IN PARTNERSHIP WITH:
Université de Grenoble Alpes

Activity Report 2018

Project-Team IBIS

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

RESEARCH CENTER
Grenoble - Rhône-Alpes

THEME
Computational Biology

Table of contents

Project-Team IBIS

Creation of the Project-Team: 2009 January 01

Keywords:

Computer Science and Digital Science:

A3.1.1. - Modeling, representation
A3.4.5. - Bayesian methods
A6.1.1. - Continuous Modeling (PDE, ODE)
A6.1.2. - Stochastic Modeling
A6.2.1. - Numerical analysis of PDE and ODE
A6.2.4. - Statistical methods
A6.3.1. - Inverse problems
A6.3.2. - Data assimilation
A6.3.3. - Data processing
A6.4.1. - Deterministic control

Other Research Topics and Application Domains:

B1. - Life sciences
B1.1.2. - Molecular and cellular biology
B1.1.4. - Genetics and genomics
B1.1.7. - Bioinformatics
B1.1.8. - Mathematical biology
B1.1.10. - Systems and synthetic biology
B4.3.1. - Biofuels

1. Team, Visitors, External Collaborators

Research Scientists

Hidde de Jong [Team leader, Inria, Senior Researcher, HDR]
Eugenio Cinquemani [Inria, Researcher]
Delphine Ropers [Inria, Researcher]

Faculty Members

Johannes Geiselmann [Univ Grenoble Alpes, Professor]
Stephan Lacour [Univ Grenoble Alpes, Associate Professor]
Yves Markowicz [Univ Grenoble Alpes, Associate Professor]
Michel Page [Univ Grenoble Alpes, Associate Professor]

Post-Doctoral Fellows

Aline Marguet [Inria]
Marco Mauri [Inria]

PhD Students

Thibault Etienne [INRA]
Antrea Pavlou [Inria, from Nov 2018]

Technical staff

Tamas Muszbek [Inria]
Célia Boyat [Univ Grenoble Alpes]

Corinne Pinel [CNRS]

Intern

Naina Goel [Inria, from Feb 2018 until Jul 2018]

Administrative Assistant

Alexandra Fitzgerald [Inria]

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 questions 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 also made contributions to research projects on different topics. 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 group of Johannes Geiselmann, formerly at the Laboratoire Adaptation et Pathogénicité des Microorganismes 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.

Univ Joseph Fourier (UJF, CNRS UMR 5163), and since September 2014 at the Laboratoire Interdisciplinaire de Physique (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. 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 its 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.

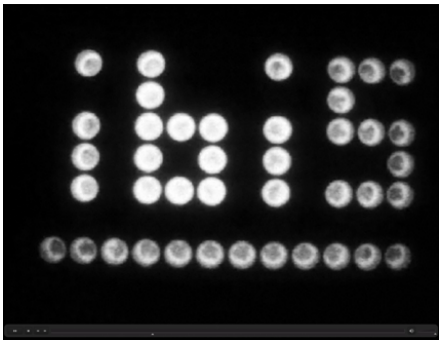


Figure 1. Display of the project-team name on a "bacterial billboard" (see http://team.inria.fr/ibis for the corresponding movie). A microplate containing a minimal medium (with glucose and acetate) is filmed during 36 hours. Wells contain E. coli bacteria which are transformed with a reporter plasmid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

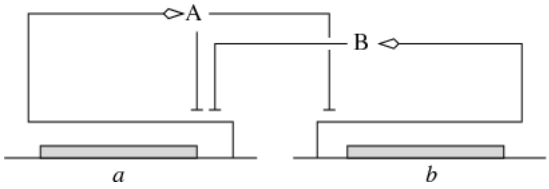
Participants: Hidde de Jong [Correspondent], Michel Page, Delphine Ropers.

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://team.inria.fr/ibis 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.



$$\dot{x}_a = \kappa_a \, s^-(x_a, \theta_a^2) \, s^-(x_b, \theta_b) - \gamma_a \, x_a$$

$$\dot{x}_b = \kappa_b \, s^-(x_a, \theta_a^1) - \gamma_b \, x_b$$

$$s^+(x, \theta) = \begin{cases} 1, & \text{if } x > \theta \\ 0, & \text{if } x < \theta \end{cases}$$

$$s^-(x, \theta) = 1 - s^+(x, \theta)$$

Figure 2. (Left) Example of a gene regulatory network of two genes (a and b), each of which codes 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, Stephan Lacour, Aline Marguet, Michel Page, Corinne Pinel, Delphine Ropers.

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.

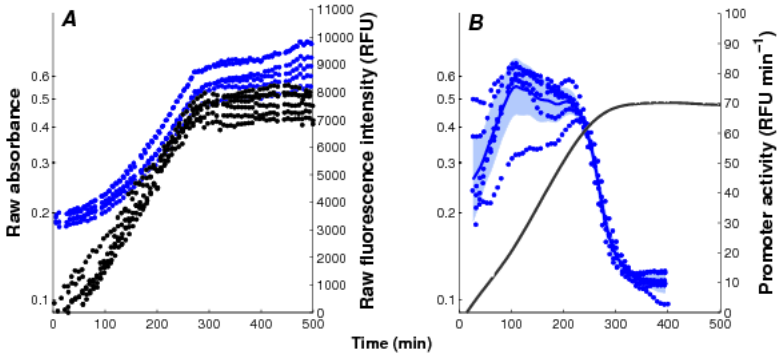


Figure 3. Monitoring of bacterial gene expression in vivo using fluorescent reporter genes (Stefan et al., PLoS Computational Biology, 11(1):e1004028, 2015). The plots show the primary data obtained in a kinetic experiment with E. coli cells, focusing on the expression of the motility gene tar in a mutant 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. 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 and EPF Lausanne (Switzerland). 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, Thibault Etienne, Johannes Geiselmann, Stephan Lacour, Yves Markowicz, Marco Mauri, Michel Page, Corinne Pinel, Delphine Ropers [Correspondent].

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 growth and gene expression

Participants: Célia Boyat, Eugenio Cinquemani, Johannes Geiselmann [Correspondent], Hidde de Jong [Correspondent], Stephan Lacour, Marco Mauri, Tamas Muszbek, Michel Page, Antrea Pavlou, Delphine Ropers.

The adaptation of bacterial physiology to changes in the environment, involving changes in the growth rate and a reorganization of gene expression, is fundamentally a resource allocation problem. It notably poses the question how microorganisms redistribute their protein synthesis capacity over different cellular functions when confronted with an environmental challenge. Assuming that resource allocation in microorganisms has been optimized through evolution, for example to allow maximal growth in a variety of environments, this question can be fruitfully formulated as an optimal control problem. We have developed such an optimal control perspective, focusing on the dynamical adaptation of growth and gene expression in response to envrionmental changes, in close collaboration with the BIOCORE project-team.

A complementary perspective consists in the use of control-theoretical approaches to modify 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. Within a number of on-going projects, IBIS is focusing on two different questions. The first concerns the development of open-loop and closed-loop growth-rate controllers of bacterial cells for both fundamental research and biotechnological applications (Figure 5). Second, we are working on the development of methods for the real-time control of the expression of heterologous proteins in communities of interacting bacterial populations. The above projects involve collaborations with, among others, the Inria

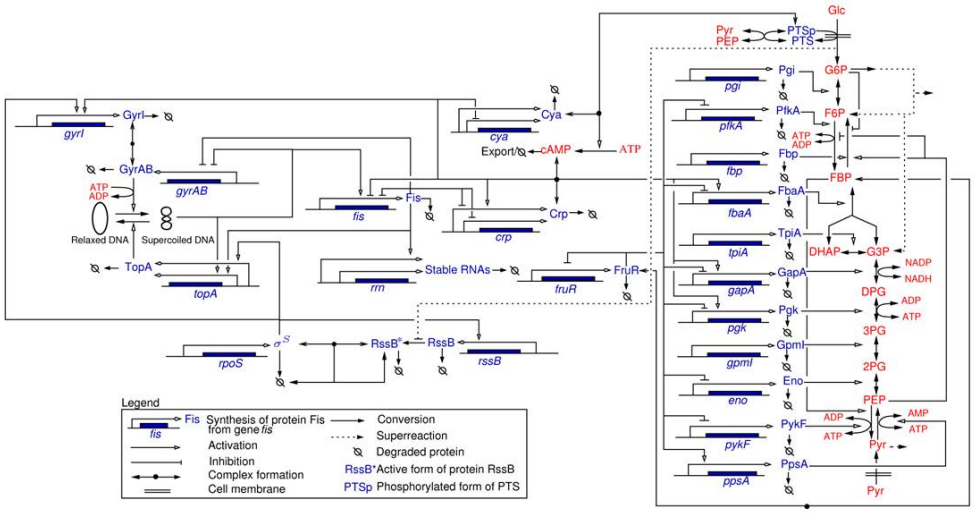


Figure 4. Network of key genes, proteins, and regulatory interactions involved in the carbon assimilation network in E. coli (Baldazzi et al., PLoS Computational Biology, 6(6):e1000812, 2010). The metabolic part includes the glycolysis/gluconeogenesis pathways as well as a simplified description of the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

project-teams LIFEWARE (INBIO), BIOCORE, and McTAO as well as with a biophysics group at Univ Paris Descartes and a mathematical modeling group at INRA Jouy-en-Josas.

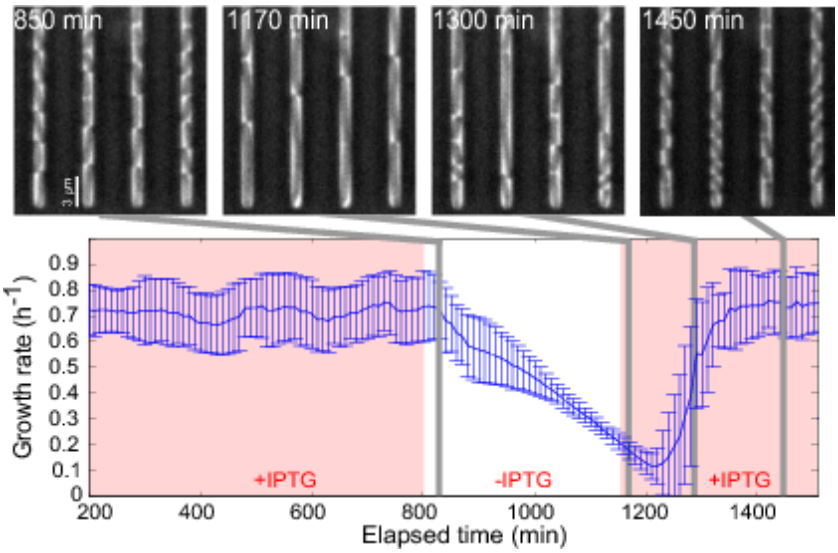


Figure 5. Growth arrest by external control of the gene expression machinery (Izard, Gomez Balderas et al., Molecular Systems Biology, 11:840, 2015). An E. coli strain in which an essential component of the gene expression machinery, the $\beta\beta'$ subunits of RNA polymerase, was put under the control of an externally-supplied inducer (IPTG), was grown in a microfluidics device and phase-contrast images were acquired every 10 min. The cells were grown in minimal medium with glucose, initially in the presence of 1 mM IPTG. 6 h after removing IPTG from the medium, the growth rate slows down and cells are elongated. About 100 min after adding back 1 mM IPTG into the medium, the elongated cells divide and resume normal growth. The growth rates in the plot are the (weighted) mean of the growth rates of 100 individual cells. The error bars correspond to $\pm$ one standard deviation. The results of the experiment show that the growth rate of a bacterial can be switched off in a reversible manner by an external inducer, based on the reengineering of the natural control of the expression of RNA polymerase.

4. Highlights of the Year

4.1. Highlights of the Year

Two new projects with participation from IBIS started this year: the ANR project RIBECO and the IXXI project MuSE (Section 7.2). The web application WellInverter was made available through the new cloud of the French Institute of Bioinformatics (IFB) (Section 5.2). A publication summarizing several conference contributions on the stochastic modeling and inference of gene regulatory networks was published in the main control journal *Automatica* this year [16].

5. New Software and Platforms

5.1. WellFARE

KEYWORDS: Bioinformatics - Statistics - Data visualization - Data modeling

SCIENTIFIC DESCRIPTION: WellFARE is a Python library implementing linear inversion methods for the reconstruction of gene expression profiles from fluorescent or luminescent reporter gene data. WellFARE form the computational core of the WellInverter web application.
FUNCTIONAL DESCRIPTION: As input, WellFARE reads the primary data file produced by a 96-well microplate reader, containing time-series measurements of the absorbance (optical density) as well as the fluorescence and luminescence intensities in each well (if available). Various functions exist to analyze the data, in particular for detecting outliers, subtracting background, estimating growth rates, promoter activities and protein concentrations, visualizing expression profiles, synchronizing replicate profiles, etc. WellFARE is the computational core of the web application WellInverter.
NEWS OF THE YEAR: Submission of a journal publication describing the new version of WellInverter and WellFARE

- Participants: Delphine Ropers, Hans Geiselmann, Hidde De Jong, Michel Page, Valentin Zulkower and Yannick Martin
- Partner: UGA
- Contact: Hidde De Jong
- Publication: Robust reconstruction of gene expression profiles from reporter gene data using linear inversion
- URL: https://github.com/ibis-inria/wellfare

5.2. WellInverter

KEYWORDS: Bioinformatics - Statistics - Data visualization - Data modeling
SCIENTIFIC DESCRIPTION: WellInverter is a web application that implements linear inversion methods for the reconstruction of gene expression profiles from fluorescent or luminescent reporter gene data. WellInverter makes the methods available to a broad audience of biologists and bioinformaticians. In particular, we have put in place a parallel computing architecture with a load balancer to distribute the analysis queries over several back-end servers, redesigned the graphical user interface, and developed a plug-in system for defining high-level routines for parsing data files produced by microplate readers from different manufacturers.
FUNCTIONAL DESCRIPTION: As input, WellInverter reads the primary data file produced by a 96-well microplate reader, containing time-series measurements of the absorbance (optical density) as well as the fluorescence and luminescence intensities in each well (if available). Various modules exist to analyze the data, in particular for detecting outliers, subtracting background, estimating growth rates, promoter activities and protein concentrations, visualizing expression profiles, synchronizing replicate profiles, etc. The computational core of the web application consists of the Python library WellFARE.
NEWS OF THE YEAR: Deployment of WellInverter on an Inria server and on the new cloud of the French Institute for Bioinformatics (see the web page for details). Submission of a journal article describing the new version of the application.

- Participants: Delphine Ropers, Hans Geiselmann, Hidde De Jong, Johannes Geiselmann, Michel Page, Valentin Zulkower and Yannick Martin
- Partner: UGA
- Contact: Hidde De Jong
- Publication: Robust reconstruction of gene expression profiles from reporter gene data using linear inversion
- URL: https://team.inria.fr/ibis/wellinverter/

5.3. FluoBacTracker

KEYWORDS: Bioinformatics - Biology - Biomedical imaging

SCIENTIFIC DESCRIPTION: FluoBacTracker is an ImageJ plugin allowing the segementation and tracking of growing bacterial cells from time-lapse microscopy movies. The segmentation and tracking algorithms used by FluoBacTracker have been developed by Lionel Moisan and colleagues at Université Paris Descartes.
FUNCTIONAL DESCRIPTION: FluoBacTracker has the following functionalities: 1) Select regions of interest in images of microcolonies 2) Denoise and renormalize the images 3) Identify each cells in each image (segmentation) 4) Follow cells through the whole movie (tracking), including the detection of cells washed out from a microfluidics channel 5) Detect divisions and construct cell lineage of the population
NEWS OF THE YEAR: Version 2 of FluoBacTracker also allows the analysis of microscopy of bacteria growing in a microfluidics device called "mother machine".

- Participants: Hugues Berry, Cyril Dutrieux, Hidde De Jong, Charles Kervrann, David Parsons and Magali Vangkeosay
- Partners: Université Descartes - UGA
- Contact: Hugues Berry
- URL: http://fluobactracker.inrialpes.fr

5.4. GNA

Genetic Network Analyzer
KEYWORDS: Model Checking - Bioinformatics - Gene regulatory networks - Qualitative simulation
SCIENTIFIC DESCRIPTION: Genetic Network Analyzer (GNA) is the implementation of methods for the qualitative modeling and simulation of gene regulatory networks developed in the IBIS project-team.
FUNCTIONAL DESCRIPTION: 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.

RELEASE FUNCTIONAL DESCRIPTION: (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.
NEWS OF THE YEAR: New mode of distribution from the IBIS web site.

- Participants: Hidde De Jong, Michel Page and Delphine Ropers
- Partner: UGA
- Contact: Hidde De Jong
- Publications: Genetic Network Analyzer: A Tool for the Qualitative Modeling and Simulation of Bacterial Regulatory Networks - Piecewise linear approximations to model the dynamics of adaptation to osmotic stress by food-borne pathogens
- URL: http://www-helix.inrialpes.fr/gna

6. New Results

6.1. Analysis of fluorescent 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). Over the years, many useful resources have appeared, such as libraries of reporter strains for model organisms and computer tools for designing reporter plasmids. Moreover, the widespread adoption of thermostated microplate readers in experimental laboratories has made it possible to automate and multiplex reporter gene assays on the population level. This has resulted in large time-series data sets, typically comprising $10^5 - 10^6$ measurements of absorbance, fluorescence, and luminescence for 10^3 wells on the microplate. In order to fully exploit these data sets, we need sound mathematical methods to infer biologically relevant quantities from the primary data and computer tools to apply the methods in an efficient and user-friendly manner.

In the past few years we developed novel methods for the analysis of reporter gene data obtained in microplate experiments, based on the use of regularized linear inversion. This allows a range of estimation problems to be solved, notably the inference of growth rate, promoter activity, and protein concentration profiles. The linear inversion methods, published in *Bioinformatics* in 2015 [12], have been implemented in the Python package WELLFARE and integrated in the web application WELLINVERTER. Funded by a grant from the Institut Français de Bioinformatique (IFB), we improved WellInverter by developing a parallel computational architecture with a load balancer to distribute the analysis queries over several back-end servers, a new graphical user interface, and a plug-in system for defining high-level routines for parsing data files produced by microplate readers from different manufacturers. This has resulted in a scalable and user-friendly web service providing a guaranteed quality of service, in terms of availability and response time. This year the web service has been redeployed on the new IFB cloud and on an Inria server, accompanied by extensive user documentation, online help, and a tutorial. Moreover, we submitted a journal paper on WELLINVERTER illustrating the use of the tool by analyzing data of the expression of a fluorescent reporter gene controlled by a phage promoter in growing *Escherichia coli* populations. We notably show that the expression pattern in different growth media, supporting different growth rates, corresponds to the pattern expected for a constitutive gene.

Compared to most reporter gene assays based on fluorescence proteins, luciferase reporters have a superior signal-to-noise ratio, since they do not suffer from the high autofluorescence background of the bacterial cell. At the same time, however, luciferase reporters have the drawback of constant light emission, which leads to undesired cross-talk between neighbouring wells on a microplate. To overcome this limitation, Marco Mauri in collaboration with colleagues from the Philipps-Universität Marburg developed a computational method to correct for luminescence bleed-through and to estimate the "true" luminescence activity for each well of a microplate. As the sole input our algorithm uses the signals measured from a calibration plate, in which the light emitted from a single luminescent well serves as an estimate for the "light-spread function". We show that this light-spread function can be used to deconvolve any other measurement obtained under the same technical conditions. Our analysis demonstrates that the correction preserves low-level signals close to the background and shows that it is universally applicable to different kinds of microplate readers and plate types. A journal article on this work was submitted this year.

6.2. Microdomain formation of bacterial membrane proteins

Fluorescent reporters can be used not only to quantify gene expression, but also to localize proteins in different compartments of the cell, In particular, in bacteria proteins within the cytoplasmic membrane display distinct localization patterns and arrangements. While multiple models exist describing the dynamics of membrane proteins, to date there have been few systematic studies, particularly in bacteria, to evaluate how protein size, number of transmembrane domains, and temperature affect their diffusion, and if conserved localization patterns exist.

Marco Mauri in collaboration with colleagues from the Philipps-Universität in Marburg has used fluorescence microscopy, single-molecule tracking (SMT), and computer-aided visualization methods to obtain a better understanding of the three-dimensional organization of bacterial membrane proteins, using the model bacterium *Bacillus subtilis*. First, we carried out a systematic study of the localization of over 200 *B. subtilis* membrane proteins, tagged with monomeric mVenus-YFP at their original gene locus. Their subcellular localization could be discriminated in polar, septal, patchy, and punctate patterns. Almost 20% of membrane proteins specifically localized to the cell poles, and a vast majority of all proteins localized in distinct structures, which we term microdomains. Dynamics were analyzed for selected membrane proteins, using SMT. Diffusion coefficients of the analyzed transmembrane proteins did not correlate with protein molecular weight, but correlated inversely with the number of transmembrane helices, *i.e.*, transmembrane radius. We observed that temperature can strongly influence diffusion on the membrane, in that upon growth temperature upshift, diffusion coefficients of membrane proteins increased and still correlated inversely to the number of transmembrane domains, following the Saffman–Delbrück relation.

The vast majority of membrane proteins were observed to localize to distinct multimeric assemblies. Diffusion of membrane proteins can be suitably described by discriminating diffusion coefficients into two protein populations, one mobile and one immobile, the latter likely constituting microdomains. Moreover, in this study published in *BMC Biology* [18], we provided a method to correct the diffusion coefficient for the membrane curvature. Our results show there is high heterogeneity and yet structural order in the cell membrane, and provide a roadmap for our understanding of membrane organization in prokaryotes. Given the exceptionally richness of the data obtained, both further analysis on membrane lateral movements and a more detailed theory on the effect of membrane curvature is possible.

6.3. 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 rather than the deterministic models underlying the linear inversion methods discussed in Section 6.6. 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 engineered) control of cell populations and even of single cells.

Work in IBIS on gene expression and interaction dynamics at the level of individual cells is addressed in terms of identification of parametric intrinsic noise models, on the one hand, and the nonparametric inference of gene expression statistics, on the other hand, from population snapshot data. Along with modelling and inference, identifiability analysis is dedicated special attention. Other problems related with single-cell modelling, extracellular variability and inheritance of traits at cell division are considered also through external collaborations, as discussed below (Section 6.4).

Concerning identification of intrinsic noise dynamics in single cells, previous results on the contribution of stochasticity to parameter identifiability and on reconstruction of unknown gene regulatory networks have been taken further. For the case of population snapshot meaurements, where the dynamics of the population statistics are observed by simple time-lapse experiments, our earlier results showing that variance measurements may provide tremendous improvement in network reconstruction relative to sole mean measurements have been developed into a full-blown method for first-order gene network reconstruction. Additionally, parameter identifiability methods and results initially developed for gene expression models have been generalized to the whole class of first-order stochastic reaction networks. These developments have been presented and demonstrated by simulation in a paper published in the journal *Processes* [15].

Reconstruction of promoter activity statistics from reporter gene population snapshot data has been further investigated, leading to a full-blown spectral analysis and reconstruction method for reporter gene systems. In

the context of the ANR project MEMIP (Section 7.2), we have characterized reporter systems as noisy linear systems operating on a stochastic input (promoter activity), and developed an inversion method for estimation of promoter activation statistics from reporter population snapshots that is nonparametric, in the sense that it does not assume any parametric model for the unknown promoter dynamics. This analysis rests on a more general, original generalization of moment equations that we have developed for stochastic reaction networks with state-affine rates subject to random input processes. The theoretical results, together with a demonstration of the reporter gene inversion method on simulated data, have been accepted for publication in *Automatica* this year [16]. In addition to utilization of the method on real gene-expression data, the results lend themselves to several additional applications, among which the study of extrinsic noise and the optimal design of reporter systems.

6.4. Modelling and analysis of cellular trait dynamics over lineage trees

The investigation of cellular populations at a single-cell level has already led to the discovery of important phenomena, such as the occurrence of different phenotypes in an isogenic population. Nowadays, several experimental techniques, such as microscopy combined with the use of microfluidic devices, enable one to take investigation further by providing time-profiles of the dynamics of individual cells over entire lineage trees. The difficulty, and at the same time the opportunity, in exploiting these data and inferring mathematical models from them is the fact that the behavior of different cells is correlated because of inheritance.

From the modelling point of view, lineage trees are well described by structured branching population models where the life cycle of each cell depends on individual characteristics, such as size and internal protein dynamics, which play a key role in the mechanisms of cell division. One important aspect in the analysis of these population models consists in the investigation of biases arising from the sampling of a finite set of observed individuals. In order to characterize bias, we studied the dynamics of a structured branching population where the trait of each individual evolves in accordance with a Markov process. We assumed that the rate of division of each individual is a function of its trait and when a branching event occurs, the trait of the descendants at birth depends on their number and on the trait of the mother. We explicitly described the Markov process, named auxiliary process, corresponding to the dynamics of the trait of a "typical" individual by deriving its associated infinitesimal generator. In particular, we proved that this process characterizes exactly the process of the trait of a uniformly sampled individual in a large population approximation. This work, carried out by Aline Marguet, has been accepted for publication in the journal *Bernoulli* [19].

We also investigated the long-time behavior of the population and proved that a typical individual in the population asymptotically behaves like the auxiliary process previously introduced. These results have been submitted for publication [22]. Structured branching processes and their analysis also provide the basis for identification tools for lineage-tree data. In particular, in the context of a bifurcating Markov chain, where each individual is characterized by a trait evolving in accordance with a scalar diffusion, we proved that the maximum-likelihood estimator of the division rate is asymptotically efficient and demonstrate the method on simulated data. This work, in collaboration with M. Hoffmann at Univ Paris-Dauphine, has also been submitted for publication [21].

Along the same lines, modelling and identification of gene expression models with mother-daughter inheritance are being investigated in the context of the ANR project MEMIP. Starting from an earlier work of the group [7], with reference to an application on osmotic shock response by yeast, the key question is to what extent leveraging an inheritance model improves inference of individual cell dynamics as well as of inheritance dynamics themselves, relative to state-of-art approaches where inheritance is not accounted for at a modelling stage.

6.5. Models of carbon metabolism in bacteria

Adaptation of bacterial growth to changes in environmental conditions, such as the availability of specific carbon sources, is triggered at the molecular level by the reorganization of metabolism and gene expression: the concentration of metabolites is adjusted, as well as the concentration and activities of enzymes, the rate

of metabolic reactions, the transcription and translation rates, and the stability of proteins and RNAs. This reprogramming of the bacterial cell is carried out by i) specific interactions involving regulatory proteins or RNAs that specifically respond to the change of environmental conditions and ii) global regulation involving changes in the concentration of RNA polymerase, ribosomes, and metabolite pools that globally affect the rates of transcription, translation, and degradation of all RNAs and proteins.

A quantitative description and understanding of this complex network, cutting across metabolism, gene expression, and signalling, can be accessed through mathematical modelling only. In collaboration with Andreas Kremling, professor at TU München and former visiting scientist in the IBIS project-team, Hans Geiselmann, Delphine Ropers and Hidde de Jong developed an ensemble of variants of a simple core model of carbon catabolite repression. The model variants, with two substrate assimilation pathways and four intracellular metabolites only, differ from one another in only a single aspect, each breaking the symmetry between the two pathways in a different manner. Interestingly, all model variants are able to reproduce the data from a reference diauxic growth experiment. For each of the model variants, we predicted the behaviour in two new experimental conditions. When qualitatively comparing these predictions with experimental data, a number of models could be excluded while other model variants are still not discriminable. The best-performing model variants are based on inducer inclusion and activation of enzymatic genes by a global transcription factor, but the other proposed factors may complement these well-known regulatory mechanisms. The model ensemble, which was described in a study pubmished in *BMC Systems Biology* this year, offers a better understanding of the variety of mechanisms that have been proposed to play a role in carbon catabolite repression, but is also useful as an educational resource for systems biology.

The same focus on the dynamics of physiological processes has shaped a project on the post-transcriptional control of carbon central metabolism in *E. coli*. In the framework of the PhD thesis of Manon Morin, supported by a Contrat Jeune Scientifique INRA-Inria, the collaboration of Delphine Ropers with Muriel Cocaign-Bousquet and Brice Enjalbert at INRA/INSA Toulouse has demonstrated the key role played by the post-transcriptional regulatory system CSR in growth transitions in a series of publications in the past few years (*e.g.*, [9]). The collaboration with INRA/INSA de Toulouse is continued in the context of the PhD thesis of Thibault Etienne, funded by an INRA-Inria PhD grant, with the objective of developing models able to explain how cells coordinate their physiology and the functioning of the transcription, translation, and degradation machineries following changes in the availability of carbon sources in the environment. This work is further supported by the ANR project ECORIB accepted this year and an IXXI grant in collaboration with the ERABLE project-team (Section 7.2).

6.6. Modelling bacterial growth

Various mathematical approaches have been used in the literature to describe the networks of biochemical reactions involved in microbial growth. With various levels of detail, the resulting models provide an integrated view of these reaction networks, including the transport of nutrients from the environment and the metabolism and gene expression allowing the conversion of these nutrients into biomass. The models hence bridge the scale between individual reactions to the growth of cell populations. Analysing the dynamics of some of these models mentioned above becomes quickly intractable, when mathematical functions are for instance given by complex algebraic expressions resulting from the mass balance of biochemical reactions. In a paper published in the *Bulletin of Mathematical Biology* [13], Edith Grac, former post-doc in IBIS, Delphine Ropers, and Stefano Casagranda and Jean-Luc Gouzé from the BIOCORE project-team, have studied how monotone system theory and time-scale arguments can be used to reduce high-dimension models based on the mass-action law. Applying the approach to an important positive feedback loop regulating the expression of RNA polymerase in *E. coli*, made it possible to study the stability of the system steady states and relate the dynamical behaviour of the system to observations on the physiology of the bacterium *E. coli*.

In another paper published in *BMC Systems Biology* [14], Delphine Ropers and BIOCORE members Stefano Casagranda, Jean-Luc Gouzé, and Suzanne Touzeau, have developed a new approach to deal with model complexity. The approach, named Principle Process Analysis, allows to identify processes playing a key role in the model dynamics and to reduce the complex dynamics to these core processes, omitting processes that are

inactive. In particular, it has allowed the reduction of a well-known model of circadian rhythms in mammals into a succession of simpler submodels. Their analysis has resulted in the identification of the source of circadian oscillations, the main oscillator being the negative feedback loop involving proteins PER, CRY, CLOCK-BMAL1, in agreement with previous modelling and experimental studies.

Recent work has shown that coarse-grained models of resource allocation can account for a number of empirical regularities relating the the macromolecular composition of the cell to the growth rate. Some of these models hypothesize control strategies enabling microorganisms to optimize growth. While these studies focus on steady-state growth, such conditions are rarely found in natural habitats, where microorganisms are continually challenged by environmental fluctuations. In recent years, in the framework of the PhD thesis of Nils Giordano, we extended the study of microbial growth strategies to dynamical environments, using a self-replicator model. In collaboration with the BIOCORE project-team, we formulated dynamical growth maximization as an optimal control problem that can be solved using Pontryagin's Maximum Principle and we compared the theoretical results thus obtained with different possible implementations of growth control in bacterial cells [5]. The extension and experimental validation of some of these results are currently being carried out by Antrea Pavlou in the framework of her PhD project, funded by the ANR project Maximic (Section 7.2).

6.7. Growth control in bacteria and biotechnological applications

The ability to experimentally control the growth rate is crucial for studying bacterial physiology. It is also of central importance for applications in biotechnology, where often the goal is to limit or even arrest growth. Growth-arrested cells with a functional metabolism open the possibility to channel resources into the production of a desired metabolite, instead of wasting nutrients on biomass production. In recent years we obtained a foundation result for growth control in bacteria [6], in that we engineered an *E. coli* strain where the transcription of a key component of the gene expression machinery, RNA polymerase, is under the control of an inducible promoter. By changing the inducer concentration in the medium, we can adjust the RNA polymerase concentration and thereby switch bacterial growth between zero and the maximal growth rate supported by the medium. The publication also presented a biotechnological application of the synthetic growth switch in which both the wild-type *E. coli* strain and our modified strain were endowed with the capacity to produce glycerol when growing on glucose. Cells in which growth has been switched off continue to be metabolically active and harness the energy gain to produce glycerol at a twofold higher yield than in cells with natural control of RNA polymerase expression.

The experimental work underlying the growth switch has been continued in several directions in the context of the Maximic project by Célia Boyat. Moreover, in collaboration with colleagues from the BIOCORE project-team, we have formulated the maximization of metabolite production by means of the growth switch as a resource reallocation problem that can be analyzed by means of the self-replicator models of bacterial growth mentioned in Section 6.6 in combination with methods from optimal control theory. In a publication accepted for the *Journal of Mathematical Biology* this year [20], we study various optimal control problems by means of a combination of analytical and computational techniques. We show that the optimal solutions for biomass maximization and product maximization are very similar in the case of unlimited nutrient supply, but diverge when nutrients are limited. Moreover, external growth control overrides natural feedback growth control and leads to an optimal scheme consisting of a first phase of growth maximization followed by a second phase of product maximization. This two-phase scheme agrees with strategies that have been proposed in metabolic engineering. More generally, this work shows the potential of optimal control theory for better understanding and improving biotechnological production processes. Extensions concerning the effect on growth and bioproduction of the (biological or technological) costs associated with discontinuous control strategies, and of the time allotted to optimal substrate utilization, are described in a contribution to a control theory conference submitted this year.

7. Partnerships and Cooperations

7.1. Regional Initiatives

Project name	MuSE: MUlti-Omics and Metabolic models integration to study growth transition in Escherichia coli
Coordinator IBIS participants Type Web page	D. Ropers D. Ropers, T. Etienne IXXI/BioSyl project (2018-2020) http://www.biosyl.org/news/muse-2013-multi-omics-and-metabolic-models-integration-to-study-growth-transition-in-escherichia-coli

Project name	RNAfluo: Quantification d'ARN régulateurs *in vivo*
Coordinator IBIS participants Type	S. Lacour S. Lacour AGIR project Univ Grenoble Alpes (2016-2019)

7.2. National Initiatives

Project name	MEMIP – Modèles à effets mixtes de processus intracellulaires : méthodes, outils et applications
Coordinator IBIS participants Type	G. Batt E. Cinquemani, A. Marguet, D. Ropers ANR project (2016-2020)

Project name	ENZINVIVO – Détermination in vivo des paramètres enzymatiques dans une voie métabolique synthétique
Coordinator IBIS participants Type	G. Truan J. Geiselmann, H. de Jong ANR project (2016-2020)

Project name	MAXIMIC: Optimal control of microbial cells by natural and synthetic strategies
Coordinator IBIS participants Type Web page	H. de Jong C. Boyat, E. Cinquemani, J. Geiselmann, H. de Jong, A. Pavlou, C. Pinel, D. Ropers ANR project (2017-2021) https://project.inria.fr/maximic

Project name	RIBECO (RIBonucleotide ECOnomy): Engineering RNA life cycle to optimize economy of microbial energy
Coordinator IBIS participants Type Web page	M. Cocaign-Bousquet E. Cinquemani, T. Etienne, D. Ropers ANR project (2018-2022) https://project.inria.fr/ribeco/

Project name	COSY: real-time COntrol of SYnthetic microbial communities
Coordinator	E. Cinquemani
IBIS participants	E. Cinquemani, H. de Jong, J. Geiselmann, M. Mauri, T. Muszbek, C. Pinel, D. Ropers
Type	Inria Project Lab (2017-2021)
Web page	https://project.inria.fr/iplcosy/

Project name	CoSoft: Control software for a system of mini-bioreactors
Coordinator	E. Cinquemani
IBIS participants	E. Cinquemani, H. de Jong, J. Geiselmann, T. Muszbek
Type	Inria Hub (2017-2018)

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, N. Giordano
Type	Inria Project Lab (2015-2019)
Web page	https://project.inria.fr/iplalgaeinsilico/

Project name	Analyse intégrative de la coordination entre stabilité des ARNm et physiologie cellulaire chez Escherichia coli
Coordinators	D. Ropers, M. Cocaign-Bousquet (Inra, LISBP)
IBIS participants	T. Etienne, D. Ropers
Type	Contrat Jeune Scientifique Inra-Inria (2016-2019)

7.3. European Initiatives

7.3.1. Collaborations with Major European Organizations

Systems biotechnology department at Technische Universität Münich (Germany), Andreas Kremling

Modeling of carbon metabolism in bacteria

Automatic control laboratory at ETH Zürich (Switzerland), John Lygeros, and Cell cycle laboratory, School of Medicine, University of Patras (Greece), Zoe Lygeros

Control theory and systems identification with applications to single-cell systems biology

8. Dissemination

8.1. Promoting Scientific Activities

8.1.1. Scientific events organisation

8.1.1.1. Member of organizing committees

IBIS members	Conference, workshop, school	Date
Hidde de Jong	CompSysBio: Advanced Lecture Course on Computational Systems Biology, Aussois	Apr 2019

8.1.2. Scientific events selection

8.1.2.1. Chair of conference program committees

IBIS member	Conference, workshop, school	Role
Eugenio Cinquemani	European Control Conference (ECC 2019)	Associate editor

8.1.2.2. Member of conference program committees

IBIS member	Conference, workshop, program
Eugenio Cinquemani Hidde de Jong	ECC 2019, CMSB 2018 and 2019, HSB 2019, SASB 2018 CMSB 2108 and 2019, FOSBE 2019, HSB 2019, MLCSB 2018, ISMB Network Biology 2018, WML 2018

8.1.3. Journal

8.1.3.1. Member of editorial boards

IBIS member	Journal
Johannes Geiselmann	Frontiers in Microbiology (review editor)
Hidde de Jong	Journal of Mathematical Biology
Hidde de Jong	Biosystems (reviews editor)
Hidde de Jong	ACM/IEEE Transactions on Computational Biology and Bioinformatics

8.1.4. Scientific evaluation and expertise

IBIS member	Organism	Role
Johannes Geiselmann	INRA	Member scientific advisory committee Microbiologie, Adaptation, Pathogénie
Johannes Geiselmann	UMR5240 CNRS-UCBL-INSA-BayerCropScience	Member scientific council
Hidde de Jong	Microbiology and Food Chain Department, Inra	Member scientific council
Hidde de Jong	Univ Grenoble Alpes	Member scientific council of Pôle MSTIC
Delphine Ropers	INRA-Inria	Member selection committee PhD grants

8.1.5. Invited talks and other presentations

Eugenio Cinquemani

Title	Event and location	Date
Estimation du bruit extrinsèque dans l'expression génique dans des cellules individuelles	Invited talk Journées Apprentissage de modèles statistiques et stochastiques à partir de données biologiques (ASSTABIO), Rennes	Mar 2018
Real-time control of synthetic microbial communities	Presentation Journée InBio, Lyon	Apr 2018
Towards automated control of synthetic E. coli communities	Presentation Journées GDR BIOSS-IA	Dec 2018

Hidde de Jong

Title	Event and location	Date
Carbon catabolite repression and diauxic growth in E. coli	Invited talk Principles of Microbial Adaptation, Lorentz Center, Leiden, the Netherlands	Mar 2018
Optimal control of bacterial growth for the maximization of metabolite production	Presentation SMAI-MODE 2018, Autrans	Mar 2018
Analysis and control of bacterial regulatory networks	Presentation Journée InBio, Lyon	Apr 2018
Global physiological effects and the analysis of gene regulatory networks	Invited talk 1st International Plant Systems Biology Meeting, Conférence Jacques Monod, Roscoff	Sep 2018
Natural and synthetic control of resource allocation in bacteria	Invited talk Molecular Logic and Computational Synthetic Biology workshop, Santiago de Chile	Dec 2018

Stephan Lacour

Title	Event and location	Date
Influence des ARNs non-codants réprimant CsgD sur la production de curli par Escherichia coli	Poster Ecole Thématique de Microbiologie Moléculaire, Carry-Le-Rouet	Oct 2018

Aline Marguet

Title	Event and location	Date
Estimation statistique dans une population structurée branchante	Seminar Institut Fourier, Grenoble	Jan 2018
Loi des grands nombres pour des processus de branchement structurés	Seminar Institut de Mathématiques de Toulouse	Mar 2018
Loi des grands nombres pour des processus de branchement structurés	Seminar Institut de Recherche Mathématique Avancée, Strasbourg	Mar 2018
Loi des grands nombres pour des processus de branchement structurés	Seminar Institut Montpelliérain Alexander Grothendieck, Montpellier	Mar 2018
Estimation statistique dans une population branchante structurée par une diffusion	Presentation journées MAS du groupe Modélisation Aléatoire et Statistique, Dijon	Aug 2018
Estimation statistique dans une population branchante structurée par une diffusion	Seminar Institut de Mathématiques de Dijon, Dijon	Aug 2018

Marco Mauri

Title	Event and location	Date
Analysis of dynamics of membrane-protein microdomains in bacteria	Poster annual Deutsche Physikalische Gesellschaft (DPG) meeting, Berlin	Mar 2018

Michel Page

Title	Event and location	Date
WellInverter: A web application for the analysis of fluorescent reporter gene data	Poster journéé Institut Français de Bioinformatique (IFB), Paris	Dec 2018

Corinne Pinel

Title	Event and location	Date
Présentation des techniques de génie génétique utilisées au sein du LIPhy	Presentation at the kick-off meeting of the network Ingénierie des Génomes des Micro-organismes (IGM), Paris	Sep 2018

Delphine Ropers

Title	Event and location	Date
Quantitative modelling of metabolism, gene expression and growth	Presentation Journée InBio, Lyon	Apr 2018

Etienne Thibault

Title	Event and location	Date
Coordination de la stabilité des ARNms et la physiologie cellulaire durant des transitions de taux de croissance	Presentation working group BIOSS of GDR IM/BIM, Marseille	Jul 2018
Coordination de la stabilité des ARNms et la physiologie cellulaire durant des transitions de taux de croissance	Poster at ICSB 2018, Lyon	Oct 2018
Coordination de la stabilité des ARNms et la physiologie cellulaire durant des transitions de taux de croissance	Seminar LISBP, INRA-INSA, Toulouse	Dec 2018

8.1.6. Research administration

IBIS member	Committee	Role
Eugenio Cinquemani	Inria Grenoble - Rhône-Alpes	Member Comité des Emplois Scientifiques (CES)
Eugenio Cinquemani	Inria Grenoble - Rhône-Alpes	Member Comité des Utilisateurs des Moyens Informatiques (CUMI)
Eugenio Cinquemani	Inria	Member Comité Administrative Paritaire (CAP)
Eugenio Cinquemani	Inria Grenoble - Rhône-Alpes	Member Comité Développement Technologique (CDT)
Hidde de Jong	Inria Grenoble - Rhône-Alpes	Member scientific council (COS)
Hidde de Jong	Inria	Member working group on International Relations of Conseil d'Orientation Scientifique et Technique (COST)
Delphine Ropers	Inria	Member of Commission d'évaluation d'Inria
Delphine Ropers	Inria	Member of Groupe de travail plan stratégique
Delphine Ropers	Inria Grenoble - Rhône-Alpes	Référente chercheurs
Delphine Ropers	Inria Grenoble - Rhône-Alpes	Member of Comité des études doctorales (CED)

8.1.7. Recruitment committees

IBIS member	Organism	Recruitment
Johannes Geiselmann		
Delphine Ropers	Inria	Chargés de recherche (jury d'admissibilité)
Delphine Ropers	Inria Grenoble - Rhône-Alpes	Chargés de recherche (jury d'admissibilité)
Delphine Ropers	INSA de Lyon	Assistant professor

8.2. Teaching - Supervision - Juries

8.2.1. Teaching

Four members of the IBIS team are either full professor or associate professor at Univ Grenoble Alpes. 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

Course: Stochastic modelling of gene regulatory networks, M2, BIM, INSA de Lyon (6 h)
Course: Statistics for systems biology, M1, Master Approches Interdisciplinaires du Vivant, CRI/Univ Paris Descartes (20 h, also in charge of 20 h of practicals)
Course: Modelling and identification of metabolic networks, M1, Phelma, INP Grenoble (4 h)

Hidde de Jong

Course and practicals: Modeling and simulation of gene regulatory networks, M2, BIM, INSA de Lyon (20 h)

Delphine Ropers

Course and practicals: Modelling in systems biology, M1, Phelma, INP Grenoble (16 h)
Course and practicals: Cell systems biology and modelling cell functions, M1, Master ingéniérie de la santé, Univ Grenoble Alpes (14 h)
Course: Modeling and simulation of genetic regulatory networks, M2, INSA de Toulouse (4 h)

Thibault Etienne

Practicals: Mathématiques et statistique appliquées aux sciences humaines et sociales (practicals), L1 and L3, Univ Grenoble Alpes (56 h)
Course and practicals: Construction et analyse de plasmides in silico, M1, Master ingéniérie de la santé, Univ Grenoble Alpes (10 h)

8.2.2. Supervision

PhD in progress: **Thibault Etienne**, Analyse intégrative de la coordination entre stabilité des ARNm et physiologie cellulaire chez *Escherichia coli*. Supervisors: Delphine Ropers and Muriel Cocaign-Bousquet (INRA Toulouse)
PhD in progress: **Joël Espel**, RNA engineering: Design of the dynamical folding of RNA and of RNA switches. Supervisors: Alexandre Dawid (Univ Grenoble Alpes) and Johannes Geiselmann
PhD in progress: **Antrea Pavlou**, Experimental and computational analysis of bacterial self-replicators. Supervisors: Hidde de Jong and Johannes Geiselmann

8.2.3. Juries

PhD thesis committees

IBIS member	Role	PhD student	University	Date
Johannes Geiselmann	Rapporteur	Clea Lachaux	Univ Paul Sabatier Toulouse	Sep 2018
Johannes Geiselmann	Président	Tomas Andersen	Univ Grenoble Alpes	Oct 2018
Hidde de Jong	Président	Alexandre Rocca	Univ Grenoble Alpes	May 2018
Hidde de Jong	Rapporteur	Jonathan Behaegel	Univ Côte d'Azur	Oct 2018

Habilitation (HDR) committees

IBIS member	Role	PhD student	University	Date
Johannes Geiselmann	Rapporteur	Fabien Letisse	Univ Paul Sabatier Toulouse	Oct 2018

PhD advisory committees

IBIS member	PhD student	University
Johannes Geiselmann	Alain Lombard	Univ Grenoble Alpes
Johannes Geiselmann	Shiny Martis	INSA de Lyon
Hidde de Jong	Céline Hernandez	ENS Paris
Hidde de Jong	Charlotte Coton	INRA Moulon
Stephan Lacour	Julien Trouillon	CEA Grenoble
Delphine Ropers	Manon Barthe	Univ de Toulouse
Delphine Ropers	Irene Ziska	Univ de Lyon

8.2.4. Teaching administration

Yves Markowicz is director of the BSc department at Univ Grenoble Alpes.

Michel Page is coordinator of the master Systèmes d'information et d'organisation at the Institut d'Adminstration des Entreprises (IAE), Univ Grenoble Alpes.

Eugenio Cinquemani organizes a module on statistics in systems biology at CRI/Univ Paris Descartes.

Delphine Ropers organizes a module on the mathematical modeling of biological systems at PHELMA, INP Grenoble.

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

8.3. Popularization

8.3.1. Creation of media or tools for science outreach

Hidde de Jong presented the work of IBIS in the framework of the communication action "Mon équipe en 180 secondes". The video is on-line at the following address: https://videotheque.inria.fr/videotheque/media/47816;jsessionid=D615F60B85E6C88DEDD129AD56E4431E

9. Bibliography

Major publications by the team in recent years

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

[2] S. BERTHOUMIEUX, M. BRILLI, H. DE JONG, D. KAHN, E. CINQUEMANI. *Identification of metabolic network models from incomplete high-throughput datasets*, in "Bioinformatics", 2011, vol. 27, n^{o} 13, pp. i186-i195

[3] S. BERTHOUMIEUX, H. DE JONG, G. BAPTIST, C. PINEL, C. RANQUET, D. ROPERS, J. GEISELMANN. *Shared control of gene expression in bacteria by transcription factors and global physiology of the cell*, in "Molecular Systems Biology", January 2013, vol. 9, n^{o} 1, 11 p. [*DOI :* 10.1038/MSB.2012.70], http://hal.inria.fr/hal-00793352

[4] E. CINQUEMANI, V. LAROUTE, M. BOUSQUET, H. DE JONG, D. ROPERS. *Estimation of time-varying growth, uptake and excretion rates from dynamic metabolomics data*, in "Bioinformatics", 2017, vol. 33, n° 14, pp. i301-i310 [*DOI :* 10.1093/BIOINFORMATICS/BTX250], https://hal.archives-ouvertes.fr/hal-01607919

[5] N. GIORDANO, F. MAIRET, J.-L. GOUZÉ, J. GEISELMANN, H. DE JONG. *Dynamical allocation of cellular resources as an optimal control problem: Novel insights into microbial growth strategies*, in "PLoS Computational Biology", March 2016, vol. 12, n° 3, e1004802 p. [*DOI :* 10.1371/JOURNAL.PCBI.1004802], https://hal.inria.fr/hal-01332394

[6] J. IZARD, C. GOMEZ-BALDERAS, D. ROPERS, S. LACOUR, X. SONG, Y. YANG, A. B. LINDNER, J. GEISELMANN, H. DE JONG. *A synthetic growth switch based on controlled expression of RNA polymerase*, in "Molecular Systems Biology", November 2015, vol. 11, n° 11, 16 p. , https://hal.inria.fr/hal-01247993

[7] A. LLAMOSI, A. GONZALEZ, C. VERSARI, E. CINQUEMANI, G. FERRARI-TRECATE, P. HERSEN, G. BATT. *What population reveals about individual cell identity: Single-cell parameter estimation of models of gene expression in yeast*, in "PLoS Computational Biology", February 2016, vol. 12, n° 2, e1004706 p. , https://hal.inria.fr/hal-01248298

[8] M. MORIN, D. ROPERS, F. LETISSE, S. LAGUERRE, J.-C. J.-C. PORTAIS, M. COCAIGN-BOUSQUET, B. ENJALBERT. *The post-transcriptional regulatory system CSR controls the balance of metabolic pools in upper glycolysis of Escherichia coli*, in "Molecular Microbiology", January 2016, vol. 4, 15 p. [*DOI :* 10.1111/MMI.13343], https://hal.inria.fr/hal-01418224

[9] M. MORIN, D. ROPERS, F. LETISSE, S. LAGUERRE, J.-C. J.-C. PORTAIS, M. COCAIGN-BOUSQUET, B. ENJALBERT. *The Csr system regulates Escherichia coli fitness by controlling glycogen accumulation and energy levels*, in "mBio", 2017, vol. 8, n° 5, pp. 1-14, https://hal.inria.fr/hal-01672038

[10] C. PEANO, J. WOLF, J. DEMOL, E. ROSSI, L. PETITI, G. DE BELLIS, J. GEISELMANN, T. EGLI, S. LACOUR, P. LANDINI. *Characterization of the Escherichia coli σ(S) core regulon by Chromatin Immunoprecipitation-sequencing (ChIP-seq) analysis*, in "Scientific Reports", 2015, vol. 5, 15 p. [*DOI :* 10.1038/SREP10469], https://hal.inria.fr/hal-01217828

[11] D. STEFAN, C. PINEL, S. PINHAL, E. CINQUEMANI, J. GEISELMANN, H. DE JONG. *Inference of quantitative models of bacterial promoters from time-series reporter gene data*, in "PLoS Computational Biology", 2015, vol. 11, n° 1, e1004028, https://hal.inria.fr/hal-01097632

[12] V. ZULKOWER, M. PAGE, D. ROPERS, J. GEISELMANN, H. DE JONG. *Robust reconstruction of gene expression profiles from reporter gene data using linear inversion*, in "Bioinformatics", 2015, vol. 31, n° 12, pp. i71-i79, https://hal.inria.fr/hal-01217800

Publications of the year

Articles in International Peer-Reviewed Journals

[13] I. BELGACEM, S. CASAGRANDA, E. GRAC, D. ROPERS, J.-L. GOUZÉ. *Reduction and stability analysis of a transcription-translation model of RNA polymerase*, in "Bulletin of Mathematical Biology", 2018, vol. 80, n° 2, pp. 294-318 [*DOI :* 10.1007/S11538-017-0372-4], https://hal.inria.fr/hal-01655367

[14] S. CASAGRANDA, S. TOUZEAU, D. ROPERS, J.-L. GOUZÉ. *Principal process analysis of biological models*, in "BMC Systems Biology", 2018, vol. 12, 68 p. [*DOI :* 10.1186/s12918-018-0586-6], https://hal.inria.fr/hal-01818033

[15] E. CINQUEMANI. *Identifiability and Reconstruction of Biochemical Reaction Networks from Population Snapshot Data*, in "Processes", August 2018, vol. 6, n° 9, pp. 1-24 [*DOI :* 10.3390/PR6090136], https://hal.inria.fr/hal-01909765

[16] E. CINQUEMANI. *Stochastic reaction networks with input processes: Analysis and application to gene expression inference*, in "Automatica", 2018, pp. 1-9, https://hal.inria.fr/hal-01925923

[17] A. KREMLING, J. GEISELMANN, D. ROPERS, H. DE JONG. *An ensemble of mathematical models showing diauxic growth behaviour*, in "BMC Systems Biology", December 2018, vol. 12, n° 1, pp. 1-16 [*DOI :* 10.1186/s12918-018-0604-8], https://hal.inria.fr/hal-01893978

[18] D. LUCENA, M. MAURI, F. SCHMIDT, B. ECKHARDT, P. L. GRAUMANN. *Microdomain formation is a general property of bacterial membrane proteins and induces heterogeneity of diffusion patterns*, in "BMC Biology", December 2018, vol. 16, n° 1 [*DOI :* 10.1186/s12915-018-0561-0], https://hal.inria.fr/hal-01966627

[19] A. MARGUET. *Uniform sampling in a structured branching population*, in "Bernoulli", 2019, https://arxiv.org/abs/1609.05678 , https://hal.archives-ouvertes.fr/hal-01362366

[20] I. YEGOROV, F. MAIRET, H. DE JONG, J.-L. GOUZÉ. *Optimal control of bacterial growth for the maximization of metabolite production*, in "Journal of Mathematical Biology", October 2018, pp. 1-48 [*DOI :* 10.1007/s00285-018-1299-6], https://hal.inria.fr/hal-01929475

Other Publications

[21] M. HOFFMANN, A. MARGUET. *Statistical estimation in a randomly structured branching population*, November 2018, working paper or preprint, https://hal.archives-ouvertes.fr/hal-01662203

[22] A. MARGUET. *A law of large numbers for branching Markov processes by the ergodicity of ancestral lineages*, March 2018, https://arxiv.org/abs/1707.07993 - working paper or preprint, https://hal.archives-ouvertes.fr/hal-01567317