IN PARTNERSHIP WITH:
Université Joseph Fourier (Grenoble 1)

Activity Report 2011

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

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Project-Team IBIS

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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]

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

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

Sara Berthoumieux, PhD student in IBIS, received the Ian Lawson Van Toch Memorial Award for the best student paper presented at the major bioinformatics conference ISMB/ECCB 2011. The paper of Sara, "Identification of metabolic network models from incomplete high-throughput datasets", has been published in the special ISMB/ECCB issue of *Bioinformatics*. An interview in which she explains her work can be found at http://www.inria.fr/centre/grenoble/actualites/estimation-de-parametres-et-donnees-biologiques-incompletes.

Several members of IBIS were involved in the supervision of the student team from Grenoble that participated in the iGEM 2011 competition. Their project "Mercuro-Coli: A new way to quantify heavy metals", won a gold medal at the iGEM regional jamboree in Amsterdam and qualified for the world jamboree that was held in Boston in December 2011. More information on the iGEM project can be found at http://www.inria.fr/centre/grenoble/actualites/une-bacterie-de-synthese-pour-doser-le-mercure.

Organization by IBIS members of the Workshop on Identification and Control of Biological Interaction Networks, INRIA Grenoble - Rhône-Alpes, February 2011. See http://ibis.inrialpes.fr/article982.html for more information.

3. Scientific Foundations

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

Participants: Sara Berthoumieux, Eugenio Cinquemani, Jérôme Izard, Johannes Geiselmann, Hidde de Jong, Stéphane Pinhal, Delphine Ropers [Correspondent], Valentin Zulkower.

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.

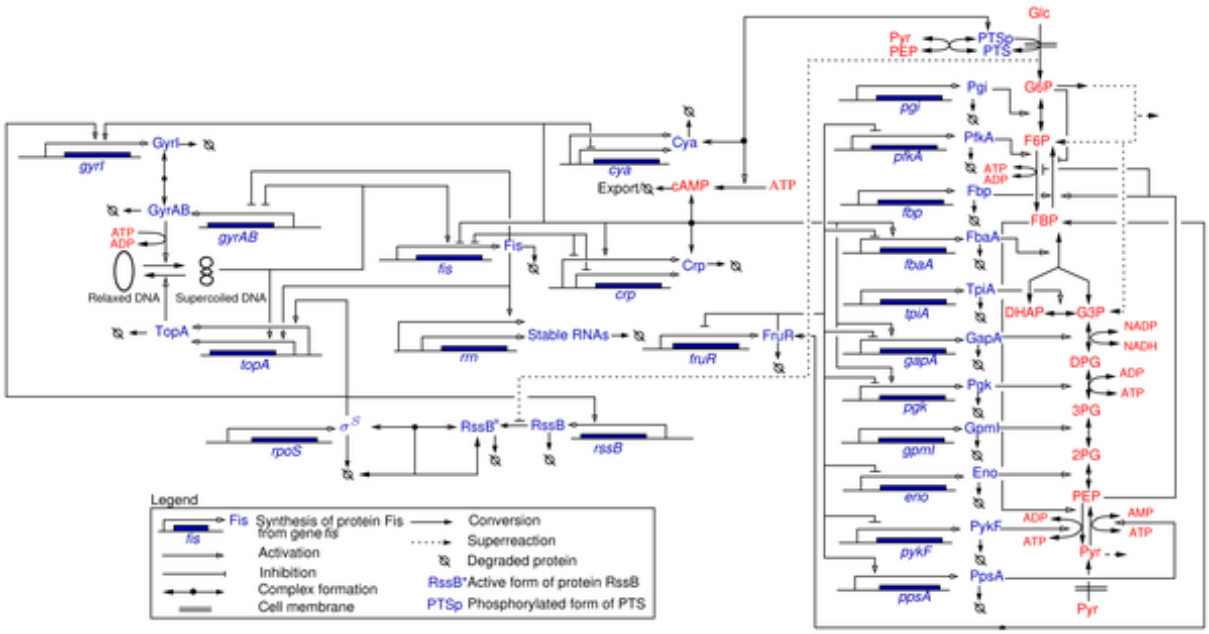


Figure 2. Network of key genes, proteins, and regulatory interactions involved in the carbon assimilation network in E. coli (Baldazzi et al., PLoS Computational Biology, 6(6):e1000812, 2010). The metabolic part includes the glycolysis/gluconeogenesis pathways as well as a simplified description of the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

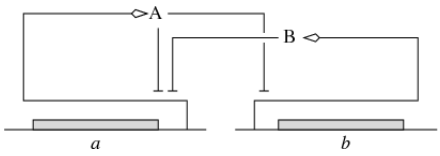
Participants: Sara Berthoumieux, Eugenio Cinquemani, Johannes Geiselmann, Hidde de Jong [Correspondent], Michel Page, François Rechenmann, Delphine Ropers, Diana Stefan, Woei-Fuh Wang, Valentin Zulkower.

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 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 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



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

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 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, Julien Demol, Johannes Geiselmann [Correspondent], Jérôme Izard, Hidde de Jong, Stephan Lacour, Yves Markowicz, Corinne Pinel, Stéphane Pinhal, Delphine Ropers, Claire Villiers.

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.

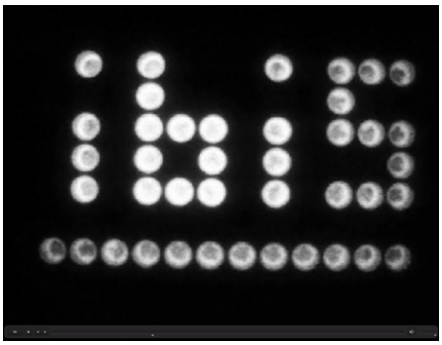


Figure 4. Playful illustration of the principle of reporter genes (see http://ibis.inrialpes.fr for the corresponding movie). A microplate containing a minimal medium (with glucose and acetate) is filmed during 36 hours. Wells contain E. coli bacteria which are transformed with a reporter plasmid containing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

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.

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. Qualitative modeling, simulation, analysis, and verification of gene regulatory networks

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 8.3 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 [15]. 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* (see also [14]). A paper on the use of temporal logic and formal verification in the context of GNA appeared in *Theoretical Computer Science* this year [7], in a special issue associated with the conference Computational Methods in System (CMSB), held in Rostock in 2008.

Notwithstanding the above improvements of the software, most of our efforts in the past year have gone into applications in collaboration with users of GNA. For example, Delphine Ropers has worked with several groups at IST Lisbon on the modeling of the FLR1 network in yeast, resulting in a paper in *IET Systems Biology* [8]. The paper reports on the qualitative modelling and simulation of the transcriptional regulatory network controlling the response of the model eukaryote *Saccharomyces cerevisiae* to the agricultural fungicide mancozeb. The model has allowed the analysis of the regulation level and activity of the components of the mancozeb-induced network controlling the transcriptional activation of FLR1. This gene is proposed to confer multidrug resistance to the cell through its putative role as a drug efflux pump. Formal verification analysis of the network allowed us to confront model predictions with experimental data and to assess the model robustness to parameter ordering and gene deletion. This analysis led to a better understanding of the mechanisms regulating the response of FLR1 to mancozeb and confirmed the need for a new transcription factor to account for the full transcriptional activation of the gene YAP1. The result is a model of the response of FLR1 to mancozeb, permitting a quick and cost-effective test of hypotheses prior to experimental validation.

As another example of the use of GNA, Hidde de Jong has contributed to the modeling of the TOL system in *Pseudomonas putida*, carried out at the Spanish National Biotechnology Center (CNB). The gene regulatory network of the TOL plasmid pWW0 of the soil bacterium *Pseudomonas putida* mt-2 for catabolism of m-xylene is an archetypal model for environmental biodegradation of aromatic pollutants. Although nearly every metabolic and transcriptional component of this regulatory system is known in detail, the complexity of its architecture is still perplexing. To gain an insight into the inner layout of this network a PL model of the TOL system was implemented, simulated and experimentally validated by measuring the expression of the genes encoding the regulators XylR and XylS when specific portions of the network were activated with selected inducers (m-xylene, o-xylene, 3-methylbenzylalcohol and 3-methylbenzoate). This analysis made sense of the specific regulatory topology on the basis of an unprecedented network motif in the genetic circuit for m-xylene catabolism. The motif appears to ensure a simultaneous expression of the upper and lower segments of the m-xylene catabolic route that would be difficult to bring about with a standard substrate-responsive single promoter. Furthermore, it is plausible that the motif helps to avoid biochemical conflicts between competing plasmid-encoded and chromosomally-encoded pathways in this bacterium. The analysis of the TOL system has been published in *BMC Systems Biology* [11].

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 are actually 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 Omaya Dudin and Stephan Lacour, the validation of a model of the network of global regulators of transcription by Sara Berthoumieux and Hidde de Jong, and the analysis of the regulation of cAMP levels in the bacterial cell by Claire Villiers.

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. We previously showed, in a paper published in the *IEEE/ACM Transactions on Computational Biology and Bioinformatics* this year, that PL models provide a good approximation of the direct and indirect interactions occurring in gene regulation [10].

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 short, preliminary paper on this work was presented at an invited session of the 18th IFAC World Congress held in Milano [12] and a long paper has been accepted for publication in the *Journal of Theoretical Biology* [2].

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 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.

On the biological side, we have applied the method to a linlog model of central metabolism in *Escherichia coli*, consisting of some 23 variables. We estimated the 100 parameters of this model from a high-throughput dataset published in the literature. The data consists of measurements of metabolic fluxes and metabolite and enzyme levels in glucose-limited chemostat under 29 different conditions such as wild-type strain and single-gene mutant strains or different dilution rates. Standard linear regression is difficult to apply in this case due to missing data, which disqualifies for 7 reactions too many datapoints, leaving a dataset of size inferior to the number of parameters to estimate. Application of our approach allows one to compute reasonable estimates for most of the identifiable model parameters even when regression is inapplicable. The method and its application to the linlog model of central metabolism in *E. coli* are the subject of a paper accepted for the ISMB/ECCB conference this year and published in a special issue of *Bioinformatics* [3]. Sara Berthoumieux received the Ian Lawson Van Toch Memorial Award for outstanding student paper at ISMB/ECCB. In the continuation of this work, we are currently preparing for submission a journal paper on the identifiability of linlog models.

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 theses of Stéphane Pinhal and Valentin Zulkower, which started at the end of this year, 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 proposed 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 which we refer to as models with unate-like structure. In Boolean network modeling, unate functions are argued to capture virtually all observable interactions in gene 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 simplify the identification task.

As described in previous work, published in *Bioinformatics* in 2010, the key idea is to divide the identification process into two steps. In the first step, different monotonicity properties of different model structures are exploited to discard those structures whose property is falsified by the observed data points (time-lapse protein concentrations and synthesis rates). In the second step, the parameters of the model structures not discarded in the first step are fitted to the data in the search of the simplest structure explaining the data with sufficient accuracy. The procedure was validated on challenging data from the literature.

On the methodological side, in the context of the same international collaboration, the identification approach has been further developed. For important subclasses of unate models, larger sets of network structures can now be discarded in the hypothesis falsification step, based on additional properties other than monotonicity (namely quasi-convexity). These improvements have been presented at the 18th IFAC World Congress held in Milan, and are reported in a journal paper that has been accepted for publication in the *International Journal of Robust and Nonlinear Control*, in a special issue on system identification for biological systems. In the framework of the PhD thesis of Diana Stefan, in collaboration with Eugenio Cinquemani, Stephan Lacour and Omaya Dudin, the method is now being applied to experimental data produced within IBIS for the study of the gene network regulating motility of *E. coli* bacteria.

Woei-Fuh Wang, who defended her PhD thesis carried out under the supervision of Johannes Geiselmann and Chung-Ming Chen in December 2011, addressed a different problem in the structural identification of gene regulatory networks. The inference of the network topology and regulatory mechanisms is complicated by the fact that we usually do not know all relevant genes that need to be taken into account for explaining the observed expression patterns. The aim of the thesis was to detect the presence of such "missing genes", as well as their regulatory roles and expression patterns. Using a well-known class of simplified kinetic models, based on power-law approximations of synthesis rate functions, an inference algorithm was developed. The algorithms are based on factor analysis, a well-developed multivariate statistical analysis approach that is used to investigate unknown, underlying features of a dataset, as well as independent component analysis. The proposed method of inferring the expression profile of a missing gene and connecting it to a known network structure has been applied to artificial networks, as well as a real network studied within IBIS: the *acs* regulatory network in *Escherichia coli*.

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 bacterial populations and even of single cells, with applications in for example biotechnology and medicine.

In the literature, much effort has been devoted to the analysis of stochastic gene expression models derived from biochemical kinetics and specific knowledge of the systems at hand. Less effort has been dedicated to developing general methods for inferring unknown parameter values of these stochastic models from single-cell experimental data. While some strategies have been proposed in the recent literature, no method of general applicability exists. IBIS recently started a new line of research dedicated to the study of stochastic modelling and identification of gene regulatory networks in single cells. This work, coordinated by Eugenio Cinquemani, focuses on simple network modules in bacterial cells. Our reference system is the regulation of the inset of arabinose uptake in *E. coli* upon depletion of glucose.

In the past year we developed a working method for the estimation of unknown network parameters of a simple stochastic model of the arabinose uptake process. The method was tested on simulated data and applied with success to time-lapse fluorescence microscopy data acquired by Guillaume Baptist. This application involved the development by Michel Page of a microscopy data processing program based on a customization of the freely accessible Matlab tool CellTracer. Preliminary results were presented in the poster session of the Conference on Stochastic Systems Biology held in Monte Verità (Switzerland). The work is currently being extended in preparation for a journal publication. A generalization of the method and the investigation of alternative stochastic modelling and identification methodologies are being pursued in parallel. Other ongoing work concerns the study of noise propagation in gene regulatory networks in collaboration with Irina Mihalcescu (Université Joseph Fourier).

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 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. 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.

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. For more information, see http://www.genostar.com.

7. Partnerships and Cooperations

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	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, 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	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
Web page	http://cluster-infectiologie.fr/

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. 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.

7.3. 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.

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, JOBIM 11, MARAMI 11, QR 11
Member Recruitment Committee	INRA Jouy-en-Josas, ingénieur de recherche INRA
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 2011 competition
Member Organization Committee	Workshop on Identification and Control of Biological Interaction Networks, INRIA Grenoble - Rhône-Alpes, February 2011
Project reviews	ANR, Région Acquitaine, Région Ile-de-France, FP7, FRS, CNRS, NSERC

Johannes Geiselmann

Type	Journal, conference, agency
Member PhD and HdR Committee	Dimitar Angelov (ENS Lyon), Ariel Lindner (INSERM U1001, Cochin, Paris), Jacques Coves (Université Joseph Fourier), Khady Sall (iRTSV and Université Joseph Fourier, Grenoble), Li-Chen Zhang (Université de la Méditerrannée, Marseille), Juliette Trepreau (Institute of Structural Biology and Université Joseph Fourier, Grenoble), Damien Goutte-Gattat (Université Joseph Fourier, Grenoble)
Member Selection Committee	Doctoral school ED-CSV, Grenoble
Member Organization Committee	Workshop on synthetic biology, University of Ottawa, Canada
Advisor	Grenoble team for iGEM 2011 competition
Project reviews	ANR, Welcome Trust

Stéphane Pinhal

Type	Journal, conference, agency
Advisor	Grenoble team for iGEM 2011 competition

Delphine Ropers

Type	Journal, conference, agency
Member Organization Committee	SeMoVi (Séminaire de Modélisation du Vivant)
Member Organization Committee	Workshop on Identification and Control of Biological Interaction Networks, INRIA Grenoble - Rhône-Alpes, February 2011
Member evaluation committee	e:Bio - innovation competition systems biology, BMBF (Germany)
Advisor	Grenoble team for iGEM 2011 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. He is also a national representative of the UNSA trade union.

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). 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

Sara Berthoumieux

Title	Event and location	Date
Identification of metabolic network models from incomplete high-throughput datasets	Presentation at ISMB/ECCB 2011, Vienna (Austria)	Jul. 2011

Eugenio Cinquemani

Title	Event and location	Date
Identification of genetic network dynamics : a model invalidation approach	Talk at Workshop on Identification and Control of Biological Interaction Networks, INRIA Grenoble - Rhône-Alpes	Feb. 2011
Identification of genetic network dynamics : a model invalidation approach	Seminar INRIA Sophia-Antipolis - Méditerranée	Mar. 2011
Identification of metabolic network models from incomplete high-throughput datasets	Seminar Automatic Control Lab, ETH Zürich (Switzerland)	May 2011
Regulation of arabinose uptake in Escherichia coli: stochastic modelling and identification using time-lapse fluorescence microscopy data	Poster presentation at Conference on Stochastic Systems Biology, Monte Veritá, Ascona (Switzerland)	Jul. 2011
Learning the structure of genetic network dynamics : A geometric approach	Talk in invited session on Hybrid Systems in Biological Networks at IFAC 2011, Milan (Italy)	Sep. 2011
Identification of approximate metabolic network models from high-throughput data	Seminar Dipartimento di Informatica e Sistemistica, Universita' degli Studi di Pavia (Italy)	Dec. 2011

Hidde de Jong

Title	Event and location	Date
Modélisation mathématique de réseaux de régulation bactériens	Seminar Centre d'Alembert, Université Paris-Sud, Orsay	Jan. 2011
Metabolic coupling in gene regulatory networks in bacteria	Invited talk at Metabolic and network modelling workshop, e-Science Institute, Edinburgh (UK)	Apr. 2011
Metabolic coupling in gene regulatory networks in bacteria	Invited talk at workshop Towards Systems Biology, Grenoble	May 2011
Metabolic coupling in gene regulatory networks in bacteria	Invited talk at Journée Bio-Maths, Université Pierre et Marie Curie and Université Paris Diderot, Jussieu	Jun. 2011
Mathematical modeling of gene regulatory networks in bacteria	Lecture at Summer School on Complex Systems (IXXI and RNSC), Paris	Jul. 2011
Metabolic coupling in gene regulatory networks in bacteria	Invited talk at Mathematical Biology workshop and IGTC Summit, Victoria (Canada)	Jul. 2011
Importance of metabolic coupling for the dynamics of gene expression	Talk in invited session on Hybrid Systems in Biological Networks at IFAC 2011, Milan (Italy)	Sep. 2011
Modeling of gene regulatory networks in bacteria	Invited talk at Journées Atip-Avenir (CNRS/INSERM), Roscoff	Oct. 2011
Mathematical modeling of gene regulatory networks in bacteria	Seminar University of Twente (the Netherlands)	Nov. 2011

Johannes Geiselmann

Title	Event and location	Date
The stress regulon of Escherichia coli	Seminar at University of Milano (Italy)	Mar. 2011
Experimental analysis and modeling of bacterial regulatory networks	Seminar at University of Geneva (Switzerland)	May 2011
Synthetic biology in Escherichia coli	Talk at Workshop on synthetic biology, University of Ottawa (Canada)	Oct. 2011

Stephan Lacour

Title	Event and location	Date
Study of the RpoS-Crl regulon in Escherichia coli using transcriptional fusions	Poster presentation at 4th FEMS Congress of European Microbiologists, Geneva (Switzerland)	Jun. 2011

Delphine Ropers

Title	Event and location	Date
Des mathématiques pleines de vie	Presentation at Olympiades de Mathématiques 2011, Lycée Roger Deschaux, Sassenage	Dec. 2011

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.

Delphine Ropers gave a presentation on mathematical modeling in the life sciences at the prize award ceremony of the Olympiades de Mathématiques 2011.

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 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.

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	8.5

Diana Stefan

Subject	Year	Location	Hours
Automatique et traitement du signal	2	INPG Phelma	33

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

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