IN PARTNERSHIP WITH:
Université Joseph Fourier (Grenoble)

Activity Report 2014

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

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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 Interdisciplinaire de Physique (CNRS UMR 5588) in Saint Martin d'Hères.

Creation of the Project-Team: 2009 January 01.

1. Members

Research Scientists

Eugenio Cinquemani [Researcher, Inria]
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François Rechenmann [Senior researcher, Inria (50%) and CEO Genostar (50%), until December 2014 (retirement), HdR]
Delphine Ropers [Researcher, Inria]

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Stéphan Lacour [Assistant professor, Université Joseph Fourier]
Yves Markowicz [Associate professor, Université Joseph Fourier]
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Engineer

Corinne Pinel [Technician, CNRS (80%) and BGene (20%)]

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Stéphane Pinhal [PhD student, Université Joseph Fourier, Grenoble, supervisors: Johannes Geiselmann, Delphine Ropers, and Hidde de Jong]
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Cindy Gomez Balderas-Barillot [Post-doctoral researcher, CNRS]

Visiting Scientists

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Antoine Charpentier [BSc student, from April until May 2014, Université Joseph Fourier, supervisor: Nils Giordano]
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2. Overall Objectives

2.1. Overview

When confronted with changing environmental conditions, bacteria and other microorganisms have a remarkable capacity to adapt their functioning. The responses of bacteria to changes in their environment are controlled on the molecular level by large and complex networks of biochemical interactions involving genes, mRNAs, proteins, and metabolites. The study of bacterial regulatory networks requires experimental tools for mapping the interaction structure of the networks and measuring the dynamics of cellular processes. In addition, when dealing with such large and complex systems, 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 project-team is to apply such a systems-biology approach to gain a deeper understanding, on the mechanistic level, of the strategies that bacteria have developed to respond to changes in their environment. [1] In particular, we focus on the enterobacterium *Escherichia coli*, for which enormous amounts of genomic, genetic, biochemical and physiological data have accumulated over the past decades. A better understanding of the adaptive capabilities of *E. coli* to nutritional limitations or other environmental changes is an aim in itself, but also a necessary prerequisite for the second and most ambitious aim of the project: interfering with the cellular responses by specific perturbations or by rewiring the underlying regulatory networks. This 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 new problems on the interface of biology, applied mathematics, and computer science. In particular, the following objectives have structured the work of the project-team: (1) the analysis of the qualitative dynamics of gene regulatory networks, (2) the inference of gene regulatory networks from time-series data, (3) the analysis of integrated metabolic and regulatory networks, and (4) natural and engineered control of regulatory networks. Although these axes cover most of the work carried out in IBIS, some members have maintained a research activity in their domain of origin (automatic control, molecular biology of HIV, immunology, ...) or made a contribution to research projects on different topics (human cancer cell genomics, plant modeling, ...). Since this usually represents a minor proportion of the overall research effort of the project-team, we will not describe this work in detail in the activity report. The publications resulting from these side-tracks have been included in the bibliography.

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. (Figure 1) 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

[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.

group of Johannes Geiselmann, formerly at the Laboratoire Adaptation et Pathogénicité des Microorganismes of the Université Joseph Fourier (UJF, CNRS UMR 5163), and since September 2014 at the Laboratoire Interdisciplinaire de Physique (UJF, CNRS UMR 5588), and the members of 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 60 peer-reviewed publications in journals, conferences, and books since 2000. [2]

Hidde de Jong is the head of the IBIS project-team and Johannes Geiselmann is the co-director. The experimental component of IBIS is also part of the Laboratoire Interdisciplinaire de Physique, and Johannes Geiselmann continues to represent this group in the interactions with the laboratory and university administration.

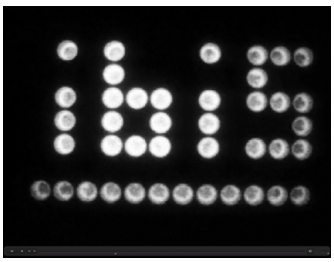


Figure 1. Display of the project-team name on a "bacterial billboard" (see http://ibis.inrialpes.fr for the corresponding movie). A microplate containing a minimal medium (with glucose and acetate) is filmed during 36 hours. Wells contain E. coli bacteria which are transformed with a reporter plasmid 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 ($FMNH_2$) 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.

3. Research Program

3.1. Analysis of qualitative dynamics of gene regulatory networks

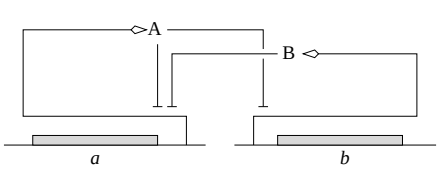
Participants: Hidde de Jong [Correspondent], Michel Page.

The dynamics of gene regulatory networks can be modeled by means of ordinary differential equations (ODEs), describing the rate of synthesis and degradation of the gene products as well as regulatory interactions between gene products and metabolites. In practice, such models are not easy to construct though, as the parameters are often only constrained to within a range spanning several orders of magnitude for most systems of biological interest. Moreover, the models usually consist of a large number of variables, are strongly nonlinear, and include different time-scales, which makes them difficult to handle both mathematically and computationally. This has motivated the interest in qualitative models which, from incomplete knowledge of the system, are able to provide a coarse-grained picture of its dynamics.

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

A variety of qualitative modeling formalisms have been introduced over the past decades. Boolean or logical models, which describe gene regulatory and signalling networks as discrete-time finite-state transition systems, are probably most widely used. The dynamics of these systems are governed by logical functions representing the regulatory interactions between the genes and other components of the system. IBIS has focused on a related, hybrid formalism that embeds the logical functions describing regulatory interactions into an ODE formalism, giving rise to so-called piecewise-linear differential equations (PLDEs, Figure 2). The use of logical functions allows the qualitative dynamics of the PLDE models to be analyzed, even in high-dimensional systems. In particular, the qualitative dynamics can be represented by means of a so-called state transition graph, where the states correspond to (hyperrectangular) regions in the state space and transitions between states arise from solutions entering one region from another.

First proposed by Leon Glass and Stuart Kauffman in the early seventies, the mathematical analysis of PLDE models has been the subject of active research for more than four decades. IBIS has made contributions on the mathematical level, in collaboration with the BIOCORE and BIPOP project-teams, notably for solving problems induced by discontinuities in the dynamics of the system at the boundaries between regions, where the logical functions may abruptly switch from one discrete value to another, corresponding to the (in)activation of a gene. In addition, many efforts have gone into the development of the computer tool GENETIC NETWORK ANALYZER (GNA) and its applications to the analysis of the qualitative dynamics of a variety of regulatory networks in microorganisms. Some of the methodological work underlying GNA, notably the development of analysis tools based on temporal logics and model checking, which was carried out with the Inria project-teams CONVEX (ex-VASY) and POP-ART, has implications beyond PLDE models as they apply to logical and other qualitative models as well.



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

3.2. Inference of gene regulatory networks from time-series data

Participants: Eugenio Cinquemani [Correspondent], Johannes Geiselmann, Hidde de Jong, Julien Demol, Stéphan Lacour, Michel Page, Corinne Pinel, Delphine Ropers, Alberto Soria-Lopéz, Diana Stefan, Valentin Zulkower.

Measurements of the transcriptome of a bacterial cell by means of DNA microarrays, RNA sequencing, and other technologies have yielded huge amounts of data on the state of the transcriptional program in different growth conditions and genetic backgrounds, across different time-points in an experiment. The information on the time-varying state of the cell thus obtained has fueled the development of methods for inferring regulatory interactions between genes. In essence, these methods try to explain the observed variation in the activity of one gene in terms of the variation in activity of other genes. A large number of inference methods have been

proposed in the literature and have been successful in a variety of applications, although a number of difficult problems remain.

Current reporter gene technologies, based on Green Fluorescent Proteins (GFPs) and other fluorescent and luminescent reporter proteins, provide an excellent means to measure the activity of a gene *in vivo* and in real time (Figure 3). The underlying principle of the technology is to fuse the promoter region and possibly (part of) the coding region of a gene of interest to a reporter gene. The expression of the reporter gene generates a visible signal (fluorescence or luminescence) that is easy to capture and reflects the expression of a gene of interest. The interest of the reporter systems is further enhanced when they are applied in mutant strains or combined with expression vectors that allow the controlled induction of any particular gene, or the degradation of its product, at a precise moment during the time-course of the experiment. This makes it possible to perturb the network dynamics in a variety of ways, thus obtaining precious information for network inference.

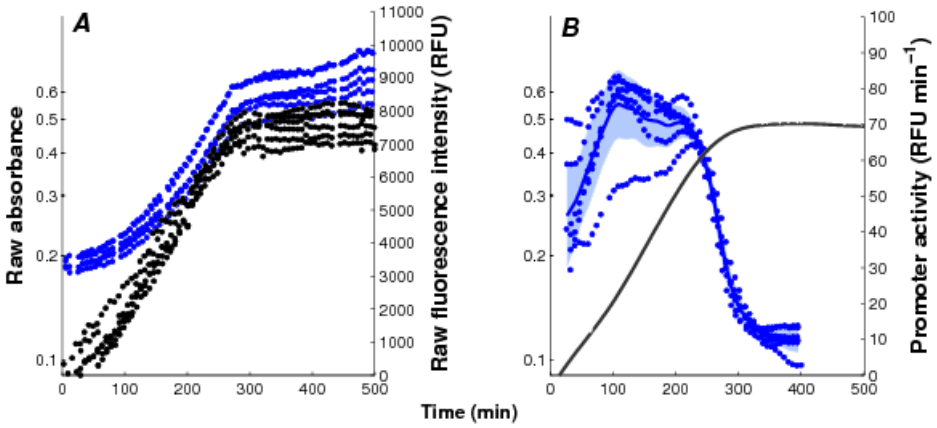


Figure 3. Monitoring of bacterial gene expression in vivo using fluorescent reporter genes (Stefan et al., PLoS Computational Biology, 11(1):e1004028, 2015). The plots show the primary data obtained in a kinetic experiment with E. coli cells, focusing on the expression of the motility gene tar in a mutant background. A: Absorbance (•, black) and fluorescence (•, blue) data, corrected for background intensities, obtained with the ΔcpxR strain transformed with the ptar-gfp reporter plasmid and grown in M9 with glucose. B: Activity of the tar promoter, computed from the primary data. The solid black line corresponds to the mean of 6 replicate absorbance measurements and the shaded blue region to the mean of the promoter activities ± twice the standard error of the mean.

The specific niche of IBIS in the field of network inference has been the development and application of genome engineering techniques for constructing the reporter and perturbation systems described above, as well as the use of reporter gene data for the reconstruction of gene regulation functions. We have developed an experimental pipeline that resolves most technical difficulties in the generation of reproducible time-series measurements on the population level. The pipeline comes with data analysis software that converts the primary data into measurements of time-varying promoter activities (Section 4.2). In addition, for measuring gene expression on the single-cell level by means of microfluidics and time-lapse fluorescence microscopy, we have established collaborations with groups in Grenoble and Paris. The data thus obtained can be exploited for the structural and parametric identification of gene regulatory networks, for which methods with a solid mathematical foundation are developed, in collaboration with colleagues at ETH Zürich (Switzerland) and the University of Pavia (Italy). The vertical integration of the network inference process, from the construction

of the biological material to the data analysis and inference methods, has the advantage that it allows the experimental design to be precisely tuned to the identification requirements.

3.3. Analysis of integrated metabolic and gene regulatory networks

Participants: Eugenio Cinquemani, Hidde de Jong, Johannes Geiselmann, Stéphan Lacour, Yves Markowicz, Manon Morin, Michel Page, Corinne Pinel, Stéphane Pinhal, Delphine Ropers [Correspondent], Diana Stefan, Valentin Zulkower.

The response of bacteria to changes in their environment involves responses on several different levels, from the redistribution of metabolic fluxes and the adjustment of metabolic pools to changes in gene expression. In order to fully understand the mechanisms driving the adaptive response of bacteria, as mentioned above, we need to analyze the interactions between metabolism and gene expression. While often studied in isolation, gene regulatory networks and metabolic networks are closely intertwined. Genes code for enzymes which control metabolic fluxes, while the accumulation or depletion of metabolites may affect the activity of transcription factors and thus the expression of enzyme-encoding genes.

The fundamental principles underlying the interactions between gene expressions and metabolism are far from being understood today. From a biological point of view, the problem is quite challenging, as metabolism and gene expression are dynamic processes evolving on different time-scales and governed by different types of kinetics. Moreover, gene expression and metabolism are measured by different experimental methods generating heterogeneous, and often noisy and incomplete data sets. From a modeling point of view, difficult methodological problems concerned with the reduction and calibration of complex nonlinear models need to be addressed.

Most of the work carried out within the IBIS project-team specifically addressed the analysis of integrated metabolic and gene regulatory networks in the context of *E. coli* carbon metabolism (Figure 4). While an enormous amount of data has accumulated on this model system, the complexity of the regulatory mechanisms and the difficulty to precisely control experimental conditions during growth transitions leave many essential questions open, such as the physiological role and the relative importance of mechanisms on different levels of regulation (transcription factors, metabolic effectors, global physiological parameters, ...). We are interested in the elaboration of novel biological concepts and accompanying mathematical methods to grasp the nature of the interactions between metabolism and gene expression, and thus better understand the overall functioning of the system. Moreover, we have worked on the development of methods for solving what is probably the hardest problem when quantifying the interactions between metabolism and gene expression: the estimation of parameters from hetereogeneous and noisy high-throughput data. These problems are tackled in collaboration with experimental groups at Inra/INSA Toulouse and CEA Grenoble, which have complementary experimental competences (proteomics, metabolomics) and biological expertise.

3.4. Natural and engineered control of regulatory networks

Participants: Cindy Gomez Balderas-Barillot, Eugenio Cinquemani, Johannes Geiselmann [Correspondent], Edith Grac, Nils Giordano, Hidde de Jong, Stéphan Lacour, Delphine Ropers, Alberto Soria-Lopéz.

In the previously-described objectives, we have focused on identifying complex regulatory networks and gaining a better understanding of how the network dynamics underlies the observable behavior of the cell. Based on the insights thus obtained, a complementary perspective consists in changing the functioning of a bacterial cell towards a user-defined objective, by rewiring and selectively perturbing its regulatory networks. The question how regulatory networks in microorganisms can be externally controlled using engineering approaches has a long history in biotechnology and is receiving much attention in the emerging field of synthetic biology.

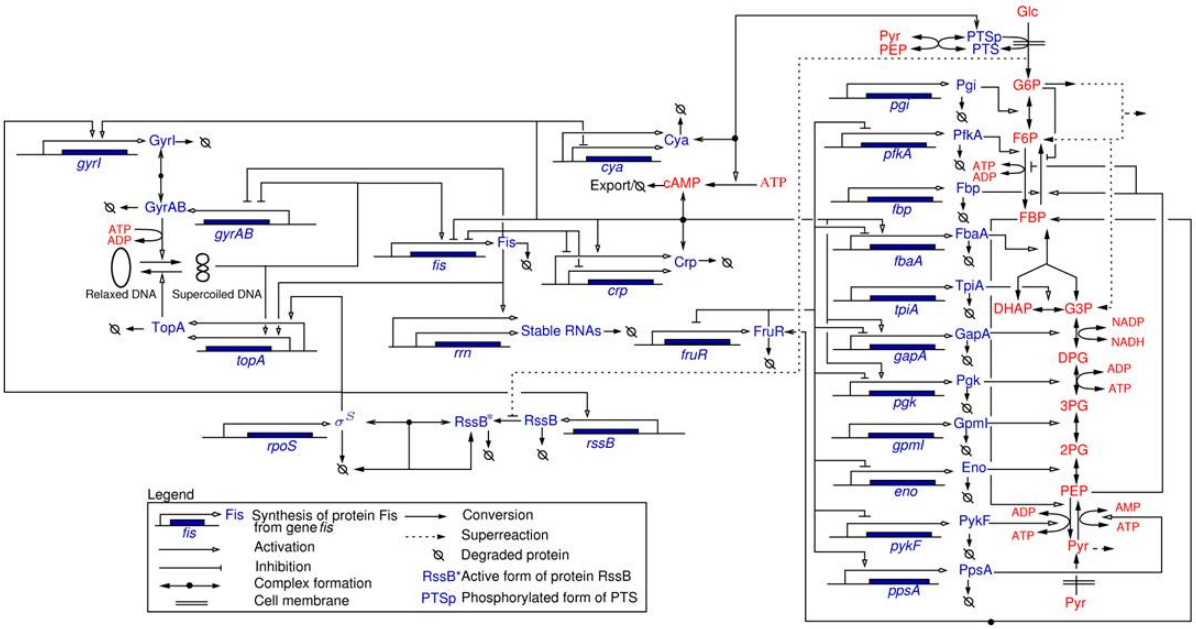


Figure 4. Network of key genes, proteins, and regulatory interactions involved in the carbon assimilation network in E. coli (Baldazzi et al., PLoS Computational Biology, 6(6):e1000812, 2010). The metabolic part includes the glycolysis/gluconeogenesis pathways as well as a simplified description of the 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.

Within a number of on-going projects, IBIS is focusing on two different questions. The first concerns the development of growth-rate controllers of bacterial cells. Since the growth rate is the most important physiological parameter in microorganisms, a better understanding of the molecular basis of growth-rate control and the engineering of open-loop and closed-loop growth-rate controllers is of major interest for both fundamental research and biotechnological applications. Second, we are working on the development of methods with a solid foundation in control theory for the real-time control of gene expression. These methods are obviously capital for the above-mentioned design of growth-rate controllers, but they have also been applied in the context of a platform for real-time control of gene expression in cell population and single cells, developed by the Inria project-team CONTRAINTES, in collaboration with a biophysics group at Université Paris Descartes.

4. New Software and Platforms

4.1. Genetic Network Analyzer (GNA)

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

Keywords. Gene regulatory networks, qualitative simulation, model checking

GENETIC NETWORK ANALYZER (GNA) is the implementation of methods 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 (PLDEs), 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 8.7. In comparison with the previously distributed versions, GNA 8.7 has the following additional functionalities: (1) it supports the editing and visualization of regulatory networks, in an SBGN-compatible format, (2) it semi-automatically generates a prototype model from the network structure, thus accelerating the modeling process, and (3) it allows models to be exported in the SBML Qual standard. For more information, see http://www-helix.inrialpes.fr/gna.

4.2. WellReader, WellFARE, and WellInverter

Participants: Johannes Geiselmann, Hidde de Jong [Correspondent], Michel Page, Delphine Ropers, Valentin Zulkower.

Keywords. 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.

In the past year, we developed novel approaches towards the analysis of reporter gene data, based on regularized linear inversion (Section 5.3). The linear inversion methods were implemented in the Python package WELLFARE, relying on the scientific Python libraries NumPy and SciPy. In addition, the package provides utilities for parsing data files and removing possible outliers from the absorbance and fluorescence signals. The WELLFARE package is available under an LGPL license, but has also been integrated into a web application called WELLINVERTER, which provides a graphical user interface allowing access to the linear inversion methods through a web browser (Figure 5). The user can upload data files by means of WELLINVERTER, remove outliers and subtract background, and launch the procedures for computing growth rates, promoter activities, and protein concentrations. For more information, see: http://ibis.inrialpes.fr/article1080.html?menu=menu4.

5. New Results

5.1. Highlights of the Year

A paper based on the PhD thesis of Diana Stefan was accepted for *PLoS Computational Biology* this year [7].

5.2. 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 PLDE models, as described in Section 4.1. GNA has been integrated with the other bioinformatics tools distributed by Genostar (http://www.genostar.com/). Version 8.7 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. Version 8.7 supports the SBML standard and is also capable of exporting its models to the newly-developed standard for qualitative models, SBML Qual. This standard has been elaborated by the community of developers of logical and related modeling tools (CoLoMoTo), in which the GNA developers participate.

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 PLDE 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 PLDE models (Section 3.1). This has motivated extensions of the PLDE 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 non-smooth mechanical, electrical and control systems can be adapted for this purpose, in a paper published in *Physica D* [1] and presented at the 21st International Symposium on Mathematical Theory of Networks and Systems (MTNS 2014) [12]. We have presented a method for the numerical analysis of one proposed extension, called Aizerman–Pyatnitskii (AP)-extension, by reformulating the PLDE models as mixed complementarity systems (MCSs). This allows the application of powerful methods developed for this class of nonsmooth dynamical systems, in particular those implemented in the SICONOS platform developed by BIPOP. We have also shown that under a set of reasonable biological assumptions, putting constraints on the right-hand side of the PLDE models, AP-extensions and classical Filippov extensions are equivalent. This means that the proposed numerical method is valid for a range of different solution concepts. We have illustrated the practical interest of our approach through the numerical analysis of three well-known networks developed in the field of synthetic biology.

5.3. Inference of bacterial regulatory networks from reporter gene data

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 3.2). 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 out of the information contained in reporter gene expression data.

Valentin Zulkower, in the framework of his PhD thesis, has developed novel methods for the analysis of reporter gene data, based on the use of regularized linear inversion. This allows a range of estimation problems in the analysis of reporter gene data, notably the inference of growth rate, promoter activity, and protein concentration profiles, to be solved in a mathematically sound and practical manner. We have evaluated the validity of the approach using *in-silico* simulation studies, and observed that the methods are more robust and less biased than indirect approaches usually encountered in the experimental literature based on smoothing and subsequent processing of the primary data, like in WELLREADER. We have applied the methods to the analysis of fluorescent reporter gene data acquired in kinetic experiments with *Escherichia coli*. The methods were shown capable of reliably reconstructing time-course profiles of growth rate, promoter activity, and protein concentration from weak and noisy signals at low population volumes. Moreover, they captured critical features of those profiles, notably rapid changes in gene expression during growth transitions. The linear inversion methods have been implemented in the Python package WELLFARE, and integrated by Michel Page in the web application WELLINVERTER (Section 4.2). This work was submitted for publication early 2015.

The above tools have been used in a series of studies directed at the experimental mapping of gene regulatory networks in *E. coli*. A first example is a study, led by Stéphan Lacour in collaboration with Akira Ishihama and Hiroshi Ogasawara in Japan, on the lifestyle adaptation of *E. coli*. The study concerns the switch between swimming motility and biofilm formation in response to changes in environmental growth conditions. The stationary phase sigma factor RpoS is an important regulator of this switch since it stimulates adhesion and represses flagellar biosynthesis. By measuring the dynamics of gene expression, we show that RpoS inhibits the transcription of the flagellar sigma factor, FliA, in exponential growth phase. RpoS also partially controls the expression of CsgD and CpxR, two transcription factors important for bacterial adhesion. We have demonstrated that these two regulators repress the transcription of *fliA*, *flgM* and *tar*, and that this regulation is dependent on the growth medium. CsgD binds to the flgM and fliA promoters around their -10 promoter element, strongly suggesting direct repression. The results show that CsgD and CpxR also affect the expression of other known modulators of cell motility. An updated structure of the regulatory network controlling the choice between adhesion and motility was proposed in the paper based on this work, published in the *Journal of Bacteriology* [2]. Stéphan Lacour also reviewed this and other work on RpoS in a publication in *Environmental Microbiology Reports* [4].

A second example derives from the PhD thesis of Diana Stefan. Although from a biological point of view the motility network of *E. coli* is also central in this work, its main thrust lies in clarifying and solving methodological issues in the automated inference of quantitative models of gene regulatory networks from time-series gene expression data, also called reverse engineering in the bioinformatics literature. The application of existing reverse engineering methods is commonly based on implicit assumptions on the biological processes under study. First, the measurements of mRNA abundance obtained in transcriptomics experiments are taken to be representative of protein concentrations. Second, the observed changes in gene expression are assumed to be solely due to transcription factors and other specific regulators, while changes in the activity of the gene expression machinery and other global physiological effects are neglected. While convenient in practice, these assumptions are often not valid and bias the reverse engineering process. In her PhD thesis, Diana Stefan systematically investigated, using a combination of models and experiments, the

importance of this bias and possible corrections. She measured with the help of fluorescent reporter genes the activity of genes involved in the FliA-FlgM module of the *E. coli* motility network. From these data, protein concentrations and global physiological effects were estimated by means of kinetic models of gene expression. The results indicate that correcting for the bias of commonly-made assumptions improves the quality of the models inferred from the data. Moreover, it was shown by simulation that these improvements are expected to be even stronger for systems in which protein concentrations have longer half-lives and the activity of the gene expression machinery varies more strongly across conditions than in the FliA-FlgM module. The approach proposed in this study is broadly applicable when using time-series transcriptome data to learn about the structure and dynamics of regulatory networks. The paper describing the work was published in *PLoS Computational Biology* [7].

5.4. Models of carbon metabolism in bacteria

All free-living bacteria have to adapt to a changing environment. Specific regulatory systems respond to particular stresses, but the most common decision bacteria have to make is the choice between alternative carbon sources, each sustaining a specific, maximal growth rate. Many bacteria have evolved a strategy that consists in utilizing carbon sources sequentially, in general favouring carbon sources that sustain a higher growth rate. As long as a preferred carbon source is present in sufficient amounts, the synthesis of enzymes necessary for the uptake and metabolism of less favourable carbon sources is repressed. This phenomenon is called Carbon Catabolite Repression (CCR) and the most salient manifestation of this regulatory choice is diauxic growth, a phenomenon discovered by Jacques Monod more than 70 years ago. Although this system is one of the paradigms of the regulation of gene expression in bacteria, the underlying mechanisms remain controversial. Carbon catabolite repression involves the coordination of different subsystems of the cell - responsible for the uptake of carbon sources, their breakdown for the production of energy and precursors, and the conversion of the latter to biomass.

The complexity of this integrated system, with regulatory mechanisms cutting across metabolism, gene expression, signaling and subject to global physical and physiological constraints, has motivated important modeling efforts over the past four decades, especially in the enterobacterium *Escherichia coli*. Different hypotheses concerning the dynamic functioning of the system have been explored by a variety of modeling approaches. In an article in *Trends in Microbiology* [3], which was initiated during the sabbatical of Andreas Kremling in Grenoble in 2013, we have reviewed these studies and summarized their contributions to the quantitative understanding of carbon catabolite repression, focusing on diauxic growth in E. coli. Moreover, we have proposed a highly simplified representation of diauxic growth that makes it possible to bring out the salient features of the models proposed in the literature and confront and compare the explanations they provide.

A bottleneck in the development of dynamic and quantitatively predictive models of bacterial metabolism, explicitly accounting for the different regulatory mechanisms on the molecular level, is information on the kinetic parameters describing the enzymatic reactions and other molecular interactions. One particularly important piece of information is knowledge of enzyme concentrations. Recent technological advances in quantitative proteomics have made mass spectrometry-based quantitative assays an interesting alternative to more traditional immuno- affinity based approaches for quantifying enzyme concentrations. In particular, these advances have improved specificity and multiplexing capabilities. In a study carried out at CEA Grenoble, a quantification workflow to analyze enzymes involved in central metabolism in *E. coli* was developed. This workflow combined full-length isotopically labeled standards with selected reaction monitoring analysis. The workflow was used to accurately quantify 22 enzymes involved in *E. coli* central metabolism in a wild-type reference strain and two derived strains, optimized for higher NADPH production. Delphine Ropers and Hidde de Jong participated in the analysis of these data. In combination with measurements of metabolic fluxes, we showed that proteomics data can be used to assess different levels of regulation, in particular enzyme abundance and catalytic rate. This is key to the development of predictive kinetic models, but also provides information that can be used for strain design in biotechnology. An article based on this work was published in *Molecular and Cellular Proteomics* [8].

Other ongoing work on the analysis of bacterial metabolism is carried out by Delphine Ropers in collaboration with Inra/INSA in Toulouse, in the framework of the PhD thesis of Manon Morin, supported by a Contrat Jeune Scientifique Inra-Inria. In their respective PhD theses, Stéphane Pinhal and Valentin Zulkower also study specific aspects of carbon metabolism, using both models and experimental data.

5.5. Stochastic modeling and identification of gene regulatory networks in bacteria

At the single-cell level, the processes that govern single-cell dynamics in general and gene expression in particular are 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 ranges from a better comprehension of the biochemical regulatory mechanisms underlying cellular phenotypes to the development of new strategies for the (computer assisted or genetically synthesized) control of cell populations and even of single cells.

Work in IBIS on the probabilistic gene expression and interaction dynamics at the level of individual cells is addressed in terms of identification of intrinsic noise models from population snapshot data, on the one hand, and the inference of models focusing on cellular variability within isogenic populations from individual cell fluorescence microscopy gene expression profiles, on the other hand. Along with modelling and inference comes analysis of the inferred models in various respects, notably in terms of single-cell state estimation and control. Other problems related with single-cell modelling and extracellular variability are considered in high-eukariotic cells through external collaborations.

In the context of yeast cell response to osmotic shocks, in collaboration with the CONTRAINTES project-team, and colleagues from Université Paris Descartes and University of Pavia (Italy), Eugenio Cinquemani has investigated the use of mixed effects-modelling and identification techniques to characterize individual cell dynamics in isogenic cell populations. Mixed-effects models are hierarchical models where parametric response profiles of individuals is subject to inter-individual parameter variability following a common population distribution. Starting from identification approaches in pharmacokinetics, we have developed and applied inference methods to the context of microfluidics data, with focus on the budding yeast response to osmotic shocks. First results presented at conference in 2013 have been taken further, both in terms of mathematical analysis of the models developed and in terms of biological interpretation. Model identification and validation were performed together with Andres Gonzales, PhD student at the University of Pavia, who has visited IBIS for six months in 2014. A journal publication is currently being prepared for publication.

In a second line of work, starting from the models inferred in the above collaboration, the problem of real-time state estimation and control of single yeast cells has been considered. Together with the BIOCORE project-team, we have put in place algorithms for state estimation in presence of hybrid random switching and continuous dynamics, and integrated them with a feedback control approach developed by collaborators at TU Delft (the Netherlands). The whole monitoring, estimation and control chain has been deployed and applied *in silico* to the stochastic control of osmosensitive genes in single yeast cells. Methods and results have been presented at the 12th international conference on Computational Methods for Systems Biology (CMSB 2014), whose proceedings have been published as a volume of the LNCS series [14]. It is shown in particular that stochastic model-based estimation and control outperforms existing methods of single-cell control based on deterministic approximations.

Additional work on identification and estimation of hidden states for intrinsic noise models of gene expression/regulation in single bacterial cells, started with reference to arabinose uptake dynamics but also applicable to other regulatory networks in *E. coli*, is being developed. In parallel, collaboration of Eugenio Cinquemani with Marianna Rapsomaniki, PhD student affiliated with the University of Patras (Greece) and ETH Zürich (Switzerland), has been devoted to the analysis of data from Fluorescence Recovery After Photobleaching (FRAP) experiments and the inference of kinetic parameters of protein dynamics in single high-eukariotic cells. As an alternative to current approximate analytical methods, we have explored inference methods based

on simulation of biological processes in realistic environments at a particle level. We introduced and demonstrated a new method for the inference of kinetic parameters of protein dynamics, where a limited number of *in-silico* FRAP experiments is used to construct a mapping from FRAP recovery curves to the parameters sought. Parameter estimates from experimental data are then computed by applying the mapping to the observed recovery curves, at virtually no additional price for any number of experiments, along with the application of a bootstrap procedure for determining identifiability of the parameters and confidence intervals for their estimates. After validation on synthetic data, the method was successfully applied to the analysis of the nuclear proteins Cdt1, PCNA and GFPnls in mammalian cells, also shedding light on cell-to-cell variability of the protein kinetics. Method and results have recently been published in *Bioinformatics* [6].

5.6. Growth control in bacteria and biotechnological applications

A bacterial cell adapts its growth rate and the level of gene expression required to sustain growth to the environment, notably to the availability of nutrients providing the molecular building blocks and the energy required for growth. This adaptive response involves the global physiological state of the cell, in particular the activity of the gene expression machinery, and 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. There is a huge literature on the molecular mechanisms coupling the activity of the gene expression machinery to changes in the nutritional quality of the environment, but a quantitative and dynamic picture of this very complicated regulatory system is still missing. Delphine Ropers and Edith Grac as well as Nils Giordano are developing models to achieve this, from bottom-up and top-down perspectives, respectively.

The quantitative models adopting the bottom-up pespective describe the molecular mechanisms controlling the activity of the gene expression machinery. The calibration and analysis of these models is made difficult by their complexity, the nonidentifiability of many parameter values, and the heterogeneity of experimental data sources. To overcome these difficulties, Delphine Ropers and Edith Grac are developing model ensembles with the same structure but different parameter values that are consistent with the experimental data. In collaboration with Jean-Luc Gouzé and Ismail Belgacem from the BIOCORE project-team at Inria Sophia-Antipolis-Méditerranée, they have analysed the dynamical behavior of a central module of these models, which controls the cellular concentration of the RNA polymerase, the key player of the transcriptional machinery. By means of model reduction approaches and monotone system theory, they have analyzed the equilibria of the system and their stability, which they could relate to biological observations on *E. coli*. This work has been published in the proceedings of the 21st International Symposium on Mathematical Theory of Networks and Systems (MTNS 2014) [9] and the 53rd IEEE Conference on Decision and Control (CDC 2014) [10]. A journal article is in preparation.

In the context of the PhD thesis of former IBIS member Jérôme Izard, we have studied the relation between the gene expression machinery, the global physiology of the cell, and the growth rate from a different perspective. Our aim was to change the mechanisms regulating the activity of the gene expression machinery in such a way so as to be able to externally control the growth rate of the cell. More precisely, we have engineered an *E. coli* strain in which the transcription of an essential component of the global gene expression machinery is under the tight control of an inducible promoter. By adjusting the inducer concentration in the medium we can adjust the activity of the gene expression machinery and thereby reversibly switch the growth rate of the bacterium between zero and the maximal growth rate. Our modified *E. coli* strain, described in a paper prepared for submission, opens new perspectives for studying the mechanisms of growth control as well as for developing biotechnological applications, the subject of the post-doctoral fellowship of Cindy Gomez Balderas-Barillot. We have submitted a patent proposing such applications, which underlies the technology transfer activities undertaken in the recently-started Reset project (Section 7.1).

6. Bilateral Contracts and Grants with Industry

6.1. Genostar

Participants: François Rechenmann, Hidde de Jong, Michel Page.

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 CEO of the company. For more information, see http://www.genostar.com.

6.2. BGene

Participants: Johannes Geiselmann, Hidde de Jong, Corinne Pinel.

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 appropriate 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). Corinne Pinel works part-time at BGene, and Johannes Geiselmann and Hidde de Jong are members of its scientific advisory board. For more information on BGene, see http://www.bgene-genetics.com/.

7. Partnerships and Cooperations

7.1. National initiatives

Project name	AlgeaInSilico: Prédire et optimiser la productivité des microalgues en fonction de leur milieu de croissance
Coordinator	O. Bernard
IBIS participants	H. de Jong
Type	Inria Project Lab (2013-)

Project name	GeMCo – Model reduction, experimental validation, and control for the gene expression machinery in *E. coli*
Coordinator	M. Chaves
IBIS participants	E. Cinquemani, J. Geiselmann, C. Gomez Balderas-Barillot, E. Grac, H. de Jong, S. Lacour, C. Pinel, D. Ropers
Type	ANR Blanc (2010-2014)
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	E. Cinquemani, J. Geiselmann, C. Gomez Balderas-Barillot, E. Grac, H. de Jong, S. Lacour, Y. Markowicz, C. Pinel, D. Ropers
Type	Bioinformatics call, Investissements d'Avenir program (2012-2016)
Web page	https://project.inria.fr/reset/

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

7.2. 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. Andres Gonzales-Vargas, PhD student of Giancarlo Ferrari-Trecate, will spend six months in IBIS in 2014.

7.3. International research visitors

Visiting PhD student	Andres Gonzalez Vargas (University of Pavia, Italy)
Subject	Stochastic modeling and identification of regulatory networks

Invited professor	Alberto Soria-Lopéz (Centro de Investigación y de Estudios Avanzados (Cinestav) of Instituto Politécnico Nacional (IPN), Mexico)
Subject	Development of an automatically-controlled system of multiplexed mini-bioreactors

8. Dissemination

8.1. Editorial, animation, and reviewing activities

Eugenio Cinquemani

Type	Journal, conference, agency
Associate editor	European Control Conference (ECC) 2015
Member PhD committee	Marianna Rapsomaniki (School of Medicine, University of Patras, Greece)
Member PhD advisory committee	Artemis Llamosi (Université Paris Descartes)

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 15, MEMICS 14, HSB 14, ISMB/ECCB 14
Member scientific advisory board	Microbiology and Food Chain Department, Inra
Member review and selection committees	International Human Frontier Science Program (HFSP)
Member evaluation committee	AERES evaluation of MIAj and MIG laboratories, INRA, Jouy-en-Josas, 2014
Member recruitment committee	Junior research scientists, INRA
Member recruitment committee	Assistant professor, INSA de Lyon
Member HDR committee	Grégory Batt (Université Paris Descartes)
Member PhD committee	Kyrill Batmanov (Université de Lille I)
Member organization committee	BEeSy Conference on Perspectives in Environmental and Systems Biology (Grenoble, 2015)
Project reviews	BBSRC

Johannes Geiselmann

Type	Journal, conference, agency
Member recruitment committee	Assistant professor, INSA de Lyon
Member PhD committee	Thomas Esquerré (Université de Toulouse, INSA), Peng Liu, (Institut Pasteur, Paris), Elisa Brambilla (ENS Cachan)
Member PhD advisory committee	Xuejiao Jiang (INSA de Lyon)
Member scientific committee	ARC1, Rhône-Alpes region
Member organization committee	BEeSy Conference on Perspectives in Environmental and Systems Biology (Grenoble, 2015)
Project reviews	ANR

Stephan Lacour

Type	Journal, conference, agency
Member PhD advisory committee	Alexandre Duprey (Université de Lyon)

Delphine Ropers

Type	Journal, conference, agency
Member organization committee	SeMoVi (Séminaire de Modélisation du Vivant)
Member recruitment committee	Assistant professor, INSA de Lyon (twice)
Member PhD committee	Anna Zhukova (Université de Bordeaux)
Member PhD advisory committee	Alice Julien-Laferrière (Université de Lyon), Martin Wannagat (Université de Lyon)

8.2. 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 member of the working group on International Relations of the Conseil d'Orientation Scientifique et Technique (COST) of Inria.

Johannes Geiselmann is member of the scientific council of the Department of Biology at Université Joseph Fourier.

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

François Rechenmann is CEO of Genostar. In addition, he 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.

Michel Page is coordinator of the master Systèmes d'information et d'organisation at the Institut d'Adminstration des Entreprises (IAE), Université Pierre Mendès-France, Grenoble.

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 and Référente Chercheurs at Inria Grenoble - Rhône-Alpes.

8.3. Seminars, presentations, and PhD thesis defenses

Eugenio Cinquemani

Title	Event and location	Date
Modeling and analysis of population systems with applications to molecular biology	Tutorial at HYCON2 workshop at European Control Conference (ECC 14), with G. Ferrari-Trecate and J. Lygeros, Strasbourg	June 2014
Modelling cellular heterogeneity of gene expression in yeast : The mixed-effects approach	CWI-Inria workshop, Paris	September 2014
Modelling dynamics and regulation of gene expression from fluorescent reporter data: Two challenges in two biological scenarios	Seminar at School of Medicine, University of Patras (Greece)	November 2014
Towards real-time control of gene expression at the single cell level: a stochastic control approach	Presentation at 12th international conference on Computational Methods for Systems Biology (CMSB 2014), Manchester (UK)	November 2014

Hidde de Jong

Title	Event and location	Date
Genetic Network Analyzer: modélisation et simulation qualitative de réseaux de régulation	Demo at Rencontre Inria-Industrie Bio-informatique et outils numériques pour les produits de santé, Lyon	February 2014
Analysis and identification of bacterial regulatory networks using real-time gene expression data	Invited presentation at Journée régionale Bioinformatique, Toulouse	June 2014
The analysis of global regulatory networks in bacteria using models and experiments	Invited presentation at International Workshop on Hybrid Systems Biology (HSB 2014), Vienna (Austria)	July 2014
Dynamic optimisation of resource allocation in microorganisms	Presentation at International Symposium on Mathematical Theory of Networks and Systems (MTNS 2014), Groningen (the Netherlands)	July 2014
Analysis and identification of bacterial regulatory networks using real-time gene expression data	Invited presentation at Colloque Biologie Synthétique & Systémique (BioSynSys), Toulouse	July 2014
Numerical simulation of piecewise-linear models of gene regulatory networks using complementarity systems	Presentation at International Symposium on Mathematical Theory of Networks and Systems (MTNS 2014), Groningen (the Netherlands)	July 2014

Johannes Geiselmann

Title	Event and location	Date
Global regulation and the control of growth rate in Escherichia coli	Invited seminar SeMoVi, Lyon	January 2014
Regulation of gene expression in E. coli: global effects and specific regulations	Seminar at Centre de Biologie Integrative, Toulouse	March 2014
Systems biology of E. coli	University of Trondheim (Norway)	May 2014
Control of gene expression in E. coli	Journée Recherche, UJF	October 2014

François Rechenmann

Title	Event and location	Date
Genostar	Demo at Rencontre Inria-Industrie Bio-informatique et outils numériques pour les produits de santé, Lyon	February 2014

Delphine Ropers

Title	Event and location	Date
Global control of gene expression in *Escherichia coli*	Seminar at MAP laboratory, Lyon	March 2014
Complex regulatory networks in bacteria	Seminar at Institute of Food Research, University of Norwich (UK)	
Complex regulatory networks in bacteria: How bacteria face the unexpected	Invited presentation at the winter school Multi-scale modelling of biological systems, INSA, Toulouse	December 2014

Diana Stefan

Title	Event and location	Date
Identification structurelle et paramétrique des réseaux de régulation bactériens	PhD thesis defense, Université Joseph Fourier, Grenoble	June 2014

Valentin Zulkower

Title	Event and location	Date
A quantitative study of the regulation of cAMP-dependent promoters in *E. coli*	Presentation at workshop PTS50, Göttingen (Germany)	September 2014

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. In the context of the Math C2+ initiative for high-school students, François Rechenmann has given the presentation "Algorithmes et génomes : analyse informatique de l'information génétique".

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	4
Statistics for biologists	4	Master Approches Interdisciplinaires du Vivant, Centre de Recherches Interdisciplinaires/Université Paris Descartes	20
Modelling and identification of metabolic networks	4	Master PHELMA, INP Grenoble	4

Hidde de Jong

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	5	INSA de Lyon	20
Modeling and simulation of genetic regulatory networks	5	ENS Paris	8

Nils Giordano

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	5	ENS Paris	8
Microbiologie	2	Université Joseph Fourier	32
Génétique des Populations	2	Université Joseph Fourier	30
Microbiologie	2	Université Joseph Fourier	32
Introduction à la bioinformatique	2	Université Joseph Fourier	12

Delphine Ropers

Subject	Year	Location	Hours
Modeling and simulation of genetic regulatory networks	4	Université Joseph Fourier	7.5
Modeling and simulation of genetic regulatory networks	5	INSA de Toulouse	4
Modeling in systems biology	4	Master PHELMA, INP Grenoble	16

François Rechenmann is preparing a MOOC on the subject "Algorithmes et génome", which is due to appear in 2015. Hidde de Jong organized with Daniel Kahn a module on the modeling of genetic and metabolic networks at INSA de Lyon. Delphine Ropers organizes a module on the mathematical modeling of biological systems at PHELMA, INP Grenoble.

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