IN PARTNERSHIP WITH:
Université de Grenoble Alpes

Activity Report 2019

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, HDR]
Aline Marguet [Inria, Researcher, from Oct 2019]
Delphine Ropers [Inria, Researcher]

Faculty Members

Johannes Geiselmann [Univ Grenoble Alpes, Professor, HDR]
Stéphan 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, Post-Doctoral Fellow, until Sep 2019]
Marco Mauri [Inria, Post-Doctoral Fellow, until Apr 2019]

PhD Students

Thibault Etienne [INRA, PhD Student]
Antrea Pavlou [Inria, PhD Student]
Maaike Sangster [Inria, PhD Student, from Oct 2019]

Technical staff

Tamas Muszbek [Inria, Engineer]
Corinne Pinel [CNRS, Engineer]
Célia Boyat [Univ Grenoble Alpes]

Interns and Apprentices

Arieta Shabani [Inria, until Jul 2019]
Emmanouil Sideris [Inria, from Feb 2019 until Apr 2019]

Administrative Assistants

Alexandra Fitzgerald [Inria, Administrative Assistant, until Sep 2019]
Diane Courtiol [Inria, Administrative Assistant, from Oct 2019]

Visiting Scientist

Tomas Gedeon [Professor in Mathematics, Montana State University, from May 2019 until Aug 2019]

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.

[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.

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 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 80 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.

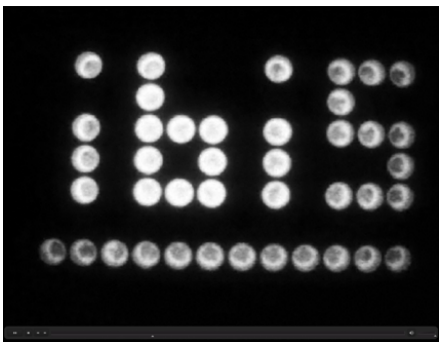


Figure 1. Display of the project-team name on a "bacterial billboard" (see http://team.inria.fr/ibis for the corresponding movie). A microplate containing a minimal medium (with glucose and acetate) is filmed during 36 hours. Wells contain E. coli bacteria which are transformed with a reporter plasmid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

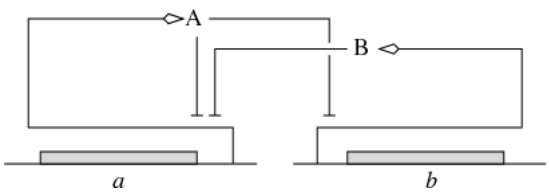
Participants: Hidde de Jong [Correspondent], Michel Page, Delphine Ropers.

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

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.

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, Stéphan 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.

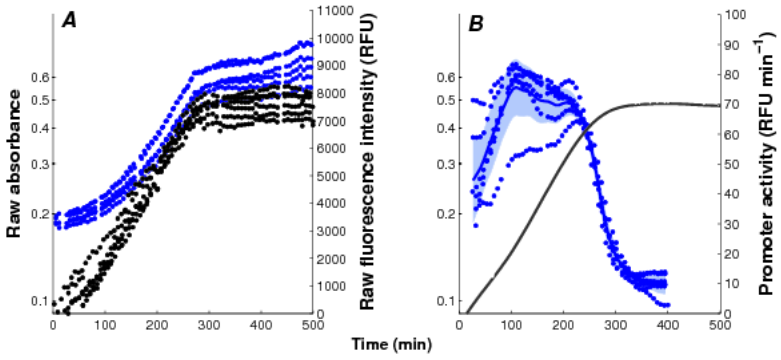


Figure 3. Monitoring of bacterial gene expression in vivo using fluorescent reporter genes (Stefan et al., PLoS Computational Biology, 11(1):e1004028, 2015). The plots show the primary data obtained in a kinetic experiment with E. coli cells, focusing on the expression of the motility gene tar in a mutant 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, Stéphan 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], Stéphan Lacour, Marco Mauri, Tamas Muszbek, Michel Page, Antrea Pavlou, Delphine Ropers, Maaike Sangster.

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.

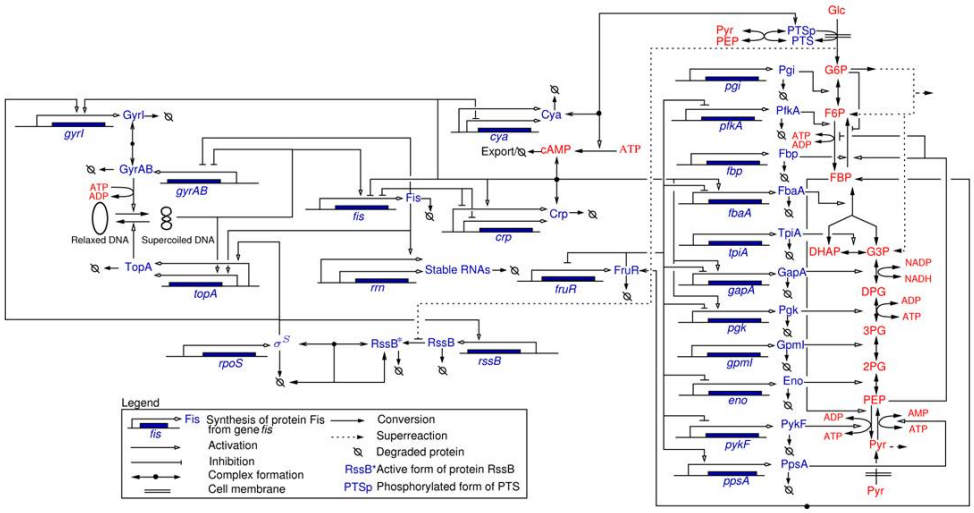


Figure 4. Network of key genes, proteins, and regulatory interactions involved in the carbon assimilation network in E. coli (Baldazzi et al., PLoS Computational Biology, 6(6):e1000812, 2010). The metabolic part includes the glycolysis/gluconeogenesis pathways as well as a simplified description of the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

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 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.

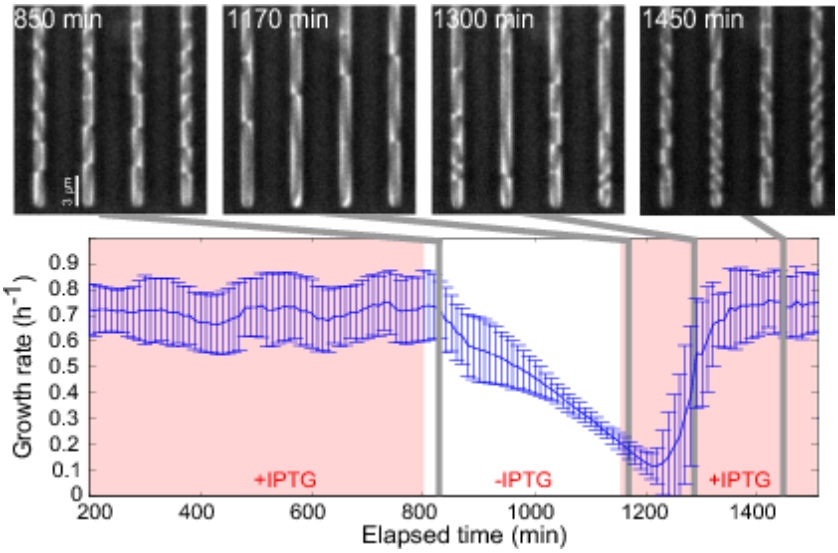


Figure 5. Growth arrest by external control of the gene expression machinery (Izard, Gomez Balderas et al., Molecular Systems Biology, 11:840, 2015). An E. coli strain in which an essential component of the gene expression machinery, the $\beta\beta'$ subunits of RNA polymerase, was put under the control 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

A publication on the use of mixed-effects models for the analysis of the inheritance and variability of gene expression parameters along lineage trees was published in a special issue of *Bioinformatics* and presented at

the major bioinformatics conference ISMB/ECCB 2020. A publication in *BMC Bioinformatics* accompanied the release of the new version of the web application WELLINVERTER for the analysis of fluorescent reporter gene data. IBIS member Michel Page launched his start-up ProLeads (https://proleads.fr/), a specialized business search engine.

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: Publication in BMC Bioinformatics describing the new version of 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). Publication in BMC Bioinformatics 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. 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. Tutorial on the use of the model formalism for analyzing synthetic genetic circuits.

- 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 [13], 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. 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. An article 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, was published in *BMC Bioinformatics* this year [22]. We notably show that the expression pattern in different growth media, supporting different growth rates, corresponds to the pattern expected for a constitutive gene.

6.2. 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 6.1. 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. The investigation of the problem of reconstructing promoter activity statistics from reporter gene population snapshot data has led 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. The method has been demonstrated on simulated data. Theoretical as well as simulation resuts have been published in *Automatica* this year [15], and will be the object of application to real data.

One of the key limitations of the method is the assumption of stationary promoter activation statistics. In the context of controlled gene expression processes, this may hamper applicability of the method. In response to this, an extension of the method for so-called modulated processes (stationary processes reshaped by a time-varying control input), has been developed and demonstrated on simulations of controlled gene expression. Results were submitted for possible presentation and publication in the proceedings of the IFAC world congress 2020.

6.3. Mathematical analysis of structured branching populations

The investigation of cellular populations at the single-cell level has led to the discovery of important phenomena, such as the co-occurrence of different phenotypes in an isogenic population. Novel experimental techniques, such as time-lapse fluorescence microscopy combined with the use of microfluidic devices (Section 3.2), enable one to take the investigation further by providing time-course profiles of the dynamics of

individual cells over entire lineage trees. The development of models that take into account the genealogy of individual cells is an important step in the study of inheritance in bacterial population. As a prerequisite, the efficient analysis of single-cell data relies on the mathematical analysis of those models.

Structured branching processes allow for the study of populations, where the lifecycle of each cell is governed by a given characteristic or trait, such as the concentration of a specific protein inside the cell. The dependence of bacterial phenotypes like cell division times or ageing on such characteristics has been investigated by Aline Marguet using mathematical analysis of the underlying processes. To understand the long-time behavior of structured branching populations, the process describing the trait of a typical individual along its ancestral lineage, called auxiliary process [21] and its asymptotic behavior play a key role. In a publication in *ESAIM: Probability and Statistics* that appeared this year [20], we proved that the empirical measure of the structured branching process converges to the mean value of this auxiliary process. The approach relies on ergodicity arguments for the time-inhomogeneous auxiliary Markov process. The novelty compared to existing spectral methods is that our method allows to consider processes with time-varying rates for the modeling of changing environments. For example, we studied the case of a size-structured population in a varying environment and proved the convergence of the empirical measure in this specific case.

In collaboration with Charline Smadi (IRSTEA Grenoble), Aline Marguet also investigated the long-time behavior of a general class of branching Markov processes. This work, which has been submitted for publication [27], aims at understanding the link between the dynamic of the trait and the dynamic of the population. In the case of a trait modelling the proliferation of a parasite infection in a cellular population, we exhibit conditions on the dynamics of the parasites to survive in the population, despite the cellular divisions that dilute the number of parasites in each cell.

The study of the asymptotic behavior of general non-conservative semigroups is important for several aspects of branching processes, especially to prove the efficiency of statistical procedures. Vincent Bansaye from École Polytechnique, Bertrand Cloez from INRA Montpellier, Pierre Gabriel from Université Versailles Saint-Quentin, and Aline Marguet obtained necessary and sufficient conditions for uniform exponential contraction in weighted total variation norm of non-conservative semigroups. It ensures the existence of Perron eigenelements and provides quantitative estimates of spectral gaps, complementing Krein-Rutman theorems and generalizing recent results relying on probabilistic approaches. This work was submitted for publication this year [26].

6.4. Inference of gene expression parameters on lineage trees

As explained in the previous section, recent technological developments have made it possible to obtain time-course single-cell measurements of gene expression as well as the associated lineage information. However, most of the existing methods for the identification of mathematical models of gene expression are not well-suited to single-cell data and make the simplifying assumptions that cells in a population are independent, thus ignoring cell lineages. The development of statistical tools taking into account the correlations between individual cells will allow in particular for the investigation of inheritance of traits in bacterial populations.

In the framework of structured branching processes, we studied the statistical reconstruction of parameters. We considered the problem of estimating the division rate from the observations of the trait of the cells at birth. Previous works on the subject considered deterministic dynamics for the evolution of the trait. In collaboration with Marc Hoffmann (Université Paris Dauphine), Aline Marguet investigated the case of a trait evolving according to a diffusion process. The study of the asymptotic behavior of the tagged-chain, corresponding to the trait of a uniformly chosen individual, allowed us to prove the convergence of the empirical measure of the branching process, and the asymptotic minmax efficiency of nonparametric estimators for the density of the transition kernel and the invariant measure of the tagged-chain. For the estimation of the division rate, we proved in a parametric framework the asymptotic efficiency of a standard maximum likelihood proxy estimation. Finally, we demonstrate the validity of our approach on simulated datasets. The results of this work were published in *Stochastic Processes and their Applications* [17].

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], Eugenio Cinquemani, Marc Lavielle (XPOP, Inria Saclay–Île-de-France) and Aline Marguet developed a new model and a method for inference from data for gene expression along tree where the kinetic expression parameters are assumed to be inherited from the mother cell in an autoregressive way. This model generalizes the state-of-the-art mixed-effect models to the case of lineage trees. We implemented the inference procedure in Julia and proved that it provides unbiased estimates of the parameters. The application to the data of osmotic shock response by yeast show that the correlation between the parameter of a cell and its daughter is of 0.6 according to our model, leading to new biological questions such as the understanding of the origin of this inheritance. The results of this study were presented at the major bioinformatics conference ISMB/ECCB 2020 and published in the associated special issue of *Bioinformatics* [19].

6.5. Modeling and inference of RNA degradation

The ability to rapidly respond to changing nutrient availability is crucial for *E. coli* to survive in many environments including the gut. Reorganization of gene expression is the first step for bacteria to adjust their metabolism accordingly. It involves fine-tuning of both transcription and mRNA stability by dedicated regulatory interactions. While transcriptional regulation has been largely studied, the role of mRNA stability during a metabolic switch is poorly understood.

This question was addressed in the framework of the PhD thesis of Manon Morin funded by an INRA-Inria grant. Using combined genome-wide transcriptome and mRNA decay analyses, Manon Morin, Delphine Ropers and colleagues from the Toulouse Biotechnology Institute (ex-LISBP, INRA/INSA Toulouse) investigated the role of mRNA stability in the response of *E. coli* to nutrient changes. They demonstrated that transcript stability increases along metabolic transitions representative of the carbon source fluctuations, the glucose-acetate-starvation transition [9], [10]. Most of the stabilization occurs at glucose-acetate transition when glucose is exhausted. Stabilized mRNAs remain stable during acetate consumption and carbon starvation. Meanwhile, expression of most genes is downregulated. Metabolic control analysis showed that most of gene expression regulation is driven by changes in transcription. Post-transcriptional regulations appear to be important for genes involved in bacterial response to nutrient starvation. These results have been further developed in a paper recently submitted to a biology journal.

The observation of a global stabilization of cellular mRNAs during adaptation to carbon source depletion raises questions about the regulatory mechanisms at work. Known regulators of mRNA stability such as the protein Hfq, the carbon storage regulator Csr, and several small regulatory RNAs, specifically target mRNAs. Are these regulatory mechanisms sufficient to explain the systematic adjustment of mRNA half-lives? The collaboration with Muriel Cocaign-Bousquet and colleagues from the Toulouse Biotechnology Institute has been pursued to answer these questions, in the context of the PhD thesis of Thibault Etienne, funded by an INRA-Inria PhD grant. The objective is to develop models able to explain how cells coordinate their physiology and the functioning of the degradation machinery following environmental changes. In a paper submitted this year, Thibault Etienne, Delphine Ropers and Muriel Cocaign-Bousquet investigate the possibility that competition between mRNAs for their binding to the degradation machinery is an important mechanism for the regulation of mRNA half-lives. They develop a mathematical model of mRNA degradation and assess the role of competitive effects on mRNA degradation kinetics by numerical simulation and sensitivity analysis. Competition appears to globally increase the stability of cellular mRNAs and to amplify the effect of post-transcriptional regulation. In a follow-up study, the model is currently being used to interpret large data sets corresponding to the degradation kinetics of 4254 mRNAs in *E. coli* cells growing in four different environmental conditions.

6.6. 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 in combination with methods from optimal control theory. In a paper published in the *Journal of Mathematical Biology* this year [24], 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, were presented at the European Control Conference (ECC 2019) in Naples this year and published in the proceedings [25].

6.7. Bacterial growth inhibition by acetate

High concentrations of organic acids such as acetate inhibit growth of *Escherichia coli* and other bacteria. This phenomenon is of interest for understanding bacterial physiology but is also of practical relevance. Growth inhibition by organic acids underlies food preservation and causes problems during high-density fermentation in biotechnology. The development of new approaches for the relief of growth inhibition by acetate during high-density fermentation of *E. coli* is one of the motivating assumptions for the work of IBIS in the IPL project COSY (Sections 7.2 and 6.8 below).

What causes growth inhibition by acetate? Classical explanations invoke the uncoupling effect of acetate and the establishment of an anion imbalance. During his PhD thesis, Stéphane Pinhal investigated an alternative hypothesis: the perturbation of acetate metabolism due to the inflow of excess acetate. In an experimental and modelling study published in the *Journal of Bacteriology* [23], Stéphane Pinhal, Delphine Ropers, Hans Geiselmann, and Hidde de Jong developed a set of isogenic strains that remove different parts of the metabolic network involved in acetate metabolism. Analysis of these strains revealed that the inflow of acetate accounts for 20% of the growth-inhibitory effect through a modification of the acetyl phosphate concentration. While the study does not provide a definite answer to the question of what accounts for the remaining 80% of the reduction in growth rate, some of the observations argue against a prominent role of uncoupling in growth inhibition by acetate in the conditions tested.

6.8. Modeling synthetic microbial communities for improving productivity

Modelling, analysis and control of microbial community dynamics is a fast-developing subject with great potential implications in the understanding of natural processes and the enhancement of biotechnological processes. Within the IPL COSY (Section 7.2), we picked up the challenge to design and investigate the dynamics of synthetically engineered microbial communities with a consortium of Inria partners. In IBIS, in

particular, we are addressing the design of a bacterial community of two *E.coli strains*, mimicking mutualistic relationships found in nature, and with the potential to outperform a single producer strain in the production of a heterologous protein. During the post-doctoral stay of Marco Mauri, we developed an ODE model of the key growth phenotypes of the community and their interactions, calibrated the model on literature data, and analysed the model for an in-depth understanding of the conditions supporting coexistence and of the tradeoffs encountered in this production process. The results are presented in a paper submitted for publication this year and will be tested experimentally in the framework of the recently-started PhD project of Maaike Sangster. Analysis of optimal community control problems as well as design and deployment of optimal control strategies will follow in synergy with other IPL COSY partners.

6.9. Detection of small non-coding RNAs

Small non-coding RNAs (sRNAs) regulate numerous cellular processes in all domains of life. Several approaches have been developed to identify them from RNA-seq data, which are efficient for eukaryotic sRNAs but remain inaccurate for the longer and highly structured bacterial sRNAs. Together with colleagues from INSA de Lyon, Stéphan Lacour developed APERO, a new algorithm to detect small transcripts from paired-end bacterial RNA-seq data. This algorithm is based on a novel approach, which does not start from the read coverage distribution, but analyzes boundaries of individual sequenced fragments to infer the 5´ and 3´ ends of all transcripts. Validation of the algorithm on *Escherichia coli* and *Salmonella enterica* datasets, based on experimentally validated sRNAs, showed it to outperform all existing methods in terms of sRNA detection and boundary precision. Moreover, APERO was able to identify the small transcript repertoire of *Dickeya dadantii* including putative intergenic RNAs, 5´ UTR or 3´ UTR-derived RNA products and antisense RNAs. This work was published in *Nucleic Acids Research* this year [18]. APERO is freely available as an open source R package (https://github.com/Simon-Leonard/APERO). In other work, together with colleagues from the University of Salento, Lecce (Italy), Stéphan Lacour contributed to RHOTERMPREDICT, an algorithm for predicting Rho-dependent transcription terminators in bacterial genomes [16].

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	D. Ropers
IBIS participants	D. Ropers, T. Etienne
Type	IXXI/BioSyl project (2018-2020)
Web page	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	S. Lacour
IBIS participants	S. Lacour
Type	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 IBIS participants Type Web page	E. Cinquemani E. Cinquemani, H. de Jong, J. Geiselmann, M. Mauri, T. Muszbek, C. Pinel, D. Ropers, M. Sangster Inria Project Lab (2017-2021) https://project.inria.fr/iplcosy/

Project name	OPTICO : OPTImal COntrol software for microbial communities in a system of minibioreactors
Coordinator IBIS participants Type	E. Cinquemani E. Cinquemani, H. de Jong, J. Geiselmann, T. Muszbek Inria ADT (2019-2021)

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 (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. International Research Visitors

7.3.1. Visits of International Scientists

Tomas Gedeon, professor in Mathematics at Montana State University (USA), visited the IBIS project-team during two months (May-July 2019) to work on modeling and analysis of resource allocation in microorganisms. His stay at Inria was funded by the Visiting researcher program of the research center Grenoble - Rhône-Alpes.

7.3.1.1. Internships

Emmanouil Sideris, enrolled in the MSc program in Computer Science at the University of Patras (Greece), did a Master internship with Eugenio Cinquemani.

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
Aline Marguet	Biohasard: Stochastic models for biology, Grenoble	Jun 2020
Delphine Ropers	Séminaire de Modélisation du Vivant (SeMoVi), Lyon and Grenoble	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 and 2020)	Associate editor

8.1.2.2. Member of conference program committees

IBIS member	Conference, workshop, program
Eugenio Cinquemani	ECC 2019 and 2020, CMSB 2018 and 2019, HSB 2019 and 2020, SASB 2019, IEEE CIBCB 2019
Hidde de Jong	CMSB 2019, FOSBE 2019, HSB 2019 and 2020
Delphine Ropers	CSBio 2019

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
Hidde de Jong	International Human Frontier Science Program (HFSP)	Member grant selection committee
Delphine Ropers	INRA-Inria	Member selection committee PhD grants
Delphine Ropers	Elixir	Member of Microbial Biotechnology Community

8.1.5. Invited talks and other presentations

Eugenio Cinquemani

Title	Event and location	Date
Estimation and control of microbial dynamics: From single-cell to microbial communities	Presentation at Inria-KAIST workshop on Applied mathematics, Paris	Mar 2019
Optimal control of bacterial growth for metabolite production: The role of timing and costs of control	Presentation at ECC 2019, Naples (Italy)	Jun 2019
Enhanced production of heterologous proteins by a synthetic microbial consortium: Conditions and tradeoffs	Invited talk at Workshop Advanced Physics for Medicine, CNR, Rome (Italy)	Sep 2019

Hidde de Jong

Title	Event and location	Date
Integrated models of the cell: metabolism, gene expression, signalling	Tutorial at CompSysBio: Advanced Lecture Course on Computational Systems Biology, Aussois	Apr 2019
Optimal control of bacterial growth: biotechnological applications	Invited talk at Workshop New Vistas in Computational Systems and Synthetic Biology, Vigo (Spain)	May 2019
Optimal control of microbial growth and bioproduction	Invited talk at SYMER workshop, Global Challenge Week, Univ Grenoble Alpes, Grenoble	Jun 2019
Reengineering bacterial metabolism using synthetic biology and optimal control theory	Invited talk in special session on Predictive approaches for biological systems engineering, JOBIM 2019, Nantes	Jul 2019
Production of heterologous proteins by a synthetic microbial consortium: conditions and tradeoffs	Invited talk at Biocore seminar, Peyresq	Sep 2019

Stéphan Lacour

Title	Event and location	Date
New insights into the regulation of curli production by E. coli	BactoGre workshop, Grenoble	Avr 2019

Aline Marguet

Title	Event and location	Date
Inheritance and variability of kinetic gene expression parameters in microbial cells: Modelling and inference from lineage tree data	Invited talk at École de printemps de la chaire MMB, Aussois	May 2019
Inheritance and variability of kinetic gene expression parameters in microbial cells: Modelling and inference from lineage tree data	Presentation at ISMB/ECCB conference, Basel (Switzerland)	Jul 2019
Inheritance and variability of kinetic gene expression parameters in microbial cells: Modelling and inference from lineage tree data	Invited talk at Biohasard 2019 workshop, Rennes	Aug 2019
Inheritance and variability of kinetic gene expression parameters in microbial cells: Modelling and inference from lineage tree data	Presentation at Journée Inria-Bio, Lyon	Oct 2019
Inheritance and variability of kinetic gene expression parameters in microbial cells: Modelling and inference from lineage tree data	Seminar Master Mathématiques pour les Sciences du Vivant, Ecole Polytechnique, Palaiseau	Oct 2019
Modelling and statistics of branching processes	Workshop on Growth and division in mathematics and medicine, London (UK)	Nov 2019
Inheritance and variability of kinetic gene expression parameters in microbial cells: Modelling and inference from lineage tree data	Seminar équipe-projet DRACULA, Lyon	Nov 2019
Inheritance and variability of kinetic gene expression parameters in microbial cells: Modelling and inference from lineage tree data	Seminar unité MaIage, Jouy-en-Josas	Dec 2019
Long time behavior of a general class of branching Markov processes	Seminar Institut Fourier, Grenoble	Dec 2019

Marco Mauri

Title	Event and location	Date

Thibault Etienne

Title	Event and location	Date
Regulatory mechanisms underlying coordination of mRNA degradation and cell physiology in Escherichia coli	Seminar during Journée annuelle du LIPhy, Grenoble	Jun 2019
Large scale modelling of mRNA decay in Escherichia coli	Seminar during Journée InriaBio, ENS de Lyon	Oct 2019

Antrea Pavlou

Title	Event and location	Date
Experimental and computational analysis of bacterial self-replicators	Poster at CompSysBio: Advanced Lecture Course on Computational Systems Biology, Aussois	Apr 2019
Experimental and computational analysis of bacterial self-replicators	Seminar for GdT MathBio, Grenoble	Nov 2019

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 Grenoble - Rhône-Alpes	Référente chercheurs
Delphine Ropers	Inria Grenoble - Rhône-Alpes	Co-coordinator of mentoring program

8.1.7. Recruitment committees

IBIS member	Organism	Recruitment
Johannes Geiselmann	University of Barcelona	Assistant professor
Delphine Ropers	Inria Bordeaux Sud-Ouest	Chargés de recherche (jury d'admissibilité)
Delphine Ropers	Inria national	Chargés de recherche (jury d'admissibilité)
Delphine Ropers	Univ de Rennes	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 (4 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)

Aline Marguet

Practicals: Biostatistics, M2, Univ Grenoble Alpes (24 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)

Thibault Etienne

Course: Construction et analyse de plasmides in silico, M1, Master ingéniérie de la santé, Univ Grenoble Alpes (4 h)

Antrea Pavlou

Practicals: Cellular biology, L1, Chemistry and biochemistry, Univ Grenoble Alpes (28 h)

8.2.2. Supervision

HdR: **Eugenio Cinquemani**, Identification, estimation and control of gene expression and metabolic network dynamics [14], Univ Grenoble Alpes, Nov 2019

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

PhD in progress: **Maaike Sangster**, Development, characterization and control of E. coli communities on an automated experimental platform. Supervisors: Eugenio Cinquemani and Johannes Geiselmann

8.2.3. Juries

PhD thesis committees

IBIS member	Role	PhD student	University	Date
Johannes Geiselmann	Président	Vanni Petrolli	Univ Grenoble Alpes	Nov 2019
Hidde de Jong	Rapporteur	Pauline Trébulle	AgroParisTech	Oct 2019
Hidde de Jong	Rapporteur	Firas Hammami	Univ d'Aix-Marseille	Dec 2019
Delphine Ropers	Examinateur	Marianyela Petrizzelli	Univ Paris Sud	Jul 2019
Delphine Ropers	Examinateur	Ronan Duchesne	Univ de Lyon	Dec 2019

Habilitation (HDR) committees

IBIS member	Role	HDR candidate	University	Date
Johannes Geiselmann	Président	Marianne Weidenhaupt	Univ Grenoble Alpes	Jan 2019
Hidde de Jong	Président	Arnaud Tonnelier	Univ Grenoble Alpes	Jun 2019
Hidde de Jong	Rapporteur	Stefan Müller	University of Vienna	Sep 2019
Hidde de Jong	Examinateur	Eugenio Cinquemani	Univ Grenoble Alpes	Nov 2019

PhD advisory committees

IBIS member	PhD student	University
Eugenio Cinquemani	Arthur Carcano	Institut Pasteur/Inria
Eugenio Cinquemani	Alexey Koshkin	ENS de Lyon
Johannes Geiselmann	Alain Lombard	Univ Grenoble Alpes
Johannes Geiselmann	Shiny Martis	INSA de Lyon
Hidde de Jong	Charlotte Coton	INRA Moulon
Hidde de Jong	Kapil Newar	Univ Grenoble Alpes
Hidde de Jong	Agustín Yabo	Univ Nice–Sophia-Antipolis
Stéphan Lacour	Julien Trouillon	CEA Grenoble
Stéphan Lacour	Camille Brunet	CHU Grenoble
Stéphan Lacour	Raphael Fourquet	INSA de Lyon/Univ de Lyon
Stéphan Lacour	Kevin Pick	Univ de Lyon
Delphine Ropers	Manon Barthe	Univ Paul Sabatier, Toulouse
Delphine Ropers	Charlotte Roux	Univ Paul Sabatier, 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. Interventions

Delphine Ropers participated in the colloquium FIGAS 2019 - Un rêve pour les filles et les garçons : la Science (Nov 2019). She was moderator of the session "Stéréotypes dans le numérique", and co-presented with Thierry Vieville "Mais comment éduquer les garçons à l'équité des genres au niveau informatique et numérique ?".

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^{o} 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^{o} 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^{o} 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^{o} 2, e1004706 p. , https://hal.inria.fr/hal-01248298

[8] A. MARGUET, M. LAVIELLE, E. CINQUEMANI. *Inheritance and variability of kinetic gene expression parameters in microbial cells: modeling and inference from lineage tree data*, in "Bioinformatics", 2019, vol. 35, n^{o} 14, pp. i586–i595 [*DOI :* 10.1093/BIOINFORMATICS/BTZ378], https://hal.archives-ouvertes.fr/hal-02317115

[9] 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

[10] 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^{o} 5, pp. 1-14, https://hal.inria.fr/hal-01672038

[11] 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

[12] 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

[13] 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

Doctoral Dissertations and Habilitation Theses

[14] E. CINQUEMANI. *Identification, estimation and control of gene expression and metabolic network dynamics*, Université Grenoble-Alpes, ED MSTII, November 2019, Habilitation à diriger des recherches, https://hal.inria.fr/tel-02424024

Articles in International Peer-Reviewed Journals

[15] E. CINQUEMANI. *Stochastic reaction networks with input processes: Analysis and application to gene expression inference*, in "Automatica", 2019, vol. 101, pp. 150-156 [*DOI :* 10.1016/J.AUTOMATICA.2018.11.047], https://hal.inria.fr/hal-01925923

[16] M. DI SALVO, S. PUCCIO, C. PEANO, S. LACOUR, P. ALIFANO. *RhoTermPredict: an algorithm for predicting Rho-dependent transcription terminators based on Escherichia coli, Bacillus subtilis and Salmonella enterica databases*, in "BMC Bioinformatics", December 2019, vol. 20, n° 1, pp. 1-11 [*DOI :* 10.1186/s12859-019-2704-x], https://hal.inria.fr/hal-02423208

[17] M. HOFFMANN, A. MARGUET. *Statistical estimation in a randomly structured branching population*, in "Stochastic Processes and their Applications", 2019, vol. 129, n° 12, pp. 5236-5277 [*DOI :* 10.1016/J.SPA.2019.02.015], https://hal.archives-ouvertes.fr/hal-01662203

[18] S. LEONARD, S. MEYER, S. LACOUR, W. NASSER, F. HOMMAIS, S. REVERCHON. *APERO: a genome-wide approach for identifying bacterial small RNAs from RNA-Seq data*, in "Nucleic Acids Research", May 2019, vol. 47, n° 15, pp. 1-12 [*DOI :* 10.1093/NAR/GKZ485], https://hal.archives-ouvertes.fr/hal-02152104

[19] A. MARGUET, M. LAVIELLE, E. CINQUEMANI. *Inheritance and variability of kinetic gene expression parameters in microbial cells: modeling and inference from lineage tree data*, in "Bioinformatics", 2019, vol. 35, n° 14, pp. i586-i595 [*DOI :* 10.1093/BIOINFORMATICS/BTZ378], https://hal.archives-ouvertes.fr/hal-02317115

[20] A. MARGUET. *A law of large numbers for branching Markov processes by the ergodicity of ancestral lineages*, in "ESAIM: Probability and Statistics", 2019, pp. 1-23, https://arxiv.org/abs/1707.07993 , forthcoming [*DOI :* 10.1051/PS/2018029], https://hal.archives-ouvertes.fr/hal-01567317

[21] A. MARGUET. *Uniform sampling in a structured branching population*, in "Bernoulli", 2019, vol. 25, n° 4A, pp. 2649-2695, https://arxiv.org/abs/1609.05678 [*DOI :* 10.3150/18-BEJ1066], https://hal.archives-ouvertes.fr/hal-01362366

[22] Y. MARTIN, M. PAGE, C. BLANCHET, H. DE JONG. *WellInverter: a web application for the analysis of fluorescent reporter gene data*, in "BMC Bioinformatics", December 2019, vol. 20, n° 1, 309 p. [*DOI :* 10.1186/s12859-019-2920-4], https://hal.inria.fr/hal-02195461

[23] S. PINHAL, D. ROPERS, J. GEISELMANN, H. DE JONG. *Acetate metabolism and the inhibition of bacterial growth by acetate*, in "Journal of Bacteriology", July 2019, vol. 201, n° 13, pp. 147 - 166 [*DOI :* 10.1128/JB.00147-19], https://hal.inria.fr/hal-02195459

[24] 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", 2019, vol. 78, n° 4, pp. 985–1032 [*DOI :* 10.1007/s00285-018-1299-6], https://hal.inria.fr/hal-01929475

International Conferences with Proceedings

[25] E. CINQUEMANI, F. MAIRET, I. YEGOROV, H. DE JONG, J.-L. GOUZÉ. *Optimal control of bacterial growth for metabolite production: The role of timing and costs of control*, in "ECC 2019 - 18th European Control Conference", Naples, Italy, IEEE, June 2019, pp. 2657-2662 [*DOI :* 10.23919/ECC.2019.8796079], https://hal.inria.fr/hal-02384034

Other Publications

[26] V. BANSAYE, B. CLOEZ, P. GABRIEL, A. MARGUET. *A Non-Conservative Harris' Ergodic Theorem*, March 2019, https://arxiv.org/abs/1903.03946 - working paper or preprint, https://hal.archives-ouvertes.fr/hal-02062882

[27] A. MARGUET, C. SMADI. *Long Time Behaviour of a General Class of Branching Markov Processes*, August 2019, working paper or preprint, https://hal.archives-ouvertes.fr/hal-02274642