RESEARCH CENTRE

Inria Paris Center

IN PARTNERSHIP WITH:

Institut Pasteur

2022

ACTIVITY REPORT

Project-Team

INBIO

Experimental and Computational Methods for Modeling Cellular Processes

IN COLLABORATION WITH: Centre de Bioinformatique, Biostatistique et Biologie Intégrative

DOMAIN

Digital Health, Biology and Earth

THEME

Modeling and Control for Life Sciences

Inria

Contents

Project-Team INBIO

Creation of the Project-Team: 2019 November 01

Keywords

Computer sciences and digital sciences

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1 Team members, visitors, external collaborators

Research Scientists

- Gregory Batt [Team leader, Inria, Senior Researcher, HDR]
- Jakob Ruess [Inria, Researcher]

Post-Doctoral Fellows

- Allyson Holmes [Institut Pasteur, from Feb 2022]
- Sara Napolitano [Institut Pasteur]
- Sebastian Sosa Carrillo [Inria, until Jul 2022]

PhD Students

- Henri Galez [Institut Pasteur, from Sep 2022]
- Viktoriia Gross [Institut Pasteur]

Technical Staff

- Achille Fraisse [Institut Pasteur, Engineer, until Jan 2022]

Administrative Assistants

- Christine Anocq [Inria]
- Mélanie Ridel [Institut Pasteur]

Visiting Scientist

- Lorenzo Pasotti [University of Pavia]

External Collaborator

- François Bertaux [Lesaffre]

2 Overall objectives

The main objective of our research is to understand, control, and optimize cellular processes in single cells and at the population level. We combine experimental and theoretical work within a single team.

Our focus is on developing methods and models that take stochasticity of intracellular processes and heterogeneity of cell populations into account. To this end, we use both mixed-effects models as well as continuous-time Markov chains and their diffusion approximations. We develop methods for efficiently calculating with such models and use them to design optimally informative experiments and to reverse engineer unknown cellular processes from experimental data. Furthermore, we deploy models in order to optimally construct and optimally control synthetic gene circuits.

We have recently started to set up our own biology laboratory at Institut Pasteur. We develop novel experimental platforms that are designed to be fully automated, controllable by our own software, and capabale of updating the experimental plan in response to incoming measurements. Optogenetic actuation of intracellular processes, coupled to real time fluorescence measurements by microscopy or flow cytometry, then allows us to connect cellular processes with models and algorithms in real time.

The spritit of our work is that experimental platforms and circuits should be constructed with our theoretical work in mind, while our mathematical methods should be usable to adress concrete experimental questions in the lab.

3 Research program

3.1 Analysis and identification of stochastic (biochemical) reaction networks

The advancement of single-cell technologies in the last decades revealed that stochasticity is an inherent feature of cellular processes. Stochastic models, governed by the **chemical master equation** (CME), are widely used in applications to shed light on the functioning of biochemical reaction networks inside single cells. However, in most cases, the analysis of such models is based exclusively on Gillespie's stochastic simulation algorithm (SSA). SSA allows one to easily forward simulate the model but cannot be used very well for many important model analysis tasks. To overcome this problem, we develop various alternative approaches for calculating with stochastic models. In particular, we derive ordinary differential equations for the time evolutions of statistical moments of species abundances from the CME. We use **moment equations** and moment closure to develop methods for various model analysis tasks for which stochastic simulation is computationally too expensive to be practically useful, ranging from **parameter inference** to **model predictive control**. Furthermore, we recently started to make use of approximations of the CME with a **Fokker-Planck equation** to be able to also calculate with models for which low order statistical moments are not sufficient statistics of the full data and do not contain enough information.

3.2 Population dynamics emerging from randomness in single cells

Dynamics of cell populations growing in isolation or as part of some ecological system are often shaped by biochemical processes inside cells, for instance when these processes convey resistance to stressors or trigger cell fate decisions in reponse to environmental conditions. **Understanding how stochastic reaction events inside single cells affect emerging population dynamics**, and how selection effects at the population level feed back to shape single cell characteristics of cells in the population, is one of the key questions in biology. We develop **multi-scale modeling approaches** that allow us to derive emerging population dynamics from mechanistic descriptions of stochastic reaction networks inside single cells. In the past, we have used such approaches to study how stochasticity in restriction-modification systems, acting as simple bacterial innate immune systems, propagates to the ecology of bacteria and bacterial viruses and shapes the dynamics of bacterial populations. More generally, we develop and use these approaches in connection with experimental work in our lab for understanding and **controlling the dynamics of populations** in cases where **controllable system inputs inherently operate at the level of single cells** (e.g. optogenetics) but the output of interest is at the level of populations (e.g. bioproduction).

3.3 Optimal experimental design

One of the major problems in reverse engineering biochemical processes inside cells is that **cellular processes are high-dimensional and complex** with many unknown parameters while the **available data is low dimensional and corrupted by measurement errors**. Such problems can be alleviated by ensuring that the experimental plan is designed to yield data that provides as much information as possible about the unknown model parameters. We develop mathematical approaches and computational tools that can be used to calculate the expected amount of information that can be gained from a given experiment given a specification of either a stochastic model of the system (described above) or a deterministic model based on ordinary differential equations. These information calculation approaches are then coupled to optimization tools and used to plan **maximally informative experiments** in our applications.

3.4 Cybergenetics – real time control of biological processes

Cells have evolved uncountable numbers of feedback circuits to regulate their functionalities in the presence of changing environmental conditions. But can such feedback control also be externalized and placed under control of scientists? Early work on this topic suggested that **optogenetic systems, allowing for external regulation of gene expression**, have the potential to serve as an interface between cells and experimental platform that gives a computer the power to stir the functioning of cells via the application of light. We develop all the tools required to realize automated computer control of intracellular processes. On the experimental side, we develop yeast strains that are equipped with optogenetic promoters to

drive various functionalities. On the mathematical side, we develop models and software to equip our experimental platforms with the appropriate programs to realize sucessful feedback control, both at the level of single cells (microscopy) and at the level of populations (bioreactors and plate reader).

3.5 Platforms for automated reactive experiments

The core scientific activity of the team is to connect mathematical methods with biological applications in our lab. The interface between the two sides, that is the experimental platforms, is therefore of crucial importance for the success of our activities. However, platforms that can be purchased by vendors are typically delivered without the capacity to adapt the experimental plan in response to incoming measurements, a functionality that is crucially needed for deploying our computational methods (e.g. feedback control). Therefore, we develop novel experimental platforms and/or extend existing platforms with additional software and hardware that allows us to perform automated reactive experiments. Concretely, we develop a **microscopy platform and control software for yeast that uses a digital micromirror device to expose single cells to targeted light signals** that can be adjusted in real time in response to measurements taken from the cell. Furthermore, we develop a platform of 16 parallel **small scale automated bioreactors**, each equipped with controllable LEDs to allow for optogenetic gene expression and long-term reactive experiments in tightly controlled conditions. Automation of the platform is achieved via a low-cost open-source **pipetting robot that samples all reactors to a benchtop cytometer** in which single cell gene expression is measured in all sampled cells of all reactors. Finally, we develop **software to take full control of a commercial plate reader with liquid injection capabilities** (Tecan Spark). This platform allows us to use a Raspberry Pi to pilot 96 parallel reactive experiments where optical density is used as a readout of bacterial growth.

4 Application domains

4.1 Preamble

Since most of our research is at the interface of mathematics and biology, there often is no clear split between mathematical reaseach objectives and applications. For instance, feedback control of gene expression is simultaneously a mathematical and an applied problem.

4.2 Understanding resistance and tolerance to antibiotic treatments

The non-susceptibility of pathogenic bacteria to antibiotic treatments is a major health problem. Bacteria might escape treatments in two ways: being resistant or being tolerant. Whereas resistant bacteria can multiply in presence of antibiotics, tolerant bacteria can merely survive. Yet, tolerance is increasingly recognized as a major player in treatment failure. In particular, an increasing fraction of commensal and pathogenic *E coli* bacteria express extended-spectrum β-lactamases and/or carbapenemases. When individual bacteria die as a consequence of antibiotic treatments, these enzymes are released and hydrolyze the antibiotic molecules in the environment, conveying a transient protection to the remaining bacteria that lasts until the enzymes are degraded themselves. Understanding how this collective antibiotic tolerance (CAT) shapes population dynamics is difficult yet important for **optimally killing bacterial populations**: when a second antibiotic dose is applied directly after a first dose it will not be effective since the antibiotics will be degraded by the enzymes released from bacteria killed after the first dose; when the second dose is applied too late the surviving bacterial population will have regrown to a large size. Our plate reader platform allows us to apply complex temporal patterns of antibiotic treatments to bacteria over nearly two days. Parallezing such treatments in the 96 well plates allows us to generate rich data sets and to calibrate population dynamics models that can be used to optimize temporal treatment plans. One of the applied objectives of our team is to use these capacities to study a collection of fully-sequenced clinical isolates treated with a broad range of clinically important antibiotics and grown in various media. Ideally, this will lead to an approach that can be used to assay tolerance to antibiotics in hospitals instead of, or in addition to, standard antibiotic susceptibility tests, detecting resistance.

4.3 Optimization of protein production in yeast

Many proteins are of technological or therapeutical importance. The yeast *S. cerevisiae* is an interesting organism for protein bioproduction since it combines a relatively fast growth rate with good capacities to perform post-translational modifications needed for protein maturation and full functionality. However, imposing a strong demand on protein production to the cell places a significant burden on its physiology, either at the protein production level or at the maturation and secretion levels. Using systems and synthetic biology approaches, we aim at **better understanding the origins of the production bottlenecks** and then using modeling and control approaches, we aim at finding **optimal control solutions** for bioproduction. Three different strategies are envisioned. In the first approach, bioproduction stress sensors are used to observe in real time the physiological state of the cell, and the demand is externally tuned based on the stress level of the cell population. In the second approach, the stress sensor is used to tune the response capacities of the cell to the external demand, thus creating an internal feedback loop. In the third approach, we control the fraction of the producing cells by engineering an artificial differentiation system that implements the partial differentiation of grower cells into producer cells. The optimization problem is then to find the optimum ratio based on the external environment of the cells.

5 Social and environmental responsibility

5.1 Footprint of research activities

A significant part of our daily research activities involves molecular biology work and consumes plasticware and various chemicals. We also work on lab automation and develop experimental platform to paralellize experiments. However, we work with small biorectors (15 to 50mL), so volumes of cell cultures remain very modest.

We also occasionally use a computer cluster, notably for optimization, but the jobs remain relatively modest on a yearly basis.

Allyson Holmes and Henri Galez are part of a committee working on the quantification of the carbon footprint of the computational works done in the Computational Biology Department of Institut Pasteur.

5.2 Impact of research results

Regarding biological developments, we have two main research directions.

The first one deals with the optimization of bioproduction. Bioproduction is a domain of strategic importance. The field is higly technological and rapidly growing at the global scale. The market for biopharmaceuticals alone, that notably include vaccines and monoclonal antibodies, is estimated to $400B to $500B. France imports > 70% of its vaccines and > 95% of its monoclonal antibodies and lacks sovereignty. Therefore this field has a strong social, medical and economical importance.

The second research direction deals with antibiotic stewardship. The spread of antimicrobial resistance is a both a health and an ecological problem of global impact. Antibiotic stewardship aims at using these drugs in more appropriate ways. To do so, one has to better understand and quantify bacterial response to antibiotic treatments.

Therefore our two main research directions are both tightly connected with important health and social issues.

6 Highlights of the year

6.1 ERC starting grant awarded to Jakob Ruess

Jakob Ruess has been awarded a prestigious ERC grant for his project entitled "From single cells to microbial consortia: bridging the gaps between synthetic circuit design and emerging dynamics of heterogeneous populations (Bridging-Scales)".

The Bridging-Scales project sets out to combine mathematical models for single-cell biological processes with microbial population dynamics to shed light on cell interdependence. The aim is to

improve our control over large microbial populations, especially so that we can optimize large-scale synthetic biology applications.

6.2 ReacSight and MicroMator both published in Nature Communications

Over the years, the team has developed software tools to facilitate the realization of smarter experiments. In 2022, two software tools, ReacSight and MicroMator, have been published in *Nature Communications*, a highly selective multi-disciplinary journal. The realization of experiments that extend the limits of what we are able to do has been essential to demonstrate the actual potential of these tools.

7 New software and platforms

We have continued our developments on **MicroMator** for enabling microscopy experiments to be reactive, and on **ReacSight** for the automation of bioreactor-based platforms.

Both software tools, together with challenging case studies, have been published this year, each in an article in *Nature Communications*.

8 New results

8.1 Enhancing bioreactor arrays for automated measurements and reactive control with ReacSight

Participants: François Bertaux, Sebastian Sosa-Carrillo, Viktoriia Gross, Achille Fraisse, Chetan Aditya, Mariella Furstenheim, Gregory Batt.

Small-scale, low-cost bioreactors provide exquisite control of environmental parameters of microbial cultures over long durations. Their use is gaining popularity in quantitative systems and synthetic biology. However, existing setups are limited in their measurement capabilities. In [2], we present ReacSight, a strategy to enhance bioreactor arrays for automated measurements and reactive experiment control. ReacSight leverages low-cost pipetting robots for sample collection, handling and loading, and provides a flexible instrument control architecture. We showcase ReacSight capabilities on three applications in yeast. First, we demonstrate real-time optogenetic control of gene expression. Second, we explore the impact of nutrient scarcity on fitness and cellular stress using competition assays. Third, we perform dynamic control of the composition of a two-strain consortium. We combine custom or chi.bio reactors with automated cytometry. To further illustrate ReacSight's genericity, we use it to enhance plate-readers with pipetting capabilities and perform repeated antibiotic treatments on a bacterial clinical isolate.

The ReacSight software can be found online https://gitlab.inria.fr/InBio/Public/reacsight. The experimental data generated in this study have been deposited on Zenodo (https://doi.org/10.5281/zenodo.4776009).

8.2 Enabling reactive microscopy with MicroMator

Participants: Zach Fox, Steven Fletcher, Achille Fraisse, Chetan Aditya, Sebastian Sosa-Carrillo, Julienne Petit, Sébastien Gilles, François Bertaux, Jakob Ruess, Gregory Batt.

Microscopy image analysis has recently made enormous progress both in terms of accuracy and speed thanks to machine learning methods and improved computational resources. This greatly facilitates the online adaptation of microscopy experimental plans using real-time information of the observed systems and their environments. Applications in which reactiveness is needed are multifarious. In [3] we report MicroMator, an open and flexible software for defining and driving reactive microscopy experiments. It provides a Python software environment and an extensible set of modules that greatly facilitate the

definition of events with triggers and effects interacting with the experiment. We provide a pedagogic example performing dynamic adaptation of fluorescence illumination on bacteria, and demonstrate MicroMator's potential via two challenging case studies in yeast to single-cell control and single-cell recombination, both requiring real-time tracking and light targeting at the single-cell level.

The MicroMator software can be found online https://gitlab.inria.fr/InBio/Public/micromator. The experimental data generated in this study have been deposited on Zenodo (https://doi.org/10.5281/zenodo.5761545 and https://doi.org/10.5281/zenodo.4616659).

8.3 Using single-cell models to predict the functionality of synthetic circuits at the population scale

Participants: Chetan Aditya, François Bertaux, Gregory Batt, Jakob Ruess.

Mathematical modeling has become a major tool to guide the characterization and synthetic construction of cellular processes. However, models typically lose their capacity to explain or predict experimental outcomes as soon as any, even minor, modification of the studied system or its operating conditions is implemented. This limits our capacity to fully comprehend the functioning of natural biological processes and is a major roadblock for the de novo design of complex synthetic circuits. A common cause of this problem is that cell-to-cell variability creates couplings between single-cell circuits and population processes such as selection or growth. Altering the circuit may then have unforeseen consequences inside growing populations. In [1], we construct a yeast optogenetic differentiation system that exploits cell-to-cell variability to enable external control of the population composition. We show that a simple deterministic model can explain the dynamics of the core system. However, modifying the context of the circuit by expressing system components from plasmids leads to failure of model predictions. Subsequently, we deploy theory from stochastic chemical kinetics to construct models of the system's components that simultaneously track single-cell and population processes and demonstrate that this allows us to quantitatively predict emerging dynamics of the plasmid-based system without any adjustment of model parameters. We conclude that carefully characterizing the dynamics of cell-to-cell variability using appropriate modeling theory may allow one to unravel the complex interplay of stochastic single-cell and population processes and to predict the functionality of complex synthetic circuits in growing populations before the circuit is constructed.

Software for numerical simulation can be found online https://gitlab.inria.fr/InBio/Public/predicting-selection-effects. The experimental data generated in this study have been deposited on Zenodo (https://doi.org/10.5281/zenodo.5155290).

8.4 Parameter inference for stochastic biochemical models from perturbation experiments parallelised at the single cell level

Participants: Andela Davidović, Gregory Batt, Jakob Ruess.

Understanding and characterising biochemical processes inside single cells requires experimental platforms that allow one to perturb and observe the dynamics of such processes as well as computational methods to build and parameterise models from the collected data. Recent progress with experimental platforms and optogenetics has made it possible to expose each cell in an experiment to an individualised input and automatically record cellular responses over days with fine time resolution. However, methods to infer parameters of stochastic kinetic models from single-cell longitudinal data have generally been developed under the assumption that experimental data is sparse and that responses of cells to at most a few different input perturbations can be observed. In [6], we investigate and compare different approaches for calculating parameter likelihoods of single-cell longitudinal data based on approximations of the chemical master equation (CME) with a particular focus on coupling the linear noise approximation

(LNA) or moment closure methods to a Kalman filter. We show that, as long as cells are measured sufficiently frequently, coupling the LNA to a Kalman filter allows one to accurately approximate likelihoods and to infer model parameters from data even in cases where the LNA provides poor approximations of the CME. Furthermore, the computational cost of filtering-based iterative likelihood evaluation scales advantageously in the number of measurement times and different input perturbations and is thus ideally suited for data obtained from modern experimental platforms. To demonstrate the practical usefulness of these results, we perform an experiment in which single cells, equipped with an optogenetic gene expression system, are exposed to various different light-input sequences and measured at several hundred time points and use parameter inference based on iterative likelihood evaluation to parameterise a stochastic model of the system.

8.5 Revisiting moment-closure methods with heterogeneous multiscale population models

Participants: Davin Lunz, J. Frédéric Bonnans, Jakob Ruess.

Stochastic chemical kinetics at the single-cell level give rise to heterogeneous populations of cells even when all individuals are genetically identical. This heterogeneity can lead to nonuniform behaviour within populations, including different growth characteristics, cell-fate dynamics, and response to stimuli. Ultimately, these diverse behaviours lead to intricate population dynamics that are inherently multiscale: the population composition evolves based on population-level processes that interact with stochastically distributed single-cell states. Therefore, descriptions that account for this heterogeneity are essential to accurately model and control chemical processes. However, for real-world systems such models are computationally expensive to simulate, which can make optimisation problems, such as optimal control or parameter inference, prohibitively challenging. In [9], we consider a class of multiscale population models that incorporate population-level mechanisms while remaining faithful to the underlying stochasticity at the single-cell level and the interplay between these two scales. To address the complexity, we study an order-reduction approximations based on the distribution moments. Since previous moment-closure work has focused on the single-cell kinetics, extending these techniques to populations models prompts us to revisit old observations as well as tackle new challenges. In this extended multiscale context, we encounter the previously established observation that the simplest closure techniques can lead to non-physical system trajectories. Despite their poor performance in some systems, we provide an example where these simple closures outperform more sophisticated closure methods in accurately, efficiently, and robustly solving the problem of optimal control of bioproduction in a microbial consortium model.

8.6 Maximizing protein production by keeping cells at optimal secretory stress levels using real-time control approaches

Participants: Sebastián Sosa-Carrillo, Henri Galez, Sara Napolitano, François Bertaux, Gregory Batt.

The production of recombinant proteins is a problem of major industrial and pharmaceutical importance. Secretion of the protein by the host cell considerably simplifies downstream purification processes. However, it is also the limiting production step for many hard-to-secrete proteins. Current solutions involve extensive chassis engineering to favor trafficking and limit protein degradation triggered by excessive secretion-associated stress. In [11], we propose instead a regulation-based strategy in which induction is dynamically adjusted based on the current stress level of the cells. Using a small collection of hard-to-secrete proteins and a bioreactor-based platform with automated cytometry measurements, we demonstrate that the regulation sweet spot is indicated by the appearance of a bimodal distribution of internal protein and of secretory stress levels, when a fraction of the cell population accumulates high amounts of proteins, decreases growth, and faces significant stress, that is, experiences a secretion burn-out. In these cells, adaptations capabilities are overwhelmed by a too strong production. With

these notions, we define an optimal stress level based on physiological readouts. Then, using real-time control, we demonstrate that a strategy that keeps the stress at optimal levels increases production of a single-chain antibody by 70

8.7 Optimal control of a two-species bioproducing microbial consortium

Participants: Davin Lunz, J. Frédéric Bonnans.

In [10], we study the optimal control of a system of nonlinear ordinary differential equations modeling microbial populations with light-inducable genetic differentiation that generates a two-species microbial consortium relevant for bioproduction. Appealing to Pontryagin's maximum principle, we find different optimal control structures within different regions of the parameter space. Explicit solutions are obtained in a subset of parameter space, while, for the remainder of parameter space, closed-form solutions are obtained that depend on a scalar value that solves a particular transcendental equation. We show that a unique solution of the scalar equation exists and lies in a known compact interval, making its numerical approximation particularly easy. The analytical results are verified against direct numerical calculations.

9 Partnerships and cooperations

9.1 International initiatives

- **ANR-FWF CyberCircuits** (2018-2022), on "Cybergenetic circuits to test composability of gene networks", co-coordinated by C. Guet (IST Austria, Austria) and Jakob Ruess.

  The objective of the Cybercircuit project is to explain and better predict how composed circuits function in vivo. To tackle this long standing question, we will construct hybrid bio-digital circuits in which a part of a network is effectively implemented as a biological genetic network whereas another part exists only virtually in the formof a model in a computer.

9.2 International research visitors

Lorenzo Pasotti, assistant professor at the Department of Electrical, Computer and Biomedical Engineering and at the Centre for Health Technologies of the University of Pavia (Italy), has been invited for three months in the InBio team.

9.3 National initiatives

- **Institut de Convergence Inception** (2016-2025) on the "Emergence of Diseases in Populations and in Individuals", coordinated by T. Bourgeron (Institut Pasteur). Partner institutes include Institut Pasteur, Paris Sciences et Lettres, Université de Paris, AP-HP, and research teams from CEA, CNRS, INSERM and INRA.

  The Inception's goal is to develop a core structure to mobilize data resources, numerical sciences, and fundamental experimental biology in a range of health issues. It uses integrative biology, social science and data science to understand the emergence of diseases in populations and in individuals. Inception provides funding for the PhD work of Viktoriia Gross.

- **PPR Antibiorésistance Anoruti** (2021-2025) on the "Analysis of non-response to antibiotics in vivo: application to Escherichia coli urinary tract infections", coordinated by I. El Meouche (Inserm).

  The objective of Anoruti is to identify the different factors involved in the fact that some bacteria sensitive to an antibiotic in vitro do not respond to treatment in vivo.

- **PPR Antibiorésistance Seq2Diag** (2021-2026) on "Whole genome sequencing and artificial intelligence to characterize and diagnose antibiotic resistance and capacity to escape treatment", coordinated by P. Glaser (Institut Pasteur).

Genomic sequencing has revolutionized microbiological surveillance and molecular epidemiology. The objective of the Seq2Diag project is to provide a proof of concept for its use in hospital and veterinary laboratories as a diagnostic tool for in silico antibiotic sensitivity testing.

- **ANR JCJC SmartSec** (2022-2024) on "Matching maximal host capacities: stress-informed, self-tuning bioproduction circuits", coordinated by F. Bertaux (Lesaffre) with G. Batt (Inria and Institut Pasteur).

  Bioproduction requires diverting resources normally used by host cells for growth and self-replication towards the production of desired molecules. Achieving maximal resource diversion without compromising the essential functions of the host is of critical importance, but is particularly challenging. To tackle this challenge, SmartSec aims at designing host-aware circuits, with application to the production of secreted proteins.

10 Dissemination

10.1 Promoting scientific activities

10.1.1 Journal and Conferences

Gregory Batt has been reviewer for *Nature Communications*.

Lorenzo Pasotti has been reviewer for *Biotechnology and Bioengineering*, and *Frontiers in Bioengineering and Biotechnology*.

Jakob Ruess has been reviewer for the *Journal of the Royal Society Interface*, *BMC Supplements*, the *13th IFAC Symposium on Dynamics and Control of Process Systems* (DYCOPS), and the *2022 European Control Conference* (ECC'22).

10.1.2 Invited talks

Gregory Batt has been keynote speaker at the the Fourteenth International Workshop on Bio-Design Automation (IWBDA'22), October 24-26, 2022, Paris.

He has also been invited to give a presentation at the Workshop on New Microscopies for Cell Biology (Dec 13, 2022, Institut Jacques Monod, Paris), at the Research Days of Digicosme (Oct 20, 2022, ENS Paris Saclay, Saclay) and at the French Optogenetics Club (June 23-24, 2022, Toulouse). He has also been invited to a round table discussion at the kickoff event of BioConvergence for Health (BioConvS), a Domain of Major Research and Innovation (DIM) initiative (Oct 27, 2022, Paris).

He also gave a seminar at the groupe de travail sur la biologie systémique symbolique (Bioss), (October 7, 2022, online) and a seminar at Biotechnology department of IFPEN (March 22, 2022, Rueil Malmaison).

Viktoriia Gross made a selected presentation at the FEBS-ENABLE-IUBMB Conference (November 16-18, 2022, Seville).

Allyson Holmes and Henri Galez presented their works at the Computational Biology Department Day at Institut Pasteur in November 28, 2022.

Sara Napolitano made a selected presentation at the Fourteenth International Workshop on Bio-Design Automation (IWBDA'22), October 24-26, 2022, Paris.

Lorenzo Pasotti gave a seminar at the Learning Planet Institute (LPI) in Paris (April 11, 2022) and at the QBIO seminar series at Institut Pasteur (July 7, 2022). He also made a selected presentation at the Fourteenth International Workshop on Bio-Design Automation (IWBDA'22), October 24-26, 2022, Paris.

Jakob Ruess gave invited presentations at Inria Grenoble (invited by team Microcosme), Inria Saclay (invited by team Lifeware) and in the biomathematics seminar at Imperial College London.

10.1.3 Scientific expertise

Gregory Batt has been a reviewer and panel member for the Roux Cantarini Grants awarded by Institut Pasteur.

He has also been part of the Thesis Advisory Committee of Lena Le Quellec (Physical Microfluidics and Bioengineering group) and of Madison Lenormand (Dynamics of the Genome and Physical Microfluidics and Bioengineering group).

Allyson Holmes and Gregory Batt have been part of the jury for the Poster Prizes at the Computational Biology Department Day at Institut Pasteur (November 28, 2022, Paris).

Lorenzo Pasotti was a reviewer for selecting Master and PhD thesis prizes by the Italian Bioengineering Group.

Sara Napolitano has been a reviewer for the IWBDA'22 conference.

Jakob Ruess has been member of the Thesis Advisory Committee of Emrys Reginato, a PhD-student at Inria Grenoble.

10.1.4 Research administration

Gregory Batt is the deputy-director of the Computational Biology Department at Institut Pasteur. He has notably been involved in hiring processes for new group leaders, in the management of the department budget, in the negociations of equipment for the department, in the evaluation of the department by the HCERES committee, and in the co-organization of a number of department-related events.

He is also a member of the Bureau du Comité des Equipes Projets at Inria Paris.

He has been in charge of the successive evaluation of the InBio team by the HCERES committee, by the Pasteur scientific council, and by the Inria evaluation committee.

Sara Napolitano was a member of the organization committee for the Computational Biology Department Day at Institut Pasteur.

Jakob Ruess organized the concluding meeting of the ANR-FWF project CyberCircuits at IST Austria. He also co-organized the Seminar Series of the Computational Biology Department at Institut Pasteur.

Jakob Ruess has been the tutor of two PhD students, and Gregory Batt has been the tutor of one PhD student, all at Institut Pasteur.

10.2 Supervision - Juries

10.2.1 Supervision

Gregory Batt is (co-)supervising two PhD students:

- PhD in progress: Viktoriia Gross, "An integrative approach to characterize the two sides of enzyme mediated antibiotic escape: resistance and tolerance. Started in Oct. 2020. Supervision by Imane ElMeouche, Erick Denamur and Gregory Batt.
- PhD in progress: Henri Galez, "Engineering an autocrine-like system for screening libraries of protein secreting strains in yeast. Started in Sept. 2022. Supervision by Gregory Batt.

Sara Napolitano, Sebastian Sosa-Carrillo, and Gregory Batt supervised the internship of Helene Philippe (M1 student, AgroParisTech).

Sebastian Sosa-Carrillo, Sara Napolitano, and Gregory Batt supervised the internship of Henri Galez (M2 student, MSSB master of Univ Paris-Saclay/Univ. Evry).

10.2.2 Juries

Gregory Batt has been "rapporteur" for the PhD work of Theo Maire (Delft University), and for the HDR of Manish Kushwaha (INRA, Paris Saclay University).

10.3 Popularization

10.3.1 Articles and contents

Jakob Ruess helped write and translate two news articles about his ERC Starting Grant "BridgingScales":

- one article for the Inria website https://www.inria.fr/fr/jakob-ruess-bourse-erc-starting-grant-biologie-cellulaire,
- and one article in the Journal de la recherche of Institut Pasteur https://www.inria.fr/fr/jakob-ruess-bourse-erc-starting-grant-biologie-cellulaire.

Gregory Batt also published a brief article in the Bulletin de l'Institut Pasteur to promote the use of smart experimental platforms in molecular biology labs.

10.3.2 Education

Viktoriia Gross participated to the popularization initiative "ma thèse en 180 secondes" with the IAME laboratory.

The whole team hosted a young intern, Alexandre Raverdy, for a "stage d'observation de 3ème" over a week in February 2022.

11 Scientific production

11.1 Major publications

[1] C. Aditya, F. Bertaux, G. Batt and J. Ruess. 'Using single-cell models to predict the functionality of synthetic circuits at the population scale'. In: *Proceedings of the National Academy of Sciences of the United States of America* 119.11 (10th Mar. 2022), e2114438119. DOI: 10.1073/pnas.211443811. URL: https://hal.inria.fr/hal-03544044.

[2] F. Bertaux, S. S. Carrillo, V. Gross, A. Fraisse, C. Aditya, M. Furstenheim and G. Batt. 'Enhancing bioreactor arrays for automated measurements and reactive control with ReacSight'. In: *Nature Communications* 13 (11th June 2022), p. 3363. DOI: 10.1038/s41467-022-31033-9. URL: https://hal.inria.fr/hal-03744485.

[3] Z. Fox, S. Fletcher, A. Fraisse, C. Aditya, S. Sosa-Carrillo, J. Petit, S. Gilles, F. Bertaux, J. Ruess and G. Batt. 'Enabling reactive microscopy with MicroMator'. In: *Nature Communications* 13 (22nd Apr. 2022), p. 2199. DOI: 10.1038/s41467-022-29888-z. URL: https://hal.archives-ouvertes.fr/hal-03261134.

11.2 Publications of the year

International journals

[4] C. Aditya, F. Bertaux, G. Batt and J. Ruess. 'Using single-cell models to predict the functionality of synthetic circuits at the population scale'. In: *Proceedings of the National Academy of Sciences of the United States of America* 119.11 (10th Mar. 2022), e2114438119. DOI: 10.1073/pnas.211443811. URL: https://hal.inria.fr/hal-03544044.

[5] F. Bertaux, S. S. Carrillo, V. Gross, A. Fraisse, C. Aditya, M. Furstenheim and G. Batt. 'Enhancing bioreactor arrays for automated measurements and reactive control with ReacSight'. In: *Nature Communications* 13 (11th June 2022), p. 3363. DOI: 10.1038/s41467-022-31033-9. URL: https://hal.inria.fr/hal-03744485.

[6] A. Davidović, R. Chait, G. Batt and J. Ruess. 'Parameter inference for stochastic biochemical models from perturbation experiments parallelised at the single cell level'. In: *PLoS Computational Biology* 18.3 (18th Mar. 2022), e1009950. DOI: 10.1371/journal.pcbi.1009950. URL: https://hal.inria.fr/hal-03625706.

[7] Z. Fox, S. Fletcher, A. Fraisse, C. Aditya, S. Sosa-Carrillo, J. Petit, S. Gilles, F. Bertaux, J. Ruess and G. Batt. 'Enabling reactive microscopy with MicroMator'. In: *Nature Communications* 13 (22nd Apr. 2022), p. 2199. DOI: 10.1038/s41467-022-29888-z. URL: https://hal.archives-ouvertes.fr/hal-03261134.

[8] D. Lunz. 'Optimizing Noisy Complex Systems Liable to Failure'. In: *SIAM Journal on Applied Mathematics* 82.1 (5th Jan. 2022), pp. 25–48. DOI: 10.1137/21M1416126. URL: https://hal.inria.fr/hal-03537040.

[9] D. Lunz, J. Frédéric Bonnans and J. Ruess. 'Revisiting moment-closure methods with heterogeneous multiscale population models'. In: *Mathematical Biosciences* 350 (Aug. 2022), p. 108866. DOI: 10.1016/j.mbs.2022.108866. URL: https://hal.inria.fr/hal-03479587.

Reports & preprints

[10] D. Lunz and J. Frédéric Bonnans. *Modelling and optimal control of a two-species bioproducing microbial consortium*. 21st June 2022. URL: https://hal.inria.fr/hal-03479385.

[11] S. Sosa-Carrillo, H. Galez, S. Napolitano, F. Bertaux and G. Batt. *Maximizing protein production by keeping cells at optimal secretory stress levels using real-time control approaches*. 4th Nov. 2022. DOI: 10.1101/2022.11.02.514931. URL: https://hal.inria.fr/hal-03940186.