upon depletion of glucose in the growth medium. For this system, Eugenio Cinquemani and Michel Page are developing and implementing methods for the inference of unknown parameters from fluorescence microscopy data. On the other hand, we investigate several alternative modelling approaches in an attempt to determine their relevance to different systems and application scenarios. This activity is being developed in collaboration

with Gregory Batt (CONTRAINTES, Inria Paris-Rocquencourt), Giancarlo Ferrari-Trecate (University of Pavia, Italy), and Alfonso Carta (COMORE, Inria Sophia-Antipolis - Méditerranée). First results connected to control applications on real and simulated data have been submitted for presentation at the European Control Conference to be held in 2013. Finally, further ongoing work concerns the study of noise propagation in gene regulatory networks, in collaboration with Irina Mihalcescu (Université Joseph Fourier), and the analysis of data from Fluorescence Recovery After Photobleaching (FRAP) experiments, in collaboration with Marianna Rapsomaniki and Zoi Lygerou (University of Patras, Greece) and John Lygeros (ETH Zürich, Switzerland).

## 5.7. Control of regulatory networks in bacteria

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.

Within the projects ColAge and GeMCo (Section 7.2), 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. Delphine Ropers is developing models of the gene expression machinery and Jérome Izard, in the context of his PhD thesis, is rewiring part of the network to enable control of the network dynamics. The results on these projects are currently being prepared for publication.

## 5.8. Shared control of gene expression by global physiological effects and specific regulators

Gene expression is controlled by the joint effect of (i) the global physiological state of the cell, in particular the activity of the gene expression machinery, and (ii) DNA-binding transcription factors and other specific regulators. While many studies have focused on networks of transcription factors, the analysis of the relative contributions of both transcription factors and global effects of the physiological state has received relatively little attention thus far.

In the framework of the PhD thesis of Sara Berthoumieux, we have developed a model-based approach to distinguish between these two effects using time-resolved measurements of promoter activities. We have demonstrated the strength of the approach by analyzing a circuit involved in the regulation of carbon metabolism in *E. coli*, consisting of two pleiotropic regulators of the cell (Crp and Fis), the gene *acs* encoding the enzyme acetyl-CoA synthetase (Acs), and the signaling metabolite cyclic AMP (cAMP) which activates Crp. *acs* is strongly expressed in the absence of glucose and is thus an excellent indicator of the transcriptional response of carbon metabolism to a growth-phase transition.

Our results show that the transcriptional response of the network is controlled by the physiological state of the cell and the signalling metabolite cAMP. The (surprising) absence of a strong regulatory effect of transcription factors suggests that they are not the main coordinators of gene expression changes during growth transitions, but rather that they complement the effect of global physiological control mechanisms. This change of perspective has important consequences for the interpretation of transcriptome data and the design of biological networks in biotechnology and synthetic biology. An article presenting the above results has been accepted for *Molecular Systems Biology* [7].

# 6. Bilateral Contracts and Grants with Industry

## 6.1. Genostar

**Participant:** François Rechenmann.

Genostar, an Inria start-up created in 2004, provides bioinformatics solutions for the comparative analysis of bacterial genomes, proteomes and metabolomes. Genostar's software suite performs the annotation of sets of genomic sequences, *i.e.*, the identification of the coding sequences and other features, followed by the prediction of the functions of the gene products. The modules which make up the software suite were originally developed within the Genostar consortium and the HELIX project team at Inria Grenoble - Rhône-Alpes. The software suite also includes the modeling and simulation tool GNA developed by members of IBIS (Section 4.1). Genostar offers a comprehensive service line-up that spans genome sequencing, read assembly, annotation, and comparison. Genostar thus works with trusted subcontractors, each specialized in state-of-the-art sequencing technologies. François Rechenmann is scientific consultant of the company. For more information, see http://www.genostar.com.

## 6.2. BGene

**Participant:** Johannes Geiselmann.

BGene is a start-up company of Université Joseph Fourier in the field of DNA engineering. BGene proposes efficient and custom-made modifications of bacterial genomes, leaving no scars or antibiotics resistance genes. The company has know-how and expertise at all stages of the development process, including the *in-silico* design of a desired construction, the choice of the right genetic tools, and the delivery of the finished product. Former IBIS-member Caroline Ranquet and Johannes Geiselmann are co-founders of BGene, together with Marie-Gabrielle Jouan (Floralis, Université Joseph Fourier).

# 7. Partnerships and Cooperations

## 7.1. Regional initiatives

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

| Project name | Motilité ou adhésion : comment les entérobactéries choisissent entres ces deux états physiologiques déterminants pour la virulence |
|---|---|
| Coordinator | S. Lacour |
| IBIS participants | J. Demol, O. Dudin, J. Geiselmann, J. Izard, S. Lacour, C. Pinel |
| Type | Grant, Cluster Infectiologie, Région Rhône-Alpes |

| Project name | Séminaire grenoblois des systèmes complexes |
|---|---|
| Coordinators | S. Achard, O. François, A. Girard, E. Prados, S. Rafai, D. Ropers |
| IBIS participants | D. Ropers |
| Type | Funding by Institut des Systèmes Complexes de Lyon (IXXI) |
| Web page | http://www.ixxi.fr/?page_id=114&lang=fr |

| Project name | Séminaire de modélisation du vivant |
|---|---|
| Coordinators | O. Gandrillon |
| IBIS participants | D. Ropers |
| Type | Funding by GdR BIM |
| Web page | http://cgphimc.univ-lyon1.fr/CGphiMC/Semovi/Semovi.php |

## 7.2. National initiatives

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

| Project name | RESET – Arrest and restart of the gene expression machinery in bacteria: from mathematical models to biotechnological applications |
|---|---|
| Coordinator | H. de Jong |
| IBIS participants | G. Baptist, E. Cinquemani, J. Geiselmann, H. de Jong, J. Izard, S. Lacour, Y. Markowicz, C. Pinel, D. Ropers |
| Type | Bioinformatics call, Investissements d'Avenir program (2012-2016) |

| Project name | Fonction du système de régulation post-transcriptionnel CSR dans la dynamique de l'adaptation métabolique chez la bactérie modèle Escherichia coli |
|---|---|
| Coordinators | M. Cocaign-Bousquet (INRA, LISBP), B. Enjalbert (INSA, LISBP), D. Ropers |
| IBIS participants | M. Morin, D. Ropers |
| Type | Contrat Jeune Scientifique INRA-Inria (2012-2016) |
| Web page | http://www.inra.fr/les_hommes_et_les_femmes/rejoignez_nous/completer_sa_formation/le_recrutement_de_doctorants/cjs__1/inra_inria |

## 7.3. International projects

| Project name | French bioinformatics contribution to ICGC |
|---|---|
| Coordinator | G. Thomas |
| IBIS participants | F. Rechenmann |
| Type | International Cancer Genome Consortium (ICGC) |
| Web page | 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 [12], [10].

## 7.4. International collaborations

IBIS has strong collaborations 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.

## 7.5. International research visitors

| | |
|---|---|
| Internship<br>Supervisor<br>Subject | Elif Köksal (Bogazici University, Turkey))<br>E. Cinquemani<br>Modeling, analysis, and identification of metabolic networks |

| | |
|---|---|
| Internship<br>Supervisor<br>Subject | Nicola Simeone (University of Pavia, Italy))<br>E. Cinquemani<br>Stochastic modeling and identification of bacterial regulatory networks |

# 8. Dissemination

## 8.1. Editorial, animation, and reviewing activities

**Guillaume Baptist**

| Type | Journal, conference, agency |
|---|---|
| Editor | French-language web site on synthetic biology, theoretical biology, and philosophy, http://www.cellule-et-futur.fr |

**Eugenio Cinquemani**

| Type | Journal, conference, agency |
|---|---|
| Associate Editor | European Control Conference (ECC) 2012 |

**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 | CMSB 12, DKRC 12, ECCB 12, IEEE BIBM 12, JOBIM 12, PAIS 12, QR 12 |
| Member Scientific Advisory Board | Microbiology and Food Chain Department, INRA |
| Member Review Committee | International Human Frontier Science Program (HFSP) |
| President Recruitment Committee | Assistant-professor ENS, Paris, department of Biology |
| Member PhD Committee | Sara Berthoumieux (Université Joseph Fourier) |
| Member PhD Advisory Committee | Matthieu Trauchessec (CEA/Metabolic Explorer and Université Joseph Fourier), Caroline Baroukh (Inria/INRA and Université de Montpellier 2) |
| Coordinator (with C. Ambroise and F. Molina) | Working group on Transcriptome, protéome, modélisation, inférence et analyse des réseaux biologiques of GDR CNRS 3003 Bioinformatique moléculaire |
| Advisor | Grenoble team for iGEM 2012 competition |
| Project reviews | ANR, Région Acquitaine, FRS, CNRS, Technical University Munich, NWO |

**Johannes Geiselmann**

| Type | Journal, conference, agency |
|---|---|
| Member Recruitment Committee | Professor in bioinformatics, INSA Lyon |
| Member Recruitment Committee | Assistant-professor, Université Joseph Fourier |
| Member PhD Committee | John Lalith (ENS Lyon), Axelle Dawidas (Université Joseph Fourier), Sara Berthoumieux (Université Joseph Fourier), Guillaume Baptist (Université Joseph Fourier), Jérôme Izard (Université Joseph Fourier) |
| Member PhD Advisory Committee | Khady Sall (CEA and Université Joseph Fourier), Xuejiao Jiang (Université Claude Bernard, Lyon) |
| Advisor | Grenoble team for iGEM 2012 competition |

**Stéphane Pinhal**

| Type | Journal, conference, agency |
|---|---|
| Advisor | Grenoble team for iGEM 2012 competition |

**Delphine Ropers**

| Type | Journal, conference, agency |
|---|---|
| Member Recruitment Committee | Ingénieur de recherche INRA |
| Member Organization Committee | SeMoVi (Séminaire de Modélisation du Vivant) |
| Member PhD Committee | Jérôme Izard (Université Joseph Fourier) |
| Member PhD Advisory Committee | Claire Villiers (Université Joseph Fourier) |
| Advisor | Grenoble team for iGEM 2012 competition |
| Project reviews | CNRS |

**Valentin Zulkower**

| Type | Journal, conference, agency |
|---|---|
| Advisor | Grenoble team for iGEM 2012 competition |

## 8.2. Other administrative activities

Eugenio Cinquemani is member of the Comité des Utilisateurs des Moyens Informatiques (CUMI) and of the Commission des Emplois Scientifiques at Inria Grenoble - Rhône-Alpes.

Hidde de Jong is local representative of the Department of International Relations of Inria at the Grenoble - Rhône-Alpes research center. He is also member of the working group on International Relations of the Conseil d'Orientation Scientifique et Technique (COST) of Inria.

Johannes 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.

Yves Markowicz is director of the BSc department at Université Joseph Fourier.

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

François Rechenmann has been commissioned by the Director of Inria Grenoble - Rhône-Alpes to help and to coach PhD students, in the research center, who encounter problems of various sorts during their thesis.

Delphine Ropers represents Inria Grenoble - Rhône-Alpes in the scientific board of IXXI, the Complex Systems Institute in Lyon (http://www.ixxi.fr). She is also member of the Commission de Formation Permanente at Inria Grenoble - Rhône-Alpes.

Diana Stefan is a representative of the PhD candidates within the committees of the Doctoral School MSTII in Grenoble.

## 8.3. Seminars, presentations, and PhD thesis defenses

**Guillaume Baptist**

| Title | Event and location | Date |
|---|---|---|
| Les réseaux de régulation chez la bactérie Escherichia coli | PhD thesis defense, Université Joseph Fourier | Aug. 2012 |

**Sara Berthoumieux**

| Title | Event and location | Date |
|---|---|---|
| Methods for identification of biochemical network models | PhD thesis defense, Université Claude Bernard, Lyon | Jun. 2012 |

**Eugenio Cinquemani**

| Title | Event and location | Date |
|---|---|---|
| Identifiability and identification of dynamic models of biochemical regulatory networks | INRIabcd seminar, Inria Grenoble - Rhône-Alpes, Villeurbanne | Feb. 2012 |
| Structural and practical identifiability of approximate metabolic network models | 16th IFAC symposium on System Identification (SYSID), Brussels (Belgium) | Jul. 2012 |

**Hidde de Jong**

| Title | Event and location | Date |
|---|---|---|
| Piecewise-linear modeling of gene regulatory networks | Invited talk at Workshop on Structural Dynamical Systems (SDS-12), Monopoli (Italy) | Jun. 2012 |
| Shared control of gene expression in bacteria by transcription factors and global physiological state | Invited talk at Workshop on design, optimization and control in systems and synthetic biology, Paris | Jun. 2012 |
| Modeling of gene regulatory networks | ICGEB Course on Advances in bioinformatics tools for the analysis of high-throughput omics data, Santiago (Chili) | Jul. 2012 |
| Identification of metabolic network models from high-throughput data sets | Invited talk at Workshop on Statistical and dynamical models in biology and medicine, Stuttgart | Oct. 2012 |
| Shared control of gene expression in bacteria by transcription factors and global physiological state | Invited talk at Journées Microbiologistes de l'INRA, Avignon | Nov. 2012 |
| Integration of high-throughput datasets through dynamical modeling of regulatory networks | Tutorial at PROSPECTOM Workshop, Grenoble | Nov. 2012 |
| Modélisation mathématique des réseaux de régulation génique | Closing conference of séminaire interacadémique des inspecteurs généraux et régionaux de mathématiques, Grenoble | Nov. 2012 |

**Johannes Geiselmann**

| Title | Event and location | Date |
|---|---|---|
| Systems biology of Escherichia coli | Seminar at Université Claude Bernard, Lyon | Feb. 2012 |
| The carbon catabolite repression network of Escherichia coli | Conference on Microbial Systems Biology, Taipei (Taiwan) | May 2012 |

**Jérôme Izard**

| Title | Event and location | Date |
|---|---|---|
| Growth control and gene regulation in Escherichia coli | PhD thesis defense, Université Joseph Fourier | Dec. 2012 |

**Delphine Ropers**

| Title | Event and location | Date |
|---|---|---|
| Modeling the gene expression machinery in Escherichia coli | Séminaire de Modélisation du Vivant (SeMoVi), Inria Grenoble - Rh?Alpes | Apr. 2012 |
| Complex regulatory networks in bacteria: How bacteria face the unexpected | Ecole de Systèmes Complexes, Rennes | Oct. 2012 |

**Diana Stefan**

| Title | Event and location | Date |
|---|---|---|
| Structural and parametric identification of bacterial regulatory networks A case study on the gene network regulating motility in E. coli | Poster presentation at journée scientifique de l'ARC6 | Nov. 2012 |

## 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 the context of the Math C2+ initiative for high-school students, François Rechenmann has led two sessions of a workshop introducing the notion of algorithm. The objective set to the students was to design an algorithm for sorting a stack of objects, such as pancakes, according to their size and through only one type of swapping operation (see http://interstices.info/algo-crepes for the presentation of the algorithm).

## 8.5. Teaching

Four members of the IBIS team are either full professor, associate professor or assistant professor at the Université Joseph Fourier or the UniversitÃ©Â Âierre 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.

**Eugenio Cinquemani**

| Subject | Year | Location | Hours |
|---|---|---|---|
| Identification of dynamical models of genetic networks | 5 | INSA de Lyon | 4 |

**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 | UniversitÃ©Â Âoseph Fourier | 7.5 |
| Modeling and simulation of genetic regulatory networks | 5 | INSA de Toulouse | 4 |

**Diana Stefan**

| Subject | Year | Location | Hours |
|---|---|---|---|
| Automatique et traitement du signal | 2 | INPG Phelma | 32 |

**Valentin Zulkower**

| Subject | Year | Location | Hours |
|---|---|---|---|
| Mathématiques générales (algèbre, géométrie, ...) | 1 | Université de Grenoble | 45 |
| Mathématiques générales (analyse, séries de Fourier, calcul matriciel) | 1 | Polytech Grenoble | 90 |

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] G. BAPTIST. *Les réseaux de régulation chez la bactérie Escherichia coli*, Université Joseph Fourier, Grenoble, 2012.

[2] S. BERTHOUMIEUX. *Méthodes pour l'identification des modèles de réseaux biochimiques*, Université Claude Bernard, Lyon, 2012.

[3] J. IZARD. *Growth control and gene regulation in Escherichia coli*, Université Joseph Fourier, Grenoble, 2012.

### Articles in International Peer-Reviewed Journals

[4] V. BALDAZZI, N. BERTIN, H. DE JONG, M. GÉNARD. *Towards multiscale plant models: integrating cellular networks*, in "Trends in Plant Science", 2012, vol. 17, n$^{o}$ 12, p. 728-736.

[5] V. BALDAZZI, D. ROPERS, J. GEISELMANN, D. KAHN, H. DE JONG. *Importance of metabolic coupling for the dynamics of gene expression following a diauxic shift in Escherichia coli*, in "Journal of Theoretical Biology", 2012, vol. 295, p. 100-115.

[6] S. BERTHOUMIEUX, M. BRILLI, D. KAHN, H. DE JONG, E. CINQUEMANI. *On the identifiability of metabolic network models*, in "Journal of Mathematical Biology", 2012, In press.

[7] S. BERTHOUMIEUX, H. DE JONG, G. BAPTIST, C. PINEL, C. RANQUET, D. ROPERS, J. GEISELMANN. *Shared control of gene expression in bacteria by transcription factors and global physiology of the cell*, in "Molecular Systems Biology", 2012, In press.

[8] P. HOKAYEM, E. CINQUEMANI, D. CHATTERJEE, F. RAMPONI, J. LYGEROS. *Stochastic receding horizon control with output feedback and bounded controls*, in "Automatica", 2012, vol. 48, n$^{o}$ 1, p. 77 - 88.

[9] R. PORRECA, E. CINQUEMANI, J. LYGEROS, G. FERRARI-TRECATE. *Invalidation of the structure of genetic network dynamics: A geometric approach*, in "International Journal of Robust and Nonlinear Control", 2012, vol. 22, n$^{o}$ 10, p. 1140–1156.

[10] F. RECHENMANN. *Data modeling: the key to biological data integration*, in "EMBnet.journal", 2012, vol. 18, n$^{o}$ Suppl. B, p. 59-60.

### International Conferences with Proceedings

[11] S. BERTHOUMIEUX, D. KAHN, H. DE JONG, E. CINQUEMANI. *Structural and practical identifiability of approximate metabolic network models*, in "Proceedings of the 16th IFAC symposium on System Identification (SYSID)", Brussels, Belgium, 2012, p. 1719-1724.

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