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# Proceedings of the 23rd International Conference on Computational Methods in Systems Biology

François Fages, Sabine Pérés

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# Computational Methods in Systems Biology

23rd International Conference, CMSB 2025  
Lyon, France, September 10–12, 2025  
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# Preface

This volume contains the regular and tool papers presented at the 23rd International Conference on Computational Methods in Systems Biology (CMSB 2025), held at the University of Lyon, France, in the period September 10–12, 2025.

The CMSB annual conference series was initiated in 2003. The webpage for the conference series is at <https://cmsb.sciencesconf.org/> and for this year's edition is at <https://cmsb2025.sciencesconf.org/>. The previous editions of the conference were held in Rovereto, Italy (2003), Paris, France (2004, 2011), Edinburgh, UK (2005, 2007), Trento, Italy (2006, 2010), Rostock, Germany (2008), Bologna, Italy (2009), London, UK (2012), Klosterneuburg, Austria (2013), Manchester, UK (2014), Nantes, France (2015), Cambridge, UK (2016), Darmstadt, Germany, (2017), Brno, Czech Republic (2018), Trieste, Italy (2019), Konstanz, Germany (2020, online), Bordeaux, France (2021, hybrid), Bucharest, Romania (2022), Luxembourg City, Luxembourg (2023), and Pisa, Italy (2024).

The conference brings together researchers from across the biological, computational, mathematical, and physical sciences who are interested in the modelling, simulation, analysis, inference, design, and control of biological systems. It covers the broad field of computational methods and tools in systems and synthetic biology and their applications in biomedicine and the environment. The conference welcomes new theoretical results with potential applications to systems and synthetic biology, as well as novel applications and case studies of existing methods, tools, or frameworks. Topics of interest to the conference include, but are not limited to: formalisms for modelling biological processes; methods and tools for biological system analysis, modelling and simulation; frameworks for model verification, validation, analysis, and simulation of biological systems; automated parameter and model inference; machine learning and data analytics in biology and biomedicine; data-driven model learning and scientific discovery; high-performance methods for computational systems biology; model integration and biological databases; data-based approaches for systems and synthetic biology; optimality and control of biological systems; design, analysis, and verification methods for synthetic biology; methods for biomolecular computing and engineered molecular devices; multi-scale modelling, multi-agent simulation and analysis methods; and modelling, analysis, and control of microbial communities and ecosystems.

CMSB 2025 received 39 regular and tool paper submissions, of which 34 were sent for reviews. 16 were accepted at first-round review and 5 were accepted after revision and second review. Each paper was reviewed by three program committee members. The tool papers are shorter papers evaluated for the quality of the tool itself, its usability and documentation, in addition to the scientific contribution of the communication. Only one tool paper was accepted this year of three submitted. The papers that were not accepted for publication were all on topic and invited for poster presentation at the conference.

We had four invited talks at CMSB 2025 given by Hervé Isambert (Curie Institute, Paris, France), Franck Picard (ENS Lyon, France), Jörg Stelling (ETH Zürich, Switzerland), and Susana Vinga (University of Lisbon, Portugal). The abstracts of their talks are included in the front matter of this volume. The regular and tool papers presented at CMSB 2025 follow with a classification in five sections: Boolean networks, continuous and hybrid models, rule-based models, model inference and machine learning, and community models and control. Of note this year is the growing number of papers involving machine learning methods in connection with our various modelling frameworks for data-driven model inference or data analysis.

We are very grateful to the members of the program committee, and to all our reviewers for the thoughtful and detailed comments they gave on the articles submitted to the conference. We thank all our sponsors for making possible the lowering of the registration fees to a minimum: the University of Lyon, Inria, CNRS, GDR BIMM, as well as Springer Nature for their sponsoring of the best paper and best student paper awards. We also want to thank the CMSB steering committee and the previous organizers of CMSB conferences for their support in organizing this edition of the conference.

June 2025

François Fages  
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## **Invited Talks**

# Causal Discovery and Network Inference for Biological and Biomedical Data

Hervé Isambert 

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Discovering causal effects is at the core of scientific investigation but remains challenging when mostly observational data is available. In essence, causal discovery infers cause-effect relations from specific correlation patterns involving at least three variables, which goes beyond the popular notion that pairwise correlation does not imply causation. In principle, causal insights may also help improve the explainability and generalizability of AI methods. Yet, in practice, causal representations have been difficult to learn and interpret, in particular, for high-dimensional data such as state-of-the-art biological and biomedical data.

Single-cell transcriptomics and bioimaging techniques produce massive amounts of gene expression and cell imaging data at single-cell resolution. However, this wealth of high-dimensional biological data remains largely under-explored due to the lack of unsupervised methods and tools to interpret them without preconceived hypotheses. This highlights the need to develop novel Machine Learning strategies to better exploit the richness and complexity of the information contained in high-dimensional biological and biomedical data.

In this talk, I will outline some network reconstruction methods and applications to a broad range of biological and clinical datasets. In particular, our group has developed a reliable and scalable causal discovery method, MIIC (<https://miic.curie.fr>), to learn cause-effect relationships in a variety of biological or biomedical data, from single-cell transcriptomic data [1–4] and live-cell imaging data [5] to clinical data from medical records of patients [6–9]. MIIC combines multivariate information analysis with interpretable graphical models [10–12] and outperforms other methods on a broad range of benchmark datasets, in particular on complex non-linear, categorical or mixed-type datasets [9, 10]. MIIC has also been recently integrated in the CausalXtract tool [5] to analyze live-cell time-lapse images of tumor-on-chip data, as well as in the CausalCCC tool [1] to reconstruct gene interaction pathways across multiple interacting cell types from single-cell or spatial transcriptomic data.

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# Kernel-Based Perturbation Testing for Single-Cell Data

Franck Picard 

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The convergence of single-cell biology and high-throughput sequencing technologies has made it possible to generate high-dimensional molecular portraits of individual cells. As a result, the field of single-cell data science has emerged, focusing on the development of statistical and computational tools to analyze the complexity of these datasets. A central challenge in single-cell analysis is the statistical comparison of datasets across conditions or tissues. Comparative analysis is crucial for distinguishing biological variation from technical noise and for detecting meaningful expression or regulatory changes. Traditionally, differential expression analysis (DEA) has been addressed through gene-wise two-sample testing frameworks, but standard approaches often fail to capture the full distributional complexity of single-cell data. Single-cell perturbation experiments add another layer of complexity and opportunity. These experiments compare perturbed and control cells, allowing for fine-grained analysis of population responses to stimuli or drugs. However, the resulting data often exhibit complex, non-linear structures that standard linear methods cannot adequately capture. This calls for methods capable of performing non-linear comparisons and modeling intricate data geometries.

To address these challenges, we propose a kernel-based framework for the differential analysis of single-cell data [1], including perturbation studies [2]. Kernel methods, widely used in machine learning, allow for non-linear operations by embedding data into a reproducing kernel Hilbert space (RKHS)—an infinite-dimensional space in which linear operations correspond to non-linear operations in the input space. This enables powerful and flexible statistical comparisons that are robust to the high dimensionality, sparsity, and noise typical of single-cell data. Our approach captures the distance between distributions by measuring the separation of mean embeddings with respect to biological variability. For perturbation experiments, we further develop a linear model in RKHS and introduce a formal hypothesis testing of high-dimensional data structures in complex experimental designs. We implement our methodology in a software package, *kaov*, available in Python. The package includes tools for visualization and interpretation, and offers a user-friendly interface modeled after popular analysis libraries.

As single-cell datasets continue to grow in size and complexity, traditional statistical methods struggle to uncover subtle but biologically important patterns. Kernel-based testing offers a robust, distribution-free, and non-linear alternative that is well suited to the unique challenges of single-cell data. By accounting for complex dependencies and offering interpretable results, it enables deeper insights into gene regulation, cell

fate, and disease progression, paving the way for biomarker discovery and precision medicine.

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# Predicting Metabolic Interactions Between Multiple Species from First Principles

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Predictive genome-scale metabolic models (GSMs) have become standard tools for systems-level analysis of metabolism in contexts ranging from biotechnology to cancer 3. They rely on incorporating mass balances and potentially other constraints on the operation of metabolic networks at steady-state. In biased methods such as flux balance analysis (FBA), assumptions on biological objectives (e.g., bacteria maximizing their growth rate) allow prediction of specific flux distributions by optimization (e.g., by linear programming in FBA). Unbiased methods for the analysis of all feasible flux distributions are subsumed under metabolic pathway analysis; they typically involve solving large combinatorial problems. However, many interactions in microbial consortia or tissues of multicellular organisms rely on networks of metabolite exchanges, where multiple species interact metabolically via a shared environment. In the multi-species setting, biased methods either require assumptions on community objectives that are biologically questionable, or model augmentations by often unknown kinetics. Unbiased methods are limited by the scalability of current metabolic pathway concepts to multi-species networks 4.

For unbiased methods, we aim to increase predictive power by relying on first principles and by systematically quantifying uncertainties. This involves integrating thermodynamic constraints via probabilistic thermodynamic analysis (PTA) 2, kinetics via ENKIE, a predictor of enzyme kinetic parameters 1, and optimization over enzyme costs. The resulting method, termed global enzyme cost minimization (GECM), combines MCMC sampling with biconvex optimization to allow predictions such as alternative metabolic strategies for *E. coli* growth that are consistent with experimental observations. GECM thereby provides predictions of single-species behaviors and their uncertainty—albeit at high computational cost.

As a potentially complementary unbiased method, we define minimal pathways (MPs) that yield compact representations of metabolic network capabilities 5. They generalize existing pathway concepts by allowing inhomogeneous constraints as well as the targeted analysis of subnetworks. Computationally, enumeration and sampling of MPs is efficient via iterative minimization and pathway graphs. This enables applications such as assessing quantitative gene essentiality in *E. coli* central metabolism, predicting metabolite exchanges associated with homeostasis and health in a host-microbe model of the human gut, and designing butyrate-producing microbial communities.


In combination, we envisage MPs and GECM to predict metabolic interaction networks between multiple species as follows: MPs restrict the set of potential metabolite exchange patterns between individual species and the environment in the community context. Application of GECMs to the reduced problem(s) could then follow to predict actual interactions and their uncertainties. However, this requires improvements to the computational efficiency and numerical stability of GECM. In perspective, this integration could enable predictions of emergent community functions and of experimentally testable interactions for applications such as the human gut microbiome.

**Keywords:** Genome-scale metabolic models · Metabolic interactions · Thermodynamics · Kinetics · Metabolic pathways · Microbiome

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# Reverse Engineering and Optimization of Metabolic Networks

Susana Vinga 

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Understanding the dynamic behavior of cells and their metabolism is a key goal in systems biology. Reverse engineering metabolic processes from data is crucial for uncovering underlying mechanisms, building predictive models, and optimizing networks for strain engineering and biotechnological applications. Here, we present three representative case studies that highlight different modeling and optimization strategies.

Among the various modeling approaches, S-systems provide a remarkably flexible and interpretable framework. They consist of systems of ordinary differential equations (ODEs) based on a power-law formalism, derived from a first-order Taylor series approximation in logarithmic space. This structure makes them well suited for approximating reaction dynamics without relying on detailed mechanistic information. We present a case study of glycolysis in *Lactococcus lactis*, modeled using in vivo NMR multivariate time-series of metabolic concentrations [6]. The structural analysis of the proposed model was initially used to assess parameter sensitivity and identifiability, thereby enhancing model interpretability and significantly improving the subsequent reverse engineering step [5].

While classical ODEs offer a powerful framework for modeling continuous nonlinear dynamics, they typically assume that model parameters remain constant over time. However, many biological processes, such as cell growth under nutrient shifts or environmental changes, may exhibit distinct phases and are therefore better modeled using time-varying parameters. Indeed, these transitions can be more effectively captured using hybrid modeling approaches, which combine continuous dynamics with discrete state changes. In this context, we developed SON-EM, a methodology for parameter identification in switched systems [2], which integrates convex optimization, clustering, and a refinement step to detect switching points and estimate local parameters. Applied to multiphasic cell growth, SON-EM successfully captured diauxic transitions and metabolic shifts using a parsimonious structure based on logistic models, with minimal manual intervention [3].

Optimization also plays a crucial role in designing metabolic interventions. We developed OptPipe, a consensus-based pipeline that integrates several algorithms (including OptKnock, RobustKnock, and RobOKoD) to identify promising gene knockouts and rank them based on growth, target production, and adaptability metrics [4]. Complementarily, we explored multi-objective optimization to identify reaction deletions that simultaneously maximize and/or minimize multiple objectives, using multi-objective

mixed-integer optimization (MOMO), and applied this approach as a proof of concept to enhance ethanol production in *Saccharomyces cerevisiae* [1].

The presented modeling and optimization methods illustrate how mathematical frameworks, algorithms, and biological insights can be combined to understand, predict, and manipulate metabolic networks.

**Keywords:** Systems Biology · Strain Design · Multi-Objective Optimization · Kinetic Modeling · Hybrid models

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